PROCEEDINGS OF THE FORTY-EIGHTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., APRIL 30, 1956

PRESIDENTIAL ADDRESS

THE INDIVIDUAL IN MEDICAL RESEARCH AND THE RÔLE OF THE UNIVERSITY CENTER IN HIS TRAINING

BY A. McGEHEE HARVEY

It is the custom that there be a Presidential address. My thesis is not new. It concerns the importance of the individual in research and of the University Medical Center in his training.

This Society was established as a meeting ground for those interested in clinical investigation in its broadest aspects. If it has a fundamental objective, it is the creation and preservation of a "Heritage of Excellence" in research. The term "heritage of excellence" was coined by Alan Gregg as a penetrating expression of the abiding influence of Dr. Welch and his associates on the spirit of *creative* scholarship at the Johns Hopkins Medical Institutions.

It is important for University clinics to provide and to preserve opportunity for the training of the individual in clinical research, to promote *creative* scholarship. What does this entail? As Dr. Gregg has expressed it: "Men of superior character and capacity should have *freedom, responsibility* and *expectation.*"

There are many men on the threshold in medicine today who have the right qualities of character and ability in abundance. It is the responsibility of University Centers to maintain in full measure the opportunity for their development as investigators and teachers. Arnott has said: "It should be a basic article of faith that critical scholarship and search for new knowledge is the primary aim of the University. Not merely actual discovery, not merely even the attempt to discover, but the creation and cultivation of the spirit of discovery." This spirit should permeate the Medical Center. Granted that the combination of circumstances is such that only a *few* will achieve outstanding discoveries, everyone of us can promote such discoveries by facilitating fruitful associations with the so-called "pure scientist" and by criticism and discussion foster the spirit of inquiry.

As was pointed out from this rostrum not long ago, medical progress is in the logarithmic phase of growth. What has been the effect of this upon the ability of University Medical Centers to provide *freedom, responsibility* and *expectation* and to maintain an environment really conducive to *creative scholarship* on the part of young physicians?

I shall draw upon a crude analogy to present the situation as I see it and take as the model of a University Medical Center the Citric Acid cycle, the final common pathway for the oxidation of all three major food stuffs. It is the major source of body energy just as the University Center is the medical storage battery for the training of investigators.

The University is represented by a single active cell. Within the University are schools indicated by mitochondria, one of which is the University Medical Center. (Fig. 1, A and B.) When the intact mitochondrium is working in the presence of molecular oxygen, oxidation of food stuffs is completed, phosphorylation occurs, and ATP is formed. To carry out *its* objectives completely, the Medical Center must have molecular oxygen in the form of strong academic and financial support. The mitachondrial membrane is *selectively* permeable; only those factors essential to operation of the cycle gain entrance. In the Medical Center it is important to *select* good candidates for a career in medicine and to *select* a faculty that will foster in the student a keenness for self education and a desire to contribute to the search for new knowledge.

Let us look upon the cycle itself as the complex format for training students and investigative recruits fed into the University Medical Center. The cycle is self-perpetuating, constantly replenishing itself and constantly furnishing energy. Just as the cycle supplies 95% of the body's energy, the University Center is the major source of trained investigators for the total realm of medicine. The fat, protein and sugar feeding the cycle represent those qualities that determine selection of the prospective candidates represented by *Acetyl-CoA.*

The candidate investigators must have intelligence, integrity, a suitable personality, and ability to communi-

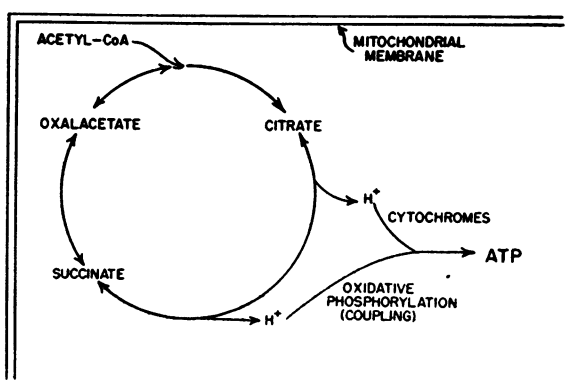

FIG. 1A

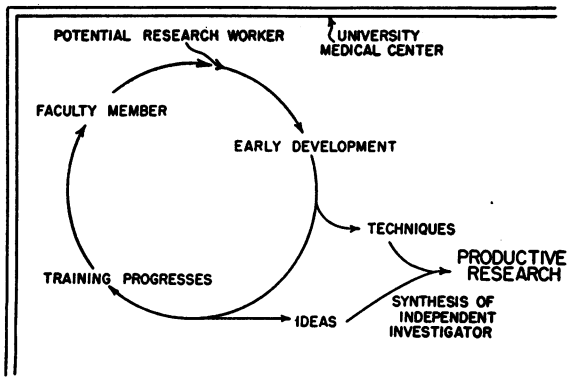

FIG. 1B

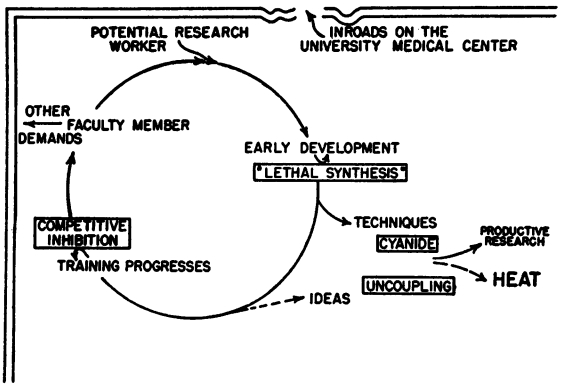

FIG. 2B

cate. Curiosity, initiative, and constructive imagination are essential. They should be resourceful and capable of self discipline. A questioning attitude to balance imaginative enthusiasm and a real measure of critical judgement complete the picture. The student or young investigator joins with the teacher (oxalacetate) and proceeds through the training cycle.

There are specific conditions which must be maintained if these integrated enzymatic reactions in the cycle are to progress optimally. Productivity requires *time* and *freedom* admixed with *responsibility—freedom* to express one's ideas, *freedom* to choose one's own research problems, and *opportunity* to teach and take *responsibility* for the care of patients without compulsion to do so at too great expense of time for research. *Freedom, responsibility* and finally *expectation;* the expectation of backing without strings, and contact with stimulating colleagues.

At several points in the cycle, electron transport to the cytochrome system occurs with oxidation and the creation of *high energy phosphate bonds.* The progress of a young investigator in developing new ideas and fostering them by well planned experiments is similarly punctuated by reports of his studies at meetings such as this one and finally culminates in a well written paper. Proper coupling is necessary for oxidative phosphorylation to occur with the greatest efficiency. The closer to optimal the conditions, the higher the P/O ratio in the cycle—the greater the amount of worthwhile research for the input of funds and facilities.

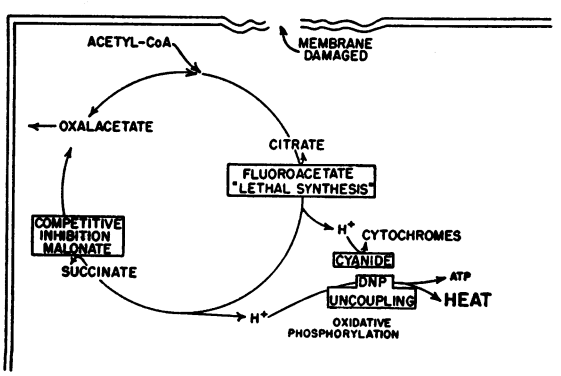

FIG. 2A

It is to be emphasized that mitochondria are the *only* elements of the cell that carry out the *complete* oxidation of carbohydrate, fat and protein just as the University Medical Center is the *only* medical environment which exists for the *complete* training of young investigators.

The ways in which the effective activity of the cycle can be blocked are many. (Fig. 2, A and B.)

The reaction rate is slowed by the removal of exalacetic acid (the teacher). The contributions of a senior staff are reduced proportionately by time spent in administration, so-called postgraduate training, society meetings, and, of course, committees.

Competitive inhibition of succinate by malonate is exemplified by the curtailment of research activities of the young investigator imposed by too many teaching or routine responsibilities necessary to supplement his remuneration. After our candidate has almost completed the cycle, the final step into a productive research career can be blocked by financial pressures to enter practice as surely and completely as cyanide can paralyze cytochrome oxidase. Furthermore, oxidation can occur without phosphorylation. Uncoupling of oxidative phosphorylation results from certain drugs such as dinitrophenol. In this circumstance there is generated unusable energy in the form of heat. Flitting from one problem to another, or taking on too many diverting functions like dinitrophenol, may produce enough heat to result in potboilers, but real research accomplishment suffers.

The recognition of these metabolic antagonists to creative scholarship is an easy matter in the gross, but there are subtle and insidious forms of inhibition that are not so obvious. *Fluoroacetate* is unusual. Its action is not like that of iodoacetate, which combines with SH groups, and no enzyme is known to be poisoned by it. Its action is one that Peters has termed *"lethal synthesis."* The non-toxic fluoroacetate is converted to fluorocitrate which blocks aconitate and the result is "jamming" of the cycle at the level of citric acid which then accumulates in the tissues. The cell fails to recognize that it is poisoning itself—too much of a good thing.

Even with good departments and a fine professional staff in a school, the production of well trained investigators can still be blocked. What are the sources of "lethal synthesis" in the Medical Center? The answers go right to the root of our research structure and affect the young investigator from his earliest medical experiences.

Common interest in the study of a given problem, with different techniques to contribute and from different points of view, that arises spontaneously from a real desire to share experiences and trade ideas, is obviously advantageous. Furthermore, the point is often reached where it is desirable and necessary to establish practical clinical applications by organized study, in patients, of experimental procedures devised in the laboratory.

When the "team" method of investigation, however, becomes a routine research activity in and of itself, the unique contributions of the individual investigator suffer a lethal blow.

Although, in many cases, the young investigator can profit greatly by apprenticeship in a team with full intellectual participation, he should be encouraged to tackle an independent problem right from the start, preferably a problem of his own choosing, not necessarily related to the group project. Only in this way can one assume an *alternative pathway* to guard against lethal synthesis.

There is a growing tendency for our training programs to become stereotyped—to turn out "board-eligible" products. The one way to assure that there will be no more Minots, or Peabodys is to load the formative years, when ideas and initiative should be given freedom to develop, with rigidly formal training.

In the training of house-offices, too much emphasis has been placed on programs of a relatively didactic nature. Here again practical experience in an atmosphere of good patient care, allowing the young man the maximum in responsibility is essential. As professors grow older, it seems harder for them to remember that knowledge gained by first-hand experience under skillful *guidance* is of far greater value than listening to the recapitulation of someone else's first-hand experience. The lethal synthesis of an excess of didactic exercises and meetings waste the time and sap the strength of the whole spectrum of medical personnel.

Real progress comes from new approaches, and new approaches come from men whose personal experience gives them an outlook different from the rest. It is easier to squelch such individuals at an early age than to make it possible for them to develop their own abilities. We seem to be heading this way with the long period of undergraduate preparation, the tightly packed medical curriculum, the years of prescribed postgraduate training for Boards, the unpredictable interruption of two years of Army Service, and the heavy clinical, administrative and teaching chores that are almost routinely expected of younger men in a department.

Are we not also calling on our Senior Faculty men to take on outside duties in far too great measure? The reasons for the decline in scientific effectiveness—the "investigative climacteric" which seems almost characteristic of graduation from this society—are many. Some no longer want to bother with the tribulations of personally conducted experimental studies. Administrative duties, membership on local and national committees, and the ever increasing demand to take part in additional educational exercises before local, state and national medical groups are obviously important but can also become socially acceptable excuses. A great scientific potential in the laboratory, the result of years of very expensive training, is soon lost. It is of utmost importance for the older investigator to maintain *active* contact with his laboratory. Without the continued intellectual stimulation of close association with young men, he is soon in no proper position to influence the training of investigators, even at the level of making so-called "policy."

The bulwark of defense against these and other factors which can cause a seemingly ideal environment for the creation of investigators to commit "lethal" synthesis, is sound and ample financial support of University Medical Centers. Acquisition of general funds for medical schools is easier said than done. A very discouraging statement appeared in the Lancet on January 7, 1956: "It is curious that in the United States, the wealthiest country in the world, medical schools seem to have financial troubles as great as, or greater than our own. At least half of them are said to have budget deficiencies or to need large sums to keep up their standards and yet there are immensely wealthy foundations in a position to spend money on a lavish scale. Sometimes, of course, the money given by the public can be used only in some specific direction such as cancer, psychiatry and poliomyelitis and the public does not necessarily give most generously to the most necessary purpose."

The money spent for national defense is more than 40 billions. Only 4 billions are spent for research and development of which only about 5% or 240 million goes to medical research in the broadest terms. Only about 80 million of this goes to support teaching and research in medical schools, and only a fraction pays its way in terms of overhead costs or can be used freely for the development of basic facilities.

Cutler recently gave figures from the Harvard Medical School which serve as an illustration. In 1954 there was expended from the funds of the Harvard Medical Center for research a total of $5,130,000. A fair overhead charge on this sum was about $1,500,000. But the various sources of this money for *direct* research expenses provided only $513,000 for *indirect* expenses. Accordingly, about a million dollars had to be taken from already strained general funds. As Cutler stated this is the way to commit medical research suicide. It is alternately the way to throw the burden to the young men who must fulfill some type of routine function for financial support making it difficult to attain the leisure and academic freedom necessary for his ideal development as an investigator.

There are several things that would help considerably. The Federal Government might adopt in the future an overall policy of more realistic reimbursement of overhead expense to grantees as it does to contractees of Federal research funds. A greater percentage of the

Federal money for *general* support of research and research training in University Centers and less of the total for *categorized* research is highly desirable. Large amounts of money are collected for medical purposes which is not utilized to the fullest extent desirable to meet these basic needs of the training institutions. I refer to the "special disease funds." These could go far towards correcting the situation by allotting 15–20% of their collections to independent schools as unrestricted or fluid funds. Important research in their specific areas of interest, far from suffering, would be enhanced by such a policy. If this practice became general, the funds might be pooled and administered under a sound formula set up by a non-partisan body such as the National Research Council.

Finally, it is to be emphasized that research is only a part of the entire medical structure. As Means put it: In serving the ultimate purposes of medicine, *research, education,* and *practice* must be sweetly blended. Within this triad there is mutual stimulation in all directions. For this reason, an optimum location for medical research and medical research training is the medical school and its affiliated teaching hospitals. It is here that the basic opportunity unfettered by regulations on research money should be provided. *The medical schools must be gotten out of their relative poverty in the midst of plenty.*

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ABSTRACTS

Protection Against Brucella with Heterologous Endotoxins. ROBERT S. ABERNATHY and WESLEY W. SPINK,* Minneapolis, Minn.

The injection into mice of sub-lethal amounts of endotoxins prepared from Gram-negative bacteria results in increased resistance to subsequent challenge with lethal amounts of endotoxin. This resistance persists for at least ten months. The specificity of this resistance has also been investigated. Groups of mice were immunized by three weekly injections of one of the following endotoxins: *Brucella melitensis, Salmonella typhi, Escherichia coli,* or *Shigella flexneri.* Thereafter, at intervals ranging from one to sixteen weeks, subdivisions of each group were challenged with lethal amounts of each of the four endotoxins. Protection against the homologous immunizing endotoxin was maximal in each group. In addition, significant protection against brucella endotoxin was conferred for sixteen weeks by immunization with the heterologous endotoxins. No other consistent heterologous protection was found. In each group of immunized animals, serum precipitins were uniformly present only for the homologous immunizing endotoxin. However, intradermal reactions to the various endotoxins were frequently nonspecific. In addition to protection against brucella endotoxin, immunization with the heterologous endotoxins also conferred resistance to massive lethal doses of viable *Br. melitensis.*

The resistance to endotoxin following preliminary injections has previously been ascribed to a nonspecific hyperactivity of the reticuloendothelial system. The duration of the observed heterologous protection and its selectivity for brucella suggest that factors other than hyperactivity of the reticuloendothelial system are important.

Formation of Tetraiodothyroacetic Acid from Thyroxine by Rat Kidney Mitochondria. EDWIN C. ALBRIGHT, FRANK C. LARSON, KENKICHI TOMITA, and HENRY A. LARDY, Madison, Wis. (Introduced by Edgar S. Gordon).

The intracellular metabolism of thyroid hormone was examined using I^{131}-labelled thyroxine as a substrate. Previous experiments with rat kidney have shown that thyroxine is metabolized by the mitochondria. A soluble enzyme was prepared by dialysis of sonic disintegrated mitochondria. The reaction mixture containing phosphate buffer (pH 7.4), ATP, DPN, succinate, and thyroxine was incubated at 37° C. for three hours. Following incubation the reaction mixture was extracted with butanol-ammonia. The butanol extracts were analyzed by paper chromatography. Three labelled reaction products uniformly appeared. The principal product (U_1) was eluted from the paper and rechromatographed. The compound gave positive diazo and Kendall reactions, negative ninhydrin and 2,4-dinitrophenylhydrazine reactions. The chromatographic mobility of U_1 was compared with several known iodinated thyronine compounds in three different solvent systems. In each system it had a mobility identical with tetraiodothyroacetic acid. Tetraiodothyropropionic acid, which has a similar chromatographic mobility in the solvent systems used, was differentiated from U_1 by determination of specific activity in recrystallization experiments.

On the basis of these findings, unknown 1 is, therefore, presumed to be tetraiodothyroacetic acid.

The other two labelled reaction products appear to be intermediaries. One of these has been tentatively identified as thyroxamine. The other has not been identified.

Pharyngeal Swallowing Mechanisms in Normal Subjects and in Patients with Neuromuscular Dysphagia. MICHAEL ATKINSON, PHILIP KRAMER, STANLEY M. WYMAN, and FRANZ J. INGELFINGER,* Boston, Mass.

The mechanisms of swallowing are controversial: some maintain that the bolus is simply advanced under positive pressure; others, finding negative intrapharyngeal pressures, believe suction occurs.

During swallowing of radioopaque liquids, we have recorded simultaneously intraluminal pressures from 3 sites in pharynx and esophagus, through open-ended tubes, whilst serial radiographs (2 to 12 per sec.) in anteroposterior and lateral planes are taken.

Basal pressure is near atmospheric in the pharynx and several millimeters of mercury lower in the esophagus. Between these zones is a 3 cm. segment where basal pressure exceeds intraesophageal pressure by 10 to 60 mm. Radiographs indicate this sphincteric segment is at the cricoid level.

On swallowing, intrapharyngeal pressure shows two major peaks: the first corresponds with the entry of the bolus; the second, which develops after the bolus has passed, with constrictor contraction. Lower in the pharynx, the interval between these peaks lengthens; the first diminishes and the second increases in size.

As the bolus approaches the high basal pressure segment, there is an abrupt fall to, but not below, basal intraesophageal pressure. After the bolus passes an equally abrupt return to initial pressure occurs. Radiography indictates these changes coincide with sphincteric opening and closing.

After bulbar poliomyelitis, deglutition pressures are low, and in the upright posture swallowing may be passive with no intrapharyngeal pressure rise. The sphincter, however, functions normally and does not delay the bolus.

As pressures lower than intraesophageal pressures do not develop before or during the passage of the bolus, swallowing does not involve suction. Although facilitated by contraction of the constrictors, swallowing is possible without constrictor activity. Achalasia of the

sphincter is apparently not a factor in dysphagia following bulbar poliomyelitis.

Studies on Phospholipid and Cholesterol Metabolism in Liver and Kidney of Rats with Experimental Nephrotic Syndrome. PETER R. BALLY, Boston, Mass. (Introduced by Kendall Emerson, Jr.).

The cholesterol and phospholipid content of liver, kidney and plasma of exsanguinated rats in which nephrotoxic serum nephrosis had been induced was determined at various intervals after serum injection. Rats injected with normal rabbit serum served as controls.

It was found that the cholesterol content of the liver increased steadily to a maximum, which was reached at about the sixth day, and was roughly 50 to 100 per cent higher than the value in the control animals. There was no significant rise in cholesterol content of the kidney. The phospholipid content showed no significant change in either liver or kidney at any stage of the disease.

Preliminary data on P^{32} uptake into rat liver phospholipid phosphorus in terms of relative specific activity (ratio of specific activities of lipid phosphorus and of acid soluble phosphorus) in intact rats and of phospholipid phosphorus specific activity in liver slices showed no significant difference in nephrotic and control animals.

These data suggest that the hyperphospholipemia which parallels hyperlipemia in these animals is not due to increased synthesis.

Inspection of paper electrophoretic strips of nephrotic rat sera taken at various intervals after toxic serum injection and stained for total lipids and for proteins respectively showed that the changes in lipid pattern occurred as early as the change in protein pattern.

The Sequential Probability Ratio Test, A New Statistical Procedure for Clinical Investigation; Its Validation in a Study of Serum Zinc Concentrations in Laennec's Cirrhosis. ANTHONY F. BARTHOLOMAY, Boston, Mass. (Introduced by Eugene C. Eppinger).

Recent experiments in this laboratory have been designed to detect differences in the serum zinc concentrations in normal and cirrhotic individuals. The decision on the number of observations required to differentiate an abnormal from a normal population is a universal problem of clinical investigation, and it arose in our study. A solution to this and the general problem was found by a new adaptation of the powerful "Sequential Probability Ratio Test" (S. P. R. T.). It is governed by the imposition of arbitrary levels of significance in advance of the experiment. After each observation a simple arithmetic procedure indicates whether or not enough evidence has accumulated to accept or reject the original hypothesis, thereby terminating the experiment. Conversely, the calculations also indicate the necessity of further experimentation.

Present methods of statistical analysis are all *"a posteriori"* in nature and therefore give no indication, *while the experiment is in progress,* of whether or not enough data are at hand. The S. P. R. T., however, is an integral part of the experimental procedure, which indicates both the accuracy and sufficiency of the data.

In the particular example studied, the mean normal serum zinc concentration was 120 ± 19 μgm. per 100 cc. After only nine observations the S. P. R. T. indicated that the corresponding concentration in cirrhosis is below 82 μgm. per 100 cc., 2σ less than the normal mean. To validate this conclusion the zinc concentration was measured in a total of 28 sera. The mean of this group was 66.7 ± 19.2 μgm. per 100 cc., a most significant deviation from the normal value, and corroborated the prediction made by the S. P. R. T.

This study demonstrates the first use of the S. P. R. T. in a clinical experimental design. The results indicate its superiority over the classical statistical construct.

The Role of Extracellular Fluid Volume in the Control of Aldosterone Secretion in Man. FREDERIC C. BARTTER,* GRANT W. LIDDLE, LEROY E. DUNCAN, and CATHERINE DELEA, Bethesda, Md.

It is established that removal of body sodium by diuretics or dietary restriction produces rises in urinary aldosterone. Conversely, in sodium-depleted subjects administration of sodium decreases aldosterone excretion. Rapid falls and gains, respectively, in body weight, and thus in body fluid volume, accompany these changes. Studies were therefore designed to clarify the role of fluid volume in mediating changes in aldosterone secretion. Fifteen normal subjects and one each with diabetes insipidus, panhypopituitarism, and hypoproteinemia were studied on balance regimen.

Sodium-depleted normal subjects were given water loads while sustained antidiuresis was produced with Pitressin®. When body fluids were thus expanded, creatinine clearance increased, but urinary aldosterone decreased, urinary sodium increased, and urinary potassium decreased—changes indicating decreases of circulating aldosterone. Normal, diabetes insipidus, and hypopituitary subjects were subjected to dehydration without sodium deprivation. When body fluids were thus contracted, creatinine clearance decreased, but urinary aldosterone increased, urinary sodium decreased, and urinary potassium increased—changes indicating increases of circulating aldosterone. Thus, aldosterone secretion can be controlled by a function of fluid volume, independent of sodium.

In any individual, simple dehydration was, volume for volume, much less effective in raising urinary aldosterone than "extracellular dehydration" produced by mercurials. Sodium-depleted normal subjects were given hypertonic saline to expand extracellular volume without weight gain, simultaneously contracting intracellular volume. Urinary aldosterone decreased.

In the subject with hypoproteinemia, expansion of circulating volume was produced, without change in sodium intake or gain in body weight, by daily intravenous administration of albumin. Urinary aldosterone decreased

markedly, and urinary sodium increased. When albumin was discontinued, these changes were reversed.

Whereas potassium loading and ACTH also regularly produce rises in urinary aldosterone, the above effects could be produced without significant changes in serum potassium or urinary corticoids, indicating a cardinal role of some extracellular fluid volume in controlling aldosterone secretion.

The Conversion of Reduced Uroporphyrin I to Coproporphyrin and Protoporphyrin by Dog Liver Homogenate. FOUAD BASHOUR, Minneapolis, Minn. (Introduced by S. Schwartz).

The conversion of porphobilinogen to uro-, copro-, and protoporphyrin by nucleated red cells and by liver homogenate has been previously demonstrated. While the interconversion of porphyrins in this order is logical, the majority of evidence has not supported such a concept. The possibility has been suggested that interconversion may occur only when the porphyrin is in the reduced "proporphyrin" or "porphyrinogen" stage. Support for this concept has recently been reported by Aldrich and co-workers who showed, by indirect means, conversion of uroporphyrinogen III to hemin.

N^{15} labelled uroporphyrin I was reduced with sodium amalgam and the porphyrinogen was immediately incubated with freshly prepared dog liver homogenate. An equal amount of homogenate was incubated similarly without added porphyrin.

Following incubation, 5.5 mgm. of crystalline coproporphyrin I methyl ester were isolated. Identification was established by the characteristic melting point (250–52° C), visible absorption spectrum, infra-red spectrum, and paper chromatography behavior.

Four and five-tenths mgm. of carrier coproporphyrin I was added for N^{15} analysis. The corrected value for the N^{15} atom per cent excess of the original 5.5 mgm. was 1.9 compared to a theoretical value of 3.1.

The extracted hemin was converted to mesoporphyrin, esterified, and purified by repeated calcium carbonate chromatography. A fraction was obtained whose melting point of 163–5° C and infra-red spectrum were in agreement with those of synthetic mesoporphyrin I. No such fraction was obtained from the control liver incubated without added uroporphyrinogen I.

These results document for the first time the finding that uroporphyrin I precursor is converted to coproporphyrin I and probably to protoporphyrin I by liver homogenate.

Abnormality of Estrogen Metabolism in Men with Myocardial Infarction. WILLIAM S. BAULD, IAN G. MILNE, and MORRIS L. GIVNER, Montreal, Canada. (Introduced by Douglas G. Cameron).

Myocardial infarction is rare in women before the menopause. This suggests a protective action of the functioning ovary. Previous investigations concerning the mechanism of this protective action have been chiefly limited to the relationship between estrogens, blood lipids and coronary atherosclerosis. In this study, a new chemical method of urinary estrogen assay was used to compare estrogen metabolism in men with and without previous myocardial infarction.

The urinary excretion of estriol, estrone and estradiol-17β was measured over a control 24-hour period. Estradiol-17β (400μg.) in oil was then injected intramuscularly. The increase in excretion of the 3 estrogens over the control levels was determined in 4 subsequent 24-hour periods.

The total amount of estrogen excreted as estriol, estrone and estradiol after injection of estradiol was similar in the two groups. However, there was marked difference in the relative proportions of estriol and estrone excreted. The urinary estriol: estrone ratio was significantly higher ($P = < 0.01$) in 15 subjects with previous myocardial infarction (mean 5.5, range 2.4 to 16 in 14 of the 15 subjects; subject 15, 0.8) than in the 14 subjects with no evidence of myocardial infarction (mean 1.5, range 0.3 to 1.9 in 13 of the 14 subjects; subject 14, 4.2).

The findings are not related to age or to the interval after infarction. None of the subjects had demonstrable renal disease. Three cases with coronary insufficiency but without evidence of myocardial infarction had normal ratios (1.7, 1.5, and 0.5). One of these has since died and autopsy showed extensive coronary atherosclerosis but no myocardial infarction.

Nephrosis in Adults: Results of Steroid Therapy. JAMES H. BAXTER,* HOWARD C. GOODMAN, and JACK ORLOFF, Bethesda, Md.

Nine adults with idiopathic nephrotic syndrome of insidious onset have been treated with steroids and followed for 6 months to 2 years. All were grossly edematous. Microscopic hematuria was observed in all. Most patients received prednisone 40 mg. or hydrocortisone 160 mg. daily for a month or more. One to 3 additional courses with higher dosage were subsequently administered to 4 patients; some further improvement in 3 could not be attributed with certainty to the increase in dosage. The responses may be divided into 4 groups: (1) Complete remissions occurred in 2 patients; one subsequently developed proteinuria but is well now. (2) Complete remissions except for some residual proteinuria were accomplished in 3 patients. One of these had a temporary relapse. (3) Substantial improvement occurred in 2 additional patients, but proteinuria and mild hypoproteinemia have persisted. Diuresis occurred during therapy in all of the patients discussed thus far. Sustained improvement in initially reduced glomerular filtration rate occurred in several. (4) The remaining 2 patients had no change in proteinuria or plasma proteins, although a diuresis occurred on discontinuing therapy.

Two additional adults were treated who differed from the above patients by the presence of gross hematuria and blood casts, and only slight hypoproteinemia and questionable edema. No dramatic change occurred with

therapy, but subsequent gradual improvement occurred in one.

The rate of clinical remissions in these adults does not differ greatly from that in a group of children treated simultaneously. We have seen little to indicate that the disease in adults is fundamentally different from that in children. These results in adults, in whom the prognosis is generally considered grave, provide some evidence that nephrosis is favorably influenced by steroid therapy.

Production of Hypertonic Urine in the Absence of Pituitary Antidiuretic Hormone. ROBERT W. BERLINER * and DOUGLAS G. DAVIDSON, Bethesda, Md.

The experiments to be reported indicate that antidiuretic hormone (ADH) is not essential for production of hypertonic urine. The rationale is as follows: If urine is rendered hypertonic by a final step, removal of water from essentially isotonic preurine, this mechanism might be independent of ADH, the latter assuring isotonicity of preurine delivered to the concentrating mechanism. Restriction of sodium and volume reaching the diluting segment should, then, so reduce the extent of dilution that even in absence of ADH a small volume of only slightly hypotonic preurine would be delivered for concentration; hypertonic urine might result.

A bladder-splitting operation in dogs permits collection of urine from the separate kidneys. A cuff, inflatable from outside through a polyethylene catheter, is placed on the right renal artery. Subsequent experiments are performed on the trained, unanesthetized animal during water diuresis induced by oral water plus infusion of dilute mannitol solution. In satisfactory preparations measured renal functions are essentially the same on the two sides during control periods. On inflation of the cuff, every measurable reduction of GFR is accompanied by a rise in osmolality on the constricted side. With adequate reduction of GFR (30 to 70 per cent) urine becomes hypertonic, sometimes exceeding 450 mOsM. Continued maximal water diuresis from the unconstricted side and similar threshold on both sides to exogenous ADH indicate ADH is not responsible for increased urine concentration on the constricted side. Hypertonic urines occur with only minimal depression of PAH clearance and no divergence of creatinine and inulin clearances. Excretion of sodium and chloride is negligible on the constricted side.

Beyond implications concerning the mechanism of action of ADH and the processes of urine dilution and concentration, the experiments are pertinent to clinical situations where failure of water diuresis is associated with low GFR and/or marked sodium retention.

The Reduction of Blood Ammonia Levels by Certain Amino Acids. SAMUEL P. BESSMAN, Baltimore, Md. (Introduced by Theodore E. Woodward).

A number of amino acids can produce a lowering of blood ammonia in the normal individual and in the patient with elevated blood ammonia due to various causes. Data will be presented showing that asparagine lowers blood ammonia in normal individuals by thirty to fifty per cent. Variable effects occur in hepatic disease, and data implying the deamidation of asparagine in the brain *in vitro* will be reported. Arginine can lower normal blood ammonia levels, and can cause a significant depression of the elevated levels in acute hepatic failure. The effectiveness of glutamate in lowering elevated blood levels of ammonia in patients with normal liver function and in those in hepatic failure will be demonstrated. It appears as if the amino acids which lower blood ammonia fall into two classes—those which participate in the synthesis of urea, and those which are involved in glutamine formation. Both of these ammonia utilizing mechanisms require enzymes which are present mainly in the liver and, for this reason, none of *these* agents for lowering blood ammonia can be expected to be markedly effective in the treatment of hepatogenous ammonemia. The greater effectiveness of glutamic acid upon the ammonemia of patients in whom coma must be provoked, than upon the ammonemia of spontaneous coma, is clearly due to the greater damage of the enzyme mechanisms in the former group and need represent no other difference. This is the only significance of the response to glutamic acid therapy. The above amino acids can be useful for the reduction of the blood ammmonia in the many other situations in which ammonia contributes to the clinical picture. This will be illustrated by several examples. The relation of these data to the mechanism of the cerebral toxicity of ammonia will be discussed.

In Vitro Studies of the Stability of Red Cell Glutathione: A New Test for Drug Sensitivity. ERNEST BEUTLER, Chicago, Ill. (Introduced by Leon O. Jacobson).

The unique susceptibility of certain individuals to the hemolytic action of drugs such as Primaquine, sulfanilamide and acetanilid has been shown to be due to an intrinsic defect of the erythrocyte.

Recently, it was demonstrated that the glutathione content of sensitive erythrocytes was lower than that of non-sensitive erythrocytes. When drug was administered to sensitive individuals, the erythrocyte glutathione level fell rapidly to about half the original value; in normal individuals the glutathione content remained unaltered. In the present study, it has been possible to reproduce this phenomenon *in vitro*. When whole blood from a non-sensitive individual is incubated with acetylphenylhydrazine, phenylhydrazine, Primaquine, or ascorbic acid, the glutathione level may fall slightly, remain the same, or even rise; when whole blood from a sensitive individual is incubated under the same conditions there is a precipitous fall in the already low glutathione level. Based on this finding a standardized method of incubation with acetylphenylhydrazine was developed. Application of this technique to the blood of a small group of known sensitive and known non-sensitive individuals indicated that its use unequivocally differentiates sensitive from non-sensitive subjects. Examination of the blood of a large number of individuals, both normal and with

a variety of diseases, confirmed the clear-cut nature of the differentiation into two groups under large scale survey conditions.

The preliminary results of further studies of this phenomenon have suggested that the acetylphenylhydrazine-induced fall in glutathione level may require the presence of oxyhemoglobin. It therefore appears that this technique should not only permit accurate field surveys of the incidence and genetics of this red cell defect but may also provide a basis for further *in vitro* study of the enzymatic mechanism of this type of drug sensitivity.

The Inactivation of Isoniazid and its Relationship to the Emergence of Isoniazid Resistance. J. PARK BIEHL and HETTIE B. HUGHES, Cincinnati, O. (Introduced by Morton Hamburger).

Isoniazid is extensively inactivated in man. Several end products are formed, none of which has significant antibacterial properties. The amount of a dose appearing in the urine as free isoniazid varies within a group of subjects from 0.6 to 25 per cent, and remains constant in any individual. The level of urinary excretion of free isoniazid has been found to correlate with the concentration of free isoniazid in the plasma. Thus it is possible that in certain patients the concentration of isoniazid at its site of action is suboptimal.

An attempt has been made to correlate this variability in excretion with the emergence of isoniazid resistant tubercle bacilli in tuberculous patients being treated with isoniazid. We have also tried to compensate for the inactivation of isoniazid by using it in higher doses, combined with pyridoxine to prevent peripheral neuritis. This work is still in progress.

Patients were assigned randomly to regimens consisting of the conventional 200 to 300 mg., or of 20 mg. per kg. per day of isoniazid, plus streptomycin. Urinary assays for isoniazid and its metabolites, and sputum cultures with drug susceptibility determinations, were done prior to treatment and monthly thereafter.

Forty-seven patients have received treatment for at least 6 months on the conventional dose of isoniazid. Eighteen excreted 4 per cent or less of their dose as free isoniazid, and in 8 isoniazid resistant tubercle bacilli emerged. Twenty-nine excreted 4.4 to 25 per cent as free isoniazid and resistance appeared in only 3. Of 22 patients reaching 3 months on 20 mg. per kg. daily, none have shown resistance, contrasted to 7 of 49 on the conventional dose who had shown isoniazid resistance by that time.

Isoniazid resistant tubercle bacilli thus may be more likely to appear in patients who inactivate isoniazid more excessively.

Leukocyte Production and Delivery in the Leukemias of Man. HOWARD R. BIERMAN,* KEITH H. KELLY, FAUNO CORDES, and RALPH L. BYRON, JR., Duarte, Calif.

Quantitative knowledge of leukopoietic activity in the normal and leukemic subject is essential for clarifying the basic nature of the leukemias and to determine the fundamental goal for therapy.

Rapid and continuous withdrawal of leukocytes from the peripheral circulation (leukophoresis) by the Cohn Blood Fractionator was employed to estimate the characteristics and capacity of leukopoiesis in the bone marrow and rate of delivery of leukocytes into the peripheral circulation of man. Nine patients (4 hematologically normal and 5 leukemic) underwent leukophoresis on 19 occasions.

In the subjects without leukemia, within 4 hours after leukopheresis was initiated, an increased leukopoietic activity in the bone marrow with increased percentage of immature granulocytes was observed and persisted for 24 to 48 hours thereafter. The discharge of leukocytes from the readily available reservoirs during leukophoresis varied from 3 to 15×10^6 per minute per Kg. body weight. Frequent differential counts permitted estimations for the various types of mature and immature leukocytes.

In the 5 leukemic patients, discharge of leukocytes from the tissue reservoir into the circulation during leukophoresis varied from 13 to 100×10^6 per minute per Kg. body weight.

Leukopheresis on 3 occasions within a 40-day period, in one individual with acute granulocytic leukemia, at control levels of 60,000; 110,000; and 220,000 WBC per cu.mm. and with rates of withdrawal of 50, 70, and 200×10^6 WBC per minute per Kg. body weight, respectively, disclosed that the rate of leukocyte production and delivery after leukophoresis was slower than observed in the normal subjects. Similar findings were observed in 3 of the remaining 4 patients.

In the leukemic patients studied, although the tissue reservoirs had accumulated more leukocytes than in the normal subjects, the rate of leukocyte production was the same or less than that found in the hematologically normal subjects.

Salt Excretion and the Control of Glomerular Filtration. WILLIAM D. BLAKE,* Portland, Ore.

Change in glomerular filtration rate (GFR) is considered one mechanism for regulating renal excretion of sodium, but little is known about control of GFR. In these studies on dogs conditions were designed specifically to cause renal sodium conservation to determine if GFR decreased and by what means. Studies were done on dogs with and without anesthesia using usual clearance techniques. In all experiments enough 5 per cent glucose in water was infused intravenously in 12 minutes to decrease plasma sodium concentration 10 to 30 mEq. per L. Some experiments were done in "normal" salt balance and others after sodium "depletion" (brought about by eliminating NaCl intake). Sympatho-adrenal system mediation of the response was evaluated by adrenergic blockade in some dogs and unilateral renal denervation in others. When dogs in salt balance received i.v. glucose, renal blood flow and GFR increased and total renal resistance decreased or did not change. When these same dogs were "salt-depleted," infusion of

glucose solution decreased renal blood flow and GFR and increased renal resistance. Change in GFR following i.v. glucose was roughly correlated with post-infusion plasma sodium concentration. Adrenergic blockade and renal denervation did not appreciably alter this correlation or completely abolish the altered hemodynamic response induced by sodium depletion. Blockade and denervation did markedly impair the ability of the kidney to conserve sodium. Conclusions: 1) alteration in GFR is one specific mechanism for regulating sodium excretion (as suggested by others); 2) it is not an exclusive mechanism (excluding adrenal cortical hormone from consideration), as indicated by the studies with adrenergic blockade and unilateral denervation; 3) control of renal hemodynamics is mediated in part by sympatho-adrenal system and possibly in part by direct action on the kidney by some function of plasma sodium concentration.

A Study of Carotenoids: Colored Lipids Which Infiltrate the Human Aorta. DAVID H. BLANKENHORN and DAVID G. FREIMAN, Cincinnati, O. (Introduced by M. A. Blankenhorn).

Carotenoid pigments are found in the human aorta where they contribute to the yellow color of atheromatous plaques. They are unsaturated paraffins ($C_{40}H_{56}$) which are known to originate exclusively from the diet. We have performed a study of their distribution in 40 aortas which provides information as to the manner in which lipids formed outside this vessel may infiltrate its wall.

We have found that the gross display of yellow color does not accurately represent the carotenoid content found upon extraction and assay. Extensive lesions which are fibrotic, ulcerated, or calcified appear grayish-white despite the fact that they contain carotenoids in amounts equal to or greater than that found in yellow lesions.

Our study has shown that up to a 250-fold parallel increase in carotenoid and cholesterol content per square centimeter is encountered when comparing an ulcerated calcified plaque with an adjacent uninvolved area. Accordingly, an infiltration of carotenoids must have occurred as cholesterol accumulated. This finding suggests that atherosclerotic plaques remain permeable to serum lipids even after extensive fibrosis and ulceration has occurred. In addition, this study demonstrates that a lipid common in the human diet, a constituent of normal serum, can infiltrate the aortic wall under conditions encountered in the usual circumstances of life.

Passive Transfer of Nonthrombocytopenic Purpura. MATTHEW BLOCK,* Denver, Colo.

Three patients with nonthrombocytopenic purpura were subjected to passive transfer studies. R and P had longstanding mitral stenosis treated by valvulotomy under hypothermic anaesthesia and quinidinization. Two weeks postoperatively both developed diffuse heat and swelling of joints and a nonthrombocytopenic coalescent petechial to purpuric rash. Another patient subjected to the same treatment had an uncomplicated course. None of the 130 other patients subjected to other cardiac operations with hypothermic anaesthesia, sometimes with quinidinization, have had a similar complication.

Two hundred and fifty cc. of plasma from each patient was transfused into each of 2 volunteers who had been taking quinidine. Both volunteers had a slightly positive Rumpel-Leed test after quinidinization, becoming clearly more strongly positive 20 hours after plasma infusion.

X had an exfoliative dermatitis, then a similar rash, hematuria and epistaxis after penicillin therapy. His plasma was infused into a receptor who received 600,000 units of penicillin intramuscularly 4 hours later. The latter noted pruritus and erythroderma from 8 to 36 hours, and purpura from 24 to 42 hours after penicillin injection.

One possible interpretation is that the vascular purpura was caused by a transferable antibody formed from the interaction of a plasma factor with quinidine under the influence of hypothermia in R and P and with penicillin in X. Other possibilities could not be eliminated since there was no opportunity to repeat the experiments using only the patients' plasma.

Pulmonary and Circulatory Effects of Acute Pulmonary Vascular Engorgement in Normal Subjects. STUART BONDURANT and JOHN B. HICKAM,* Durham, N. C.

To investigate the respiratory and circulatory effects of acute pulmonary vascular congestion, observations have been made on normal subjects during inflation of a "G-suit" or submersion in water.

In 11 seated subjects, G-suit inflation (2 psi) raised central venous pressure (CVP) by 26.5 ± 9.0 (S.D.) cm. H_2O and markedly increased radiographic density of the lungs. Simultaneously, the lungs became much stiffer; pulmonary compliance fell from $.218 \pm .035$ to $.100 \pm .033$ L. per cm. H_2O. Thereafter, despite maintained suit pressure, central vascular engorgement appeared to diminish. During 2 minutes radiographic density decreased, CVP fell by 7.3 ± 1.5 cm. H_2O, and compliance increased to $.173 \pm .049$ L. per cm. H_2O. After releasing suit pressure, CVP and compliance returned to control levels within 10 seconds. Similar effects on CVP and compliance were produced by lowering 8 seated subjects below the surface of a pool while they continued to breathe against atmospheric pressure.

The initial effects of suit inflation and the subsequent changes were altered by pre-treatment with drugs which change vascular tone. Intravenous nor-epinephrine (0.4 to 0.6 microgram per Kg. per min.) in 3 subjects caused a rise in CVP of 4.4 cm. H_2O and a fall of compliance from .198 to .141 L. per cm. H_2O. On suit inflation, CVP rose further by 32.5 ± 2.4 cm. H_2O, and compliance fell to $.061 \pm .007$ L. per cm. H_2O. These values did not change during the subsequent 2 minutes. Large doses of C_6 significantly reduced the rise in CVP on subsequent suit inflation, but there was still a large compliance fall.

Acute pulmonary vascular engorgement in normal

subjects causes lung stiffening quite comparable to that of congestive failure. The initial vascular engorgement is quickly followed by some apparent redistribution of blood to the periphery. This compensation is blocked by nor-adrenaline, possibly because it prevents relaxation of venous tone.

The technique can be applied both to the study of circulatory influences on lung mechanics and to venomotor reactions which are significant in congestive failure.

A Defect in Steroid Biogenesis in Man Associated with Hypertension. ALFRED M. BONGIOVANNI* and WALTER R. EBERLEIN, Philadelphia, Penna.

During the course of a study of patients with the adrenogenital syndrome, a subject with this disorder, complicated by hypertension, was investigated. In all previous patients there was evidence for a failure of the synthesis of Compound F. The low levels of plasma Porter-Silber chromogens and high urinary 17-ketosteroids and pregnanetriol pointed to a specific inability to hydroxylate the steroid nucleus at carbon-21. The subject of this report was an eight-year-old female with marked virilization who had been reared as a male. The urinary 17-ketosteroids were 20 to 47 milligrams per day. The pregnanetriol excretion (6 to 7 milligrams per day), although elevated, was not as high as in other subjects. Surprisingly, the plasma Porter-Silber chromogens were 24.7 to 26.2 micrograms per cent (normal in this laboratory, 8.0 ± 1.0). Paper chromatography revealed the substance to be not Compound F but Compound S, which lacked an oxygen function at carbon-11. In addition, the major metabolite in the urine of this subject was tetrahydro-S (pregnane-3γ, 17γ, 21-triol-20-one). This suggested a different defect than hitherto described, namely the inability to hydroxylate at carbon-11. A metabolite of desoxycorticosterone, namely the tetrahydro derivative, was also found in the urine and was attributable to this same defect and probably accounted for the hypertension. The entire picture, including the hypertension, was reversed by small doses of Compound F.

Regulation of Iron Transport. THOMAS H. BOTHWELL, WALTHER PRIBILLA, ARNOLD V. HURTADO, and CLEMENT A. FINCH,* Seattle, Wash.

Factors influencing the transport of iron through the plasma and its passage through reticulo-endothelial cells, mucosal cells and placenta have been studied in animals and man, using a variety of approaches and isotopic methods.

Loading of the plasma compartment with iron derived from the breakdown of injected non-viable red cells caused an increase in the plasma iron to the point of saturation, but did not increase normal plasma iron turnover. These data indicated that although unsaturated transferrin can act as an immediate receptacle for iron loading, there are normally no secondary receptors capable of taking up this excess iron from the plasma rapidly. However, in conditions of increased marrow activity, and especially when the iron needs of accelerated erythropoiesis are not fully met, iron loading caused an increased reticulo-endothelial and plasma iron turnover. In addition, there was a relative increase in iron absorption in both experimental and clinical states associated with accelerated erythropoiesis. Corroborative evidence for the role of marrow activity in regulating iron transport was found in studies on pregnant rabbits. During the latter part of pregnancy there was an increased uptake of plasma iron by the fetuses so that over 80 per cent of the plasma turnover was finally directed towards the fetuses. Calculations of the fetal rate of blood formation from hemochromogen determinations suggested that the distribution of iron was related to the rates of erythropoiesis in fetuses and mother.

Further information concerning alterations in transport mechanisms produced by changes in marrow function, in body stores and by injection will be discussed.

The results have led to a central hypothesis for the regulation of iron transport: the transport of iron through reticulo-endothelial cells, placenta and possibly mucosal cells is limited by the receptor capacity of the erythroid marrow. This implies a self-regulating system in which the needs of the marrow influence the uptake and availability of iron.

A Study of the Intermittent Character of Fever During Infection. A. I. BRAUDE* and M. ZALESKY, Dallas, Tex.

The intermittent but regular character of fever curves suggest that the fever-producing mechanism may undergo periods of non-responsiveness to the steady stimulus provided by persistent infections. In order to determine whether such a refractory period exists, fever was studied in rabbits receiving 2 successive intravenous injections of equal doses of purified endotoxin (*Escherichia coli*) at various intervals.

After one injection of endotoxin, normal rabbits exhibited fever lasting 6 hours with peaks at 1 and 4 hours. Despite a second injection at 2 hours, their fever still lasted only 6 hours, although the second peak was higher. To determine whether this non-responsiveness reflected a deficiency of endogenous pyrogen from circulating neutrophils, which are virtually absent at 2 hours in normals, a second injection was given at 5 hours when leukocytosis begins. Because leukocytosis begins in tolerant animals at 2 hours, they also received a second injection then. In both groups the refractory response was again encountered although brief elevations occurred.

The 6-hour febrile response of non-tolerant animals to their initial injection could not be explained by persistence of circulating endotoxin because studies with Cr^{51} labelled endotoxin disclosed that it had almost disappeared from their blood within 20 minutes. Instead, these results suggest 1) that a febrile response of fixed duration is initiated by the action of endotoxin immediately after injection, 2) that the febrile response to further pyrogen is abortive until the refractory period has passed,

and 3) that fever depends therefore not only on the combined stimulation of endogenous and exogenous pyrogens, but also on the responsiveness of the fever-producing apparatus.

Studies on the Functional Capacity of a Denervated Homotransplanted Kidney with Parallel Observations on the Identical Twin Donor. NEAL S. BRICKER, WARREN R. GUILD, JOHN B. REARDAN, and JOHN P. MERRILL,* Boston, Mass.

The first successful transplantation of a human kidney has been accomplished in a pair of male monozygotic twins. The recipient experienced immediate reversal of the manifestations of uremia and malignant hypertension and has to date enjoyed over a year of total well-being. Twenty-three experiments were performed on the recipient (who possessed only the transplanted kidney during the studies) and the donor over a ten-month period.

The donor's pre-transplant filtration rate (C_{In}) was 147 ml. per min. Following transplantation, C_{In} (recipient) remained stable at 60 to 70 ml. per min. whereas C_{In} (donor) increased slightly from 68 to 84 ml. per min.

Manifestations of the functional integrity of the transplanted kidney included: (1) concentration: osm U/P = 2.9, $T^mC_{H_2O}/C_{In} = 5.8\%$; (2) dilution: osm U/P = 0.36; (3) acidification: pH 5.5 following 4 gm. NH_4Cl; (4) alkalinization: pH 5.5 to 7.7 following 250 mg. Diamox®; (5) K^+, Na^+, and HCO_3^- diureses following Diamox®; (6) elaboration of Na-poor urine following sodium deprivation and hydrocortisone; and (7) prolonged maintenance of normal ECF volume and composition.

The recipient's responses (C_{In} and C_{PAH}) to orthostatic hypotension, intravenous aminophylline and l-nor-epinephrine infusion were normal. The pattern of sodium excretion was abnormal following: (1) hypotonic expansion of ECF; (2) isotonic expansion of ECF (with and without prehydration); (3) rapid hyperoncotic albumin infusion; (4) venous congestion of lower extremities; (5) orthostatic hypotension; and (6) l-nor-epinephrine infusion. In the majority of these experiments the donor responded normally.

It is concluded that: (1) Most parameters of renal function remained normal in a transplanted, irrefutably denervated kidney. This lends strong support to the conviction that the integrity of these functions (including renal hemodynamic regulation) is not dependent upon renal nerves. (2) Abnormalities in sodium excretion occurred following a group of heterogeneous stimuli. The possibility that these arose from a specific defect in sodium transport secondary to denervation cannot presently be excluded.

The Problem of Isotonic Versus Hypertonic Cell Fluid in the Light of Cryoscopic Measurements of Tissue Homogenates. WILLIAM A. BRODSKY,* WARREN H. DENNIS, WARREN S. REHM, JOHANNES W. APPELBOOM, and ISRAEL DIAMOND, Louisville, Ky.

The freezing point depression of undiluted liver or kidney homogenates prepared in a Carver press from freshly excised frozen tissue is hypertonic to plasma. Even at 0° C, the osmotic activity of such homogenates increases with time, and extrapolation of data to zero time yields an osmotic activity higher than that of plasma. On the other hand, Conway found that the calculated freezing point values of saline-diluted tissues prepared in the mortar would extrapolate to a zero time value isotonic with plasma. He concluded that cell fluid *in vivo* is isotonic with plasma. Assumptions required in such calculations were: complete destruction of the cells during preparation, and complete mixing of cell fluid with ambient solution. However, microscopic examination of frozen tissue powder revealed many clumps of intact cells. In a system of cell clumps diffusion equilibrium with an ambient fluid might not be instantaneous. If hypertonic or isotonic cells are suspended in isotonic saline, the F.P. measured instantaneously must be that of isotonic saline, since it is the phase which freezes first. Hence calculations, like those of Conway, would yield isotonic values for the tissues. Conversely, if cells are suspended in a hypertonic ambient, the instantaneous F.P. depression must be that of cell fluid which would freeze first. Then calculations on the raw F.P. data of the mixture (on the assumption of complete and instantaneous mixing) would yield aberrantly low values for the tissue. Experiments herein concern the F.P. data on tissue powder mixed with distilled water, 0.15 M NaCl and 0.47 M NaCl. Calculated osmotic activity of tissues in hypertonic diluents were 120 to 150 mOsm per L. after five minutes at 0° C, and extrapolated to a value of zero at zero time. These data confirmed the predictions of a postulate of delayed diffusion. The basis for such observations could depend on 1) the number of intact cells or particulate bodies; 2) the area of diffusion barriers; and 3) the osmotic activity of the diluent.

"Uncertainty" Measurements in Psychopharmacology. ENOCH CALLAWAY, III and JACOB E. FINESINGER,* Baltimore, Md.

The study of reactions to drugs in human beings brings up many methodological problems. It is difficult to devise measurements for assessing these complex reactions and for separating and comparing variables which interact with each other. Our previous studies on the effects of methamphetamine have shown that this drug (1) decreases the intensity of responses to environmental stimuli and/or (2) causes the subject to depend more on thoughts and other subjective factors in decision making. There has been no method available for determining whether reactions to methamphetamine are the result of either or both effects.

One method of determining the effect of environmental stimuli in eliciting responses might be to correlate sequences of stimuli with the subsequent response. In general, we infer the thoughts and other subjective factors influencing a person by observing his patterns of behavior. The influence of these thoughts and subjective factors on

a response will be at least indicated by a correlation between that response and the pattern of behavior (*i.e.*, sequence of responses) that preceded it. Unfortunately, conventional statistics cannot be legitimately applied to such data. Information theory provides a method for the calculation of "uncertainties" from these data. "Uncertainty" is mathematically identical with entropy, and as such is an index of probability for events of a given class. The data we will present are computations of "uncertainties" from experiments with methamphetamine. The method of setting up the necessary matrices, the calculations of "uncertainties," and implication of these measures will be discussed. The calculations of our data imply that methamphetamine reduces the information from stimuli and also increases the information from subjective or internal patterns.

Metabolic Function of Human Platelets. EDMUND W. CAMPBELL and WALTER J. SMALL, Boston, Mass. (Introduced by William Dameshek).

The metabolic activity of human platelets has been studied by the assay of oxidase and dehydrogenase activity and lactic acid accumulation. The relationship of these functions to the role of platelets in coagulation has been correlated. Platelets were separated by differential centrifugation at 4°C. and assayed by manometric techniques. Samples of platelet rich plasma and washed platelets resuspended in various media were studied. A normal range of activity and a respiratory quotient of 0.95 were established. The effects of system variables and methods of blood collection, separation and preservation were evaluated to establish standard conditions.

The metabolic activities of platelets in a variety of hematologic disorders were compared with normal values. Emphasis was directed toward the study of polycythemia, myeloid metaplasia, and leukemia. Striking platelet metabolic activity differences were observed in the rate, duration, total activity and response to additives in hematologic diseases. In polycythemia the oxidase activity was normal but the dehydrogenase activity was decreased by 50 per cent. No response to the addition of glucose occurred as demonstrated in platelets from normal individuals. Various responses were obtained in myeloid metaplasia suggesting differences in the platelets in this disease. Platelets obtained from individuals having chronic granulocytic leukemia showed normal metabolic rates and responses to additives but a striking decrease in duration of activity not present in the other diseases. These indices appear to be affected by therapy.

The ratio of lactic acid accumulation to oxygen consumption was greatly increased in chronic granulocytic leukemia, moderately increased in myeloid metaplasia and slightly increased in polycythemia.

Platelet metabolic activity studies may be an additional technique for the evaluation of platelets as to preservation, collection, storage and hematologic disorders.

Hemodynamics of Congestive Failure Associated with Peripheral Arteriovenous Fistulae. JAMES A. CAMPBELL, JOHN S. GRAETTINGER, JOSEPH J. MUENSTER, and CARLO S. CHECCIA, Chicago, Ill. (Introduced by Theodore B. Schwartz).

Changes in the total cardiovascular system which result from the "hole in the periphery" caused by arteriovenous fistulae have been studied in five patients two of whom were in frank congestive failure. Cardiac competence was restored by surgical correction of the peripheral vascular defect.

The cardiac indices at rest were elevated in all patients and ranged from 4.88 to 6.59 liters per minute per square meter of body surface. Postoperatively these indices decreased, ranging from 2.75 to 4.39. During exercise, preoperatively, cardiac outputs rose in those patients not in evident heart failure. In the two patients in whom congestive failure was apparent, the outputs decreased 14 per cent and 33 per cent. Postoperatively their cardiac outputs returned to normal and during exercise increased significantly.

Vascular resistance in both the systemic and pulmonary circulations was normal to low in the three patients not in failure. In the decompensated patients, however, the locally low resistance at the site of the shunt demonstrated during surgery was far overshadowed by the markedly increased resistance in both the greater and lesser circulations. During exercise the patients without heart failure demonstrated no significant change, while the patients with heart failure showed still further increase in both pulmonary and peripheral resistance. This increased vascular resistance is found in patients with cardiac decompensation regardless of etiology.

Patients in cardiac failure from the added circulatory load of peripheral arteriovenous fistulae differ from patients with congestive failure due to most other causes only in that they have a high rather than a low cardiac output.

The Serum Dihydroxy-Trihydroxy Bile Acid Ratio in Liver and Biliary Tract Disease. JAMES B. CAREY, JR., Minneapolis, Minn. (Introduced by C. J. Watson).

Since there are three principal bile acids formed exclusively by the liver cell, a comparison of the variation in their serum concentrations might be expected to yield important evidence concerning liver cell function. In the present study this is found to be true.

Serum bile acid concentrations were determined from the appropriate ultraviolet absorption maxima after extraction by Josephson's method and hydrolysis. The mean trihydroxy bile acid (cholic acid) value for 35 healthy subjects was 1.1 ± 0.4 mg. per cent, and for the dihydroxy bile acids (deoxycholic and chenodeoxycholic acid) was 0.47 ± 0.2 mg. per cent, giving a ratio of 2.3.

Of 70 jaundiced patients thus far studied, 12 had severe liver damage and in this group the trihydroxy-dihydroxy bile acid ratio was completely reversed to less than 1 for

every patient, the average being 0.71. This reversal is accounted for by the greater than 5-fold increase in the dihydroxy acid value, the group average being 2.7 mg. per cent. The average trihydroxy value was, however, only slightly increased. The severity of liver disease indicated by this finding was demonstrated when six of these patients soon expired. An additional four are in the terminal stages of their illness. A reversed ratio has reverted and approached normal in a patient recovering from infectious hepatitis and in several patients with portal cirrhosis after recovery from an episode of acute alcoholism.

Among 15 patients having extrahepatic biliary obstruction without appreciable hepatocellular damage the concentrations of trihydroxy and dihydroxy bile acids were greatly increased, as much as 10 fold in some cases, while the ratio between the two remained normal. A similar increase was observed in a case of chlorpromazine jaundice in which hepatocellular damage was relatively slight as compared to cholangiolar impairment.

The present results demonstrate the importance of a differential quantitative analysis of the serum bile acids in studies of hepatocellular function.

Variations in Gastrointestinal Cholesterol Exchange in Man with Acute Changes in Diet. SAMUEL CHENG and MALCOLM STANLEY,* Boston, Mass.

Daily gastrointestinal cholesterol secretion, absorption, and excretion were determined by an inert indicator method and simultaneous use of C^{14}-cholesterol (Gastroenterology 1956, **30**, 62). Nineteen studies were performed on 11 normal young adults consuming basic diet (2,530 calories, C. 250 g., P. 118 g., F. 101 g., cholesterol 60 mg.) or some variation of it.

1. Basic diets were given to 4 males (serum cholesterols 200 to 219 mg. per cent) in 5 tests. Cholesterol excretion, secretion, absorption were 0.51 g. (0.43 to 0.57), 2.34 g. (2.15 to 2.54), 1.90 g. (1.70 to 2.07) or 79 per cent (77 to 81), respectively.

2. Half basic diets were eaten by 3 males (serum cholesterols 212 to 250 mg. per cent), including one above. Decreased amounts excreted, secreted, absorbed were 0.38 g. (0.25 to 0.57), 1.57 g. (1.5 to 1.66), 1.22 g. (0.99 to 1.44) or 76 per cent (63 to 85).

3. Basic diet plus 2 g. cholesterol daily, preceded by 3 days when 2 g. cholesterol daily were added to regular diet, was taken by a man in first group. Absorption considerably increased to 3.25 g., excretion to 1.83 g., secretion to 3.01 g. Percentage absorption decreased to 64.

4. Three quarters basic diets were eaten by 5 young women (serum cholesterols 154 to 225 mg. per cent). Excretion was 0.45 g. (0.31 to 0.62), secretion 2.10 g. (1.94 to 2.44), absorption 1.70 g. (1.45 to 1.89) or 79 per cent (73 to 86). Three (serum cholesterols averaged 221 mg. per cent) absorbed 83 per cent; 2 (serum cholesterols averaged 168 mg. per cent) absorbed 74 per cent.

5. Low-fat three quarters basic diets (75 per cent of fat replaced isocalorically by carbohydrate) were given to same 5 nurses. Excretion, secretion and absorption decreased by 31 per cent, 26 per cent and 23 per cent to 0.31 g. (0.16 to 0.51), 1.56 g. (0.90 to 2.20) and 1.30 g. (0.78 to 1.96). Percentage absorption was unchanged. In 2 of 3 with higher serum cholesterols exchange was altered only slightly.

Relative Renal Excretion Patterns of Glomerular Substances, Sodium and Water; Effects of Mercurial Diuretics. FRANCIS P. CHINARD,* THEODORE ENNS, and MARY F. NOLAN, Baltimore, Md.

Renal excretion patterns of various substances in anaesthetized dogs are determined as follows. Injection of test substances is made "instantaneously" into the left renal artery, 30 to 60 successive samples of urine are collected from each ureter separately over a total period of 15 to 20 minutes, the fraction of each test substance excreted in each period is determined. To correct for recirculation the fractions excreted by the right kidney are subtracted, period by period, from the fractions excreted by the left kidney; these differences, plotted against time after injection, give the renal excretion patterns. In earlier studies, with infusions of 0.85 per cent NaCl, 15 per cent mannitol, or 5 per cent glucose to produce adequate urine flow, the mean and modal transit times of sodium (Na^{22}) were less by approximately 30 seconds and the transit times of water (tritium oxide) were significantly greater than the corresponding transit times of simultaneously injected creatinine. With mercurial diuretics, similar differences of the modal transit times of sodium and of creatinine obtain but the differences of the mean transit times are less. In the later periods after injection, the fractions of sodium excreted per period are nearly equal to the fractions of creatinine excreted; the fractions of water excreted per period exceed the fractions of creatinine excreted. There appear to be different pathways from renal artery to uretero-pelvic junction for sodium, creatinine and water. In mercurial diuresis, the previously reported precession of sodium is observed, suggesting a bypass of segments of the nephrons; however, a substantial portion of the sodium appears to be excreted by escape from tubular reabsorption.

The Effect of Cortisone and Hydrocortisone on Hepatic Excretory Function. JAMES A. CLIFTON, Boston, Mass. (Introduced by Louis Weinstein).

To examine the choleretic properties of adrenal glucocorticoids, normal volunteers and patients with T-tubes in the common bile duct were studied before and after cortisone or hydrocortisone was given.

In 10 normal subjects sulfobromophthalein clearance (C_{BSP}) was measured. Following cortisone (200 mgm. per day for 3 days) C_{BSP} was unaltered in six subjects and decreased by more than 200 ml. per minute in two. These two, and two others, had progressively rising plasma dye levels during the test period; whereas, no such rise was evident in the pre-cortisone tests. One hundred to 150 milligrams of hydrocortisone given by

single injection or infusion during determination of C_{BSP} in five subjects did not accelerate clearance.

In eight subjects given 200 mgm. cortisone per day for 3 days the disappearance rate of radioactive iodine labeled rose bengal (RIRB) from the blood was unchanged after administration of the hormone. Similarly, uptake of the dye by the liver and its disappearance from that organ, as estimated by external counting, was unaffected.

Two patients with T-tubes in the common bile duct but without additional hepatic complications were given 150 mgm. hydrocortisone intravenously during constant infusion of RIRB. T-tube collections were made at 15-minute intervals for two hours before hydrocortisone and for 2 to 3 hours afterwards. Bile flow and bilirubin output (oxidation method of Malloy and Evelyn) decreased in both cases. The slope of the progressive increase of RIRB in blood, bile, and liver throughout the infusion period did not change after hydrocortisone.

A choleretic action has been ascribed to adrenal glucocorticoids, but our observations have not shown any such effect on the several aspects of hepatic excretion studied. It is even possible that these hormones may depress certain hepatic excretory functions.

Metabolism of Folic Acid in Man. PAUL T. CONDIT, Bethesda, Md. (Introduced by Douglas G. Carroll).

Following the intravenous administration of folic acid (PGA) to normal subjects and to patients with chronic leukemia, 60 to 70 per cent of a 0.3 to 0.4 mg. per kg. dose appeared in the urine in twenty-four hours, while somewhat less than 0.1 per cent was excreted as the citrovorum factor (CF). No important differences were noted in the excretion of PGA or of CF by the normal subjects or by the patients with chronic lymphocytic leukemia. The patient with chronic myeloid leukemia, however, excreted four times as much CF as the others, without showing any difference in PGA excretion.

When the PGA infusion was repeated after the administration of Aminopterin, the urinary excretion of PGA was increased, while that of CF was greatly reduced. The increased urinary excretion of PGA was due largely to a marked reduction in the extrarenal uptake and only partially to an increase in the renal clearance. Two patients with chronic lymphocytic leukemia appeared to be more responsive to Aminopterin than were the normal subjects or the patient with chronic myeloid leukemia, because greater decreases in the extrarenal uptake of PGA occurred following comparable doses of Aminopterin. The effects of Aminopterin persisted for three to four weeks.

A study of two patients with Laennec's cirrhosis provided some information about the site of the extrarenal uptake of PGA. In the presence of reduced hepatic function the extrarenal uptake PGA was negligible, as was the urinary excretion of CF derived from PGA.

These results suggest that Aminopterin interferes with the uptake of PGA as well as with its conversion to CF, and that a major part of PGA metabolism occurs in the liver.

Radiopotassium Dilution Studies from the "Left" Circulation in Patients with Rheumatic Mitral Valvular Disease. HADLEY L. CONN, JR., DONALD F. HEIMAN, WILLIAM S. BLAKEMORE, PETER T. KUO, and STEPHEN B. LANGFELD, Philadelphia, Pa. (Introduced by Calvin F. Kay).

Evaluation of the status of mitral valve function in patients with rheumatic mitral valvular disease has not always been satisfactory, even with the aid of numerous specialized diagnostic techniques. Furthermore, there is little quantitative information on left-heart volumes and circulation times in these patients. It appeared likely that information pertinent to these matters could be derived from analysis of indicator-dilution curves obtained by multiple, "left-heart" injections and femoral arterial sampling. Accordingly, following direct atrial puncture, radiopotassium injections were made consecutively into the left atrium, left ventricle, and aorta of ten patients with mitral valvular disease.

For analytical purposes these vascular segments were considered as a three-compartment, series system with normally no feedback and no mixing in the atrium, but complete mixing in ventricle and aorta. Cardiac output, differential circulation times, total vascular volumes between injection and sampling points, chamber mixing volumes, and left atrial volumes were measured directly or calculated from the curves.

With "pure" mitral insufficiency 1) circulation times from atrium and ventricle were identical, 2) total volumes (C_V) were also identical and elevated (average 1.1 L.), 3) "ventricular" mixing volumes (V_R) were markedly elevated (0.85 L.), 4) accordingly the ratio (V_R/C_V) approached unity (0.8). With "pure" stenosis 1) atrial circulation times were disparately longer than ventricular, 2) total volumes differed, the atrial being moderately increased and the ventricular normal, 3) "ventricular" mixing volumes were normal, 4) accordingly, the ratio (V_R/C_V) was subnormal (0.13). With combined lesions intermediate values were obtained. Resulting evaluations of valvular function correlated well with those ascertained by surgical and other evaluations.

Conclusions are 1) With mitral insufficiency the left heart is, to varying extent, transformed into a single, enlarged mixing chamber. 2) With mitral stenosis left-heart mixing is normal, confined to ventricle, while left atrial (non-mixing) volume is increased. 3) "Left-heart" indicator-dilution curves appear to provide a good method for evaluating mitral valve functional state.

The Etiology of Hypercalcemia Associated with Lung Carcinoma. THOMAS B. CONNOR, WILLIAM C. THOMAS, JR., and JOHN EAGER HOWARD, Baltimore, Md. (Introduced by Samuel P. Asper, Jr.).

Carcinoma of the lung is occasionally accompanied by hypercalcemia. It is generally believed that this hypercalcemia is due to excess bone resorption in skeletal metastases, whether or not such metastases can be demonstrated roentgenologically or at postmortem examination. Our observations on two patients with broncho-

genic carcinoma suggest another explanation for the hypercalcemia. A 69-year-old male preoperatively had hypercalcemia and hypophosphatemia. Resection of the tumor was followed by return of the serum calcium and phosphorus concentrations to normal levels by the fourth postoperative day. Four months later hypercalcemia recurred and local tumor growth on the chest wall was evident. No bone metastases were demonstrable radiologically. Shortly before death the serum calcium level returned to normal, and at postmortem examination the tumor mass was almost entirely necrotic. Except for extension of the tumor to a single rib, no involvement of bone by the tumor could be found, but there was evidence of excessive bone resorption at sites distant from the tumor recurrence. The second patient, a 62-year-old male with no demonstrable bone metastases also had hypercalcemia and hypophosphatemia preoperatively. Following surgical removal of the tumor, serum calcium and phosphorus levels returned promptly to normal. Rib biopsy performed at operation showed histological evidence of increased bone resorption. These observations suggest that the hypercalcemia was not dependent on bone metastases, but was due to excessive bone resorption resulting from substances introduced into the circulation by the tumors.

Effect of a Sodium Chloride-Load on Renal Hemodynamics and Electrolyte Excretion in Essential Hypertension. P. T. COTTIER, S. W. HOOBLER,* and J. M. WELLER, Ann Arbor, Mich.

Changes in renal plasma flow, glomerular filtration rate, electrolyte clearance and water excretion following sodium chloride loading were studied in normotensive patients and in patients having varying degrees of essential hypertension. Normotensives (Group I) had a mean arterial blood pressure of 110/70 mm. Hg; mild hypertensives (Group II), 160/99; moderately severe hypertensives (Group III), 175/118, and severe hypertensives (Group IV), 201/131. Five were in each group.

Patients were placed on 3 to 5 g. NaCl diet for 3 weeks. Three 15-minute basic periods were followed by 6 load periods. Sustaining (0.9 per cent NaCl) and load infusions (500 ml. per 1.73 m^2 of 2.5 per cent NaCl) were given at a rate of 8 ml. per min. per 1.73 m^2. RPF (C_{PAH}) and GFR (C_{Inulin}) of Groups I, II, and III were within normal range. Group IV had 58 per cent reduction of RPF and 50 per cent lowering in GFR. RPF, GFR or filtration fraction did not change under load. In Groups I, II, and III water excretion and sodium, chloride and potassium clearances paralleled mean arterial pressure (C_{Na} in I was 1.7 ml. per minute; II, 3.0 ml. per minute; and III, 4.4 ml. per minute). Group IV had a diminished water and electrolyte excretion (C_{Na} of 2.0 ml. per minute) apparently due to reduced filtered load, not increased tubular reabsorption, as excreted water and electrolytes in per cent of filtered load were similar in Groups III and IV. Increment of water excretion and electrolyte clearance during load is not related to mean arterial blood pressure.

These studies demonstrate that renal tubular rejection of water and electrolytes parallels the blood pressure if filtration rate is not depressed. The mechanism of action is not understood.

A Comparison of the Effect of Human Plasma and Albumin on the In Vitro Uptake by Human Erythrocytes of I^{131} Labeled Thyroid Hormones. K. R. CRISPELL, JOSEPH COLEMAN, and HARRY J. HYER, Charlottesville, Va. (Introduced by William Parson).

Previous studies from our laboratory have shown that the uptake by washed human erythrocytes of I^{131} L-triiodothyronine in saline solution is greater than that of I^{131} L-thyroxine. When the labeled hormones are in a plasma-saline solution (1 to 11 dilution) the uptake of L-triiodothyronine is decreased by 34 per cent, and L-thyroxine by 80 per cent. This was interpreted as supporting the concept that "thyroxine binding globulin" in plasma binds L-triiodothyronine to a much lesser degree than L-thyroxine.

The labeled hormones were dissolved in an albumin saline-solution (equivalent to the albumin content of the plasma-saline solution). The uptake of L-triiodothyronine by erythrocytes was decreased by 8 per cent and L-thyroxine by 30 per cent as compared to the uptake from a saline solution. Thus, the uptake of the labeled hormones from the albumin solution is three to four times greater than from the plasma solution.

The addition of 0.5 gamma of stable L-thyroxine to plasma before incubation with I^{131} L-thyroxine will cause a 60 per cent increase in the uptake by erythrocytes. The addition of stable thyroxine to albumin before incubation has no effect on the uptake of I^{131} L-thyroxine by the erythrocytes.

Studies by others have shown that (1) the uptake by erythrocytes of L-triiodothyronine from "thyrotoxic plasma" is greater than normal, (2) the proportion of thyroxine bound to albumin is greater in the thyrotoxic patient. The studies to be reported suggest (1) the greater uptake of the thyroid hormones from thyrotoxic plasma could be a "spill over" of excess thyroid hormone on to albumin and hence a greater cell uptake. (2) The equilibrium between plasma proteins and cell may determine the availability of thyroid hormones to the cell. (3) The erythrocyte uptake appears to be a physical chemical phenomenon.

An Abnormal Hemolytic System Associated with Disseminated Malignant Disease. WILLIAM H. CROSBY* and NAOMI R. BENJAMIN, Washington, D. C.

When defibrinated blood from certain patients with leukemia or disseminated neoplasms is incubated under oil for 24 hours at 37° C the red cells undergo hemolysis, and 50 to 500 mg. of hemoglobin per 100 ml. of plasma may be released. The serum or plasma may be modified a number of ways to decrease or prevent this hemolysis: 1. Remove or chelate the calcium, 2. Lower the pH, 3. Add glucose, 4. Add metal-binding globulin (Cohn fraction IV-7). Hemolysis can be increased by several

means: 1. Artificially increase the hematocrit of incubated blood, 2. Add 3-methyl glucose, 3. Aerate the blood before incubation, 4. Add calcium ions.

The manipulations that affect hemolysis are those that alter the amount of calcium that is not bound to protein: A low hematocrit permits better glycolysis in the sedimented red cells; glycolysis lowers pH; lowered pH increases the binding of calcium; hemolysis is impeded. Hemolysis appears to be due to some effect of unbound calcium on the red cells. The red cells in incubated normal blood are also susceptible to hemolysis by calcium, and this hemolysis can be demonstrated by artificially increasing the concentration of calcium in incubated blood.

In patients whose blood has demonstrated this phenomenon intensely, the total serum calcium was found to be low (8 mg. per 100 ml.) as was the beta globulin (3 per cent of total protein versus normal 8 per cent). The protein-bound calcium was about half that found in normal serum. This suggests that a deficit of metal-binding beta globulin results in a high proportion of unbound calcium; this calcium hemolyzes the incubating red cells. Cross incubation experiments with normal serum indicate that red cells of the patients are damaged *in vivo* by this hemolytic system. Transfusion does not correct the fault, but remission of the leukemia is associated with disappearance of the hemolytic phenomenon.

Tissue Transplantation: Prolonged Skin Homograft Survival in Recipients with Chronic Uremia. GUSTAVE J. DAMMIN,* JOSEPH E. MURRAY, and NATHAN P. COUCH, Boston, Mass.

A study of the survival of skin homografts in recipients with chronic uremia was undertaken because, (1) in some patients with chronic uremia, renal homotransplants have shown functional survival for periods ranging from five to twenty-five weeks, (2) in the normal dog, renal homotransplants seldom function for more than one week regardless of the manner in which donor and/or recipient have been modified, (3) in a single instance in man, in which the recipient did not have chronic uremia, a renal homotransplant functioned for three weeks, then ceased abruptly, the homotransplant showing a morphologic rejection pattern resembling that in the dog, and (4) an antigenic relationship between skin and kidney has been demonstrated in the dog.

In chronic uremia, some skin homografts were observed to survive for as long as 115 days. Chronic glomerulonephritis, chronic pyelonephritis and polycystic disease were represented in the group studied. Chronic uremia was the common denominator as in recipients with prolonged functional survival of renal homotransplants. In normal recipients, evidence of rejection of skin homografts usually appears by the 14th day and rejection is usually complete by the 28th day.

Newer criteria for determining the duration of survival of skin homografts were established and/or applied for this study (pattern of sex chromatin, elastic fiber and mucopolysaccharide distribution).

If an immune response is the basis for rejection of homotransplanted tissues, then this response appears impaired in the uremic state. Prolonged survival of skin homografts also occurs in hypogammaglobulinemia. However, the uremic state differs in that normal isoagglutinin titers, lymphoid tissue and plasma cell responses have been observed.

Understanding of the impairment of the immune response in chronic uremia may lead to methods of modifying man to make him more receptive to homotransplantation and constitute another approach to such problems as tissue and organ loss or insufficiency.

Radioactive Vitamin B_{12} Absorption Studies: Results of Direct Measurement of Radioactivity in the Blood. ALFRED DOSCHERHOLMEN and PAUL S. HAGEN, Minneapolis, Minn. (Introduced by E. B. Flink).

The absorption of vitamin B_{12} was determined by measurement of radioactivity of blood samples collected after the oral administration of a test dose of radioactive vitamin B_{12}. Radioactivity measurements of 20-ml. samples of blood or plasma were made with a well-type scintillation counter. A net of seven counts per minute equalled 3 times the standard deviation of the background. Counts above this figure were considered significant.

Test doses consisted of 0.46 μg. and 0.92 μg. of vitamin B_{12} containing 0.5 μc. and 1.0 μc. of Co^{60}, respectively. Peak radioactivity in the blood samples did not appear until 8 to 12 hours after the test dose was given.

Seventeen of 20 non-pernicious anemia patients were given the 0.46-μg. dose. In 6 tests whole blood net counts ranged from 18 to 41 per minute while in 14 instances plasma counts ranged from 19 to 54. In 3 given the larger test dose whole blood net counts ranged from 33 to 55 while plasma counts were 56 to 108. In 4 patients with pernicious anemia given the 0.46 μg., 0.5 μc. dose the blood and plasma counts were not significant, ranging from 2 to 6 per minute. In one pernicious anemia patient given the dose containing 1.0 μc. of Co^{60} net counts of 10 and 11 per minute were obtained in the whole blood and plasma, respectively. However, with potent intrinsic factor concentrate the blood or plasma radioactivity was within the range found in the non-pernicious anemia patients.

These studies show that the measurement of radioactivity in 20-ml. samples of blood or plasma is a practical means to determine the absorption of test doses of vitamin B_{12}. This method appears to be useful in the diagnosis of pernicious anemia and in the evaluation of intrinsic factor activity.

Blood Lipoprotein Patterns in Preclinical and in Clinical Coronary Artery Disease. JOSEPH T. DOYLE, LOUIS S. DELALLA, WINFIELD H. BAKER, A. SANDRA HESLIN, and RAY K. BROWN, Albany, N. Y. (Introduced by Richard T. Beebe).

Autopsy data suggest that coronary atherosclerosis is almost universally prevalent in middle aged American men. Since coronary atherosclerosis is not clinically

diagnosable until the advent of symptoms or signs of myocardial ischemia, the demonstration of a pathognomonically disordered blood lipid pattern would be of obvious importance.

To this end, 115 white men over the age of 40 and with few exceptions under the age of 55 years were selected from a carefully studied group of 2,000 office workers. Sixty-seven men were considered to be clinically free of coronary artery disease, although 27 were regarded as predisposed because of poor vascular heredity, obesity, hypertension or diabetes mellitus. All had normal electrocardiograms after standard exercise. Forty-eight men had clinically manifest coronary artery disease: 20 had angina pectoris and 28 had survived an acute myocardial infarction.

The following studies were done on blood obtained from these men in the fasting state: measurement of total cholesterol; separation of α and β lipoprotein by Cohn's Method 10; measurement of α and β lipoprotein cholesterol and phospholipid; measurement of total lipids; and ultracentrifugation by the technique of Lewis and Page.

The only statistically significant differences found were increases in the total cholesterol and phospholipid in the healed myocardial infarction group. These increases were accounted for by increases in the β lipoprotein cholesterol and phospholipid without the reciprocal decrease in the α lipoprotein fractions described as characteristic by others. The overlap in all groups nullified the diagnostic value of any single determination or combination of chemical determinations.

It is concluded that in a biologically homogeneous population universally susceptible to coronary atherosclerosis, the blood lipoprotein pattern is neither qualitatively nor quantitatively a satisfactory index of atherogenesis.

A Clinical Correlative Study of the Electrocardiogram in Electrolyte Imbalance. L. DREYFUSS and A. PICK, Chicago, Ill. (Introduced by Louis N. Katz).

In order to test the practical value and the limitations of the electrocardiographic diagnosis of specific electrolyte disorders, chemical laboratory findings were correlated, in part in serial determinations, with electrocardiograms in patients with suspected or established disturbance of the potassium a/o calcium serum concentration. Full agreement of electrocardiographic and laboratory findings was noted only in the lowest and highest potassium ranges, but no such definite correlation could be established with regards to Na/K ratios, the serum pH and the serum calcium concentrations. In some cases electrocardiographic abnormalities characteristic of hypo- and hyperkalemia preceded corresponding alterations in the serum concentration. Some difficulties in diagnosing electrocardiographically mixed electrolyte disorders, and in distinguishing drug effects from electrolyte effects, are illustrated. In addition to intracellular/extracellular distribution of electrolytes, the function of the ground substance as a third intermediate compartment, and the presence or absence of renal function, may be factors determining the degree to which electrolyte imbalance is reflected in the electrocardiogram.

Comparative Effects of Free and Esterified Sitosterol on Serum and Liver Cholesterol in Rats. CHARLES H. DUNCAN and MAURICE M. BEST, Louisville, Ky. (Introduced by J. Murray Kinsman).

Groups of 6 male rats subjected to prior radiation destruction of the thyroid by Iodine-131 were fed one of several sterol containing diets for 2 weeks, and serum and liver total cholesterol then determined. The addition of 1 per cent cholesterol to the diet resulted in mean liver cholesterol concentration of 1625 ± 296 mg. per 100 gm. (wet weight) as compared to 262 ± 27 mg. per 100 gm. on a cholesterol-free diet. When the cholesterol was added to the diet as cholesteryl palmitate the mean liver cholesterol was only 357 ± 54 mg. per 100 gm.

The further addition of 5 per cent beta-sitosterol to the 1 per cent cholesterol diet prevented the increase in liver cholesterol, the mean concentration being 281 ± 28 mg. per 100 gm. When the sitosterol was administered as an ester of a long-chain saturated fatty acid its effectiveness in preventing accumulation of cholesterol in the liver was greatly reduced. Thus with a diet containing 1 per cent cholesterol and 5 per cent sitosterol as the palmitic acid ester the mean liver cholesterol was 1259 ± 415 mg. per 100 gm. That the decreased effect of sitosteryl palmitate as compared to the free sterol was not due merely to the presence of the palmitic acid is indicated by a mean liver cholesterol of 249 ± 38 mg. per 100 gm. on a diet containing 1 per cent cholesterol, 5 per cent sitosterol (free) and 3.1 per cent palmitic acid (free). A diet containing 1 per cent cholesterol and 5 per cent sitosterol as a short-chain ester (sitosteryl acetate) resulted in mean liver cholesterol of 362 ± 35 mg. per 100 gm., only slightly higher than with free sitosterol. Changes in mean serum cholesterol concentrations were in the same direction but of lesser magnitude than those in the liver.

These observations are compatible with the hypothesis that esterification is a step in the transport mechanism by which cholesterol is absorbed, and that sitosterol interferes with the absorption of cholesterol by competing for esterification.

Cerebral Blood Flow and Metabolism in Mentally Confused Subjects with Congestive Heart Failure. SEYMOUR EISENBERG and WILLIS SENSENBACH, Dallas, Tex. (Introduced by Edward L. Pratt).

Cerebral blood flow and metabolism were studied in 13 mentally confused patients with severe congestive heart failure; in six instances, the studies were repeated when the subjects had become lucid. The findings in the patients with mental confusion were also compared with results obtained in 8 patients with congestive heart failure of comparable severity but without mental symptoms.

Results: 1. CBF was profoundly decreased in each of

the confused subjects—mean of 27 cc. per 100 gm. per min. as compared with a mean of 42 cc. in 8 lucid subjects with comparable failure.

2. Similar differences were encountered in the 6 subjects studied prior to and following the development of mental symptoms.

3. A commensurate increase in CVR accompanied the decline in flow.

4. Cerebral oxygen utilization, although moderately depressed, did not differ significantly from the values in lucid subjects.

It is suggested that the mental symptoms were consequent to inadequate cerebral perfusion and reflect a generalized diminution of organ blood flow in terminal congestive heart failure.

The Relationship of Chemotherapeutic Specificity to Metabolic and Cytochemical Changes in Patients with Leukemia and Lymphosarcoma. LEONARD P. ELIEL* and ROBERT P. HEANEY, Oklahoma City, Okla.

The nature and degree of chemotherapeutic specificity of a number of agents for leukemic and lymphosarcomatous tissues in man have been investigated by means of simultaneous balance studies and cytochemical analyses of neoplastic tissue. Changes in the mass of normal and neoplastic tissues, in tumor desoxyribose and ribose nucleic acids (DNA and RNA), and in the tumor nuclear-cytoplasmic ratios of nitrogen, phosphorus, and potassium have been determined.

Administration of 6-mercaptopurine, triethylenemelamine, myleran, and demicolcin (Group I agents) to patients with leukemia or lymphosarcoma, was followed by losses of tumor tissue ranging from 0.3 to 2.4 Kg. (average 1.2 Kg.) and by *gains* in normal tissue ranging from 0.4 to 1.1 Kg. (average 0.6 Kg.).

Administration of cortisone, delta-1-cortisone, and triiodothyronine (Group II agents), on the other hand, resulted in tumor losses ranging from 0.4 to 0.8 Kg. (average 0.6 Kg.) and in *losses* of normal tissue ranging from 0.5 to 3.5 Kg. (average 1.3 Kg.).

Group I agents resulted in reductions of tumor DNA averaging 18 per cent or 3.2 mg. per gm. of wet tissue mince, while those in Group II resulted in no significant or consistent changes. No significant change in nuclear RNA was observed except in the case of delta-1-cortisone where increases of 13 to 30 per cent were found.

The nuclear-cytoplasmic ratios of nitrogen, phosphorus, and potassium were reduced uniformly by Group I agents and increased uniformly by Group II agents.

The findings indicate that the agents (Group I), inducing tumor shrinkage and permitting simultaneous normal tissue growth, also show effects on DNA and on nuclear-cytoplasmic ratios which are uniform and distinct from those of agents (Group II) which produce catabolism of both normal and tumor tissues. The results suggest that there probably exist in human lymphoid tumors approximately unique synthetic pathways which confer the chemotherapeutic specificity demonstrated in these studies.

Quantitative Aspects of Osmotic Hemolysis and Its Significance in Hereditary Spherocytosis. CHARLES P. EMERSON,* Boston, Mass.

It has been established that in dilute saline suspensions the volume of red cells bears a reciprocal relationship to the tonicity of the system and to the pH. The intersection of the curve representing the ratio between cell volume and reciprocal tonicity with the coordinate corresponding to the original volume establishes the reciprocal of isotonicity ($1/T^{i_o}$), this volume usually having been equivalent to 0.85 to 0.88 gm. NaCl per 100 ml. at pH 7.4. By extrapolation to the coordinate $1/T = 0$ (infinite tonicity) it is possible to derive the theoretical volume of cells which are desiccated, *i.e.*, devoid of mobile water (V^e), while extrapolation to the tonicity at which hemolysis occurs (T^{hem}) indicates the cell volume at maximum capacity (V^c).

The presence, or degree, of hypochromia or spherocytosis in any specimen is reflected by the volume of mobile fluid in the erythrocytes, relative to their maximum capacity, *i.e.*, their saturation at isotonicity. This value may be computed as follows: $(V^{i_o} - V^e)/(V^c - V^e) \times 100 = 100/0.87 \div 1/T^{hem} = T^{hem} \times 115$. Thus, hypochromic cells hemolyzing in 0.20 per cent NaCl solution as 23 per cent saturated, normocytes hemolyzing in 0.40 per cent NaCl, 46 per cent saturated, and hereditary spherocytes hemolyzing in 65 per cent NaCl, 75 per cent saturated at isotonicity.

As previously demonstrated, distribution curves representing gradients of hemolysis in relation to the negative logarithm of tonicity are symmetrical for most cell populations, including samples from splenectomized patients with hereditary spherocytosis. Incubation at 37° C *in vitro* produces, as a reflection of cellular metabolism, an increase in T^{hem} but fails to distort this symmetry of distribution. An analysis of such charts indicates that the changes in mean and extreme values for T^{hem} after incubation, when computed on a percentile basis, are precisely similar in samples of hypochromic, normochromic and spherocytic blood, providing no support for the concept that the metabolism of hereditary spherocytes is defective.

Ammonia Metabolism in Cirrhotic Patients with Portacaval Shunts. WILLIAM W. FALOON, J. HOWLAND AUCHINCLOSS, ROBERT EICH, and ROBERT GILBERT, Syracuse, N. Y. (Introduced by Paul A. Bunn).

The occurrence of episodic stupor in patients with portacaval shunts has stimulated a study of ammonia metabolism by use of venous catheterization in three patients with shunts. One of these was studied while in deep stupor and another rapidly developed stupor when subsequently given ammonium chloride. Similar studies have been carried out in four non-cirrhotic patients with cardiac disease.

By venous catheterization, blood ammonia levels were determined in: 1) inferior vena cava (IVC) below the renal veins, 2) IVC above the renal veins, 3) IVC above the level of the shunt, 4) hepatic vein, 5) right auricle.

Arterial blood ammonia was determined simultaneously with most venous specimens.

An A-V decrease in blood ammonia was found in the peripheral tissue (IVC below renals) in the cirrhotics but not in the cardiacs. Hepatic vein ammonia was normal in all patients and an A-V decrease was present in this vascular bed. Total body (artery to right auricle) A-V difference in blood ammonia was present in all patients but was much more marked in the cirrhotics; these data showed an increase in ammonia in blood traversing the lungs. The highest elevations in blood ammonia were found in cirrhotics above the shunt representing a marked rise over arterial and other venous levels. Ammonia in the IVC above the renal veins was usually but inconstantly higher than arterial ammonia in both groups of patients.

The data indicate that shunting of blood is of greater significance in the production of elevated blood ammonia in cirrhotics with portacaval shunts than is defective hepatic extraction. Also, in cirrhotics but not in cardiacs peripheral tissue appears to remove ammonia. An increase in ammonia levels as blood traverses the lungs is apparent in all patients but is more marked in cirrhotics.

The Arterio-Venous Difference of Potassium in a Patient with Periodic Paralysis. SAUL J. FARBER * and HUGH J. CARROLL, New York, N. Y.

A male adult with familial periodic paralysis was studied during four hypokalemic paralytic episodes induced by feeding a carbohydrate meal. Electrolyte determinations during induction of attacks indicated that potassium was removed from arterial plasma and probably entered skeletal muscles. The reverse occurred during recovery induced by the ingestion of KCl; potassium left the muscles and entered venous plasma.

The arterio-venous difference for K at the beginning of an attack was as high as 2 mEq. per L. ($A = 3.3$; $V = 1.3$) and seemed to be correlated with the state of weakness. During one attack when the upper extremities were paralyzed and the lower extremities weak, the A–V difference was 1.8 (femoral artery minus antecubital vein) and 1.5 (femoral artery minus femoral vein) mEq. per L., respectively. As the paralysis progressed arterial and venous concentration became equal. During recovery, the concentration of K in venous plasma was greater than arterial. There was no A–V difference in glucose, Na, pH, or hematocrit. Red blood cell K decreased when plasma K was maximally decreased.

To produce a stable and higher concentration of K in arterial plasma, K may have been added to the blood before it entered the aorta. Catheterization of central venous channels was attempted to determine which organs may have been contributing potassium. K concentration in internal jugular vein plasma was slightly higher at a time when the antecubital vein plasma was lower than arterial plasma. Unmixed hepatic vein blood was not obtained but blood from a location close to the entrance of the hepatic vein into the inferior vena cava had a plasma K concentration equal to venous plasma obtained from the femoral vein.

Periodic paralytic attacks involve shifts of K into skeletal muscles and out of another organ. The latter probably contributes to the observed greater arterial than venous concentration of potassium.

Toxoplasma Antibody and the Properdin System. HARRY A. FELDMAN * and LOUIS PILLEMER, Syracuse, N. Y. and Cleveland, O.

In 1948 Sabin and Feldman reported that extracellular toxoplasma suspended in fresh normal serum were stained readily by alkaline methylene blue but that when incubated with fresh serum containing antibody, the parasites lost their affinity for the dye. This is the basis for the standard dye test for toxoplasma antibodies. If the antibody containing serum was inactivated prior to the addition of the parasites, the toxoplasma were stained blue. Thus, it was apparent that a heat-labile component ("activator") of human serum was required for the antibody to be active against the parasite. Although much has been learned about the "activator" including that it was not entirely interchangeable with hemolytic complement and that it was not present in mouse serum, its composition, until now, has been something of a mystery. Experiments recently conducted by us and by Gronroos in Finland have demonstrated that toxoplasma antibody "activator" is the properdin system. This is the first instance in which it has been shown that the properdin system is required in order for a specific antibody to exert its adverse effects upon a parasite. The implications of this will be discussed. Studies conducted with serum collected just before the death of a toxoplasma infected human, in spite of a high antibody titer, failed to demonstrate any lack of "activator."

Clinical Study of Host-Parasite Relationship in Loa-loa. WILLIAM R. FELTS, JR., and EDMUND J. TALBOTT, Washington, D. C., and Bethesda, Md. (Introduced by Thomas McPherson Brown).

Hypersensitivity to parasitic antigen has been suggested as important in the pathogenesis of filarial infection. Coordinated objective laboratory and clinical data to support this concept are still inadequate. Such data have become available by the study of a case of loiasis of eleven years' standing.

The reaction of a sensitized subject to antigen release induced by an anti-parasitic substance (Hetrazan®) was observed. A severe exacerbation of the illness was noted comparable to the Jarisch-Herxheimer reaction. This was accompanied by transient electrocardiographic abnormalities, abnormal liver function tests, increase in sedimentation rate, leucocytosis, eosinophilia, a rise in total globulin, and a fall in serum albumin. Of particular interest was the rise in gamma globulin and the development for the first time of a positive filarial complement-fixation test. The clinical and laboratory expression suggesting an induced antigen-antibody reaction was paralleled by a sharp drop in circulating microfilariae. The

subsequent acceptance of increasing doses of the same anti-filarial substance with the production of progressive clinical improvement is significant in the support of the concept that the reaction was due to antigen release rather than to drug sensitivity.

The balance between host and parasite in diseases where immune mechanisms are important appears to determine the course and the expression of the disease. The present studies stress the importance of the proper interpretation of the underlying mechanisms responsible for the expressions of alteration of this balance.

Observations on the Causes and Mechanism of Insulin Resistance During Diabetic Acidosis. JAMES B. FIELD and DEWITT STETTEN, JR., Bethesda, Md. (Introduced by Joseph Edward Rall).

Diabetic acidosis is almost always associated with some degree of insulin resistance. Both increased adrenal cortical activity and decreased serum pH have previously been suggested as the cause of this phenomenon.

In six out of eight patients studied, humoral insulin antagonist activity was demonstrable by *in vitro* techniques. The basis of the method employed was the measurement of extra glycogen accumulation in rat hemidiaphragm after exposure to insulin. Sera from normal persons, patients in uremic acidosis and patients with a high plasma level of adrenal corticosteroids did not contain any insulin antagonist. In general, there was good correlation between the amount of insulin received in the first twenty-four hours of therapy and the presence of insulin antagonist activity in the serum. There did not seem to be any relation between the level of blood sugar or CO_2 and the insulin antagonist. None of the patients had exhibited insulin resistance before or after the episode of acidosis. In one case, insulin antagonist had disappeared from the serum ten hours after therapy for the acidosis was started, while in several others it was gone within a few days of treatment.

Insulin antagonist activity was non-dialyzable and was associated electrophoretically with the α globulin fraction of the serum proteins. Studies utilizing I^{131} labeled insulin indicated that the antagonist did not interfere with the binding of insulin by the rat diaphragm and did not possess any insulinase activity.

Effects of Purified Streptokinase Injected Intravenously into Patients. ANTHONY P. FLETCHER, ALAN J. JOHNSON,* and W. ROSS MCCARTY, New York, N. Y.

Studies of the physiological effects and therapeutic use of intravenously administered streptokinase have been considerably advanced by developing methods of purification. Methods of fractionation, to be described elsewhere, have raised the purity of streptokinase from its former value of 90 units/gamma N to 600 units/gamma N. Ultracentrifuge and electrophoretic examination suggest that the new material is largely homogenous, though immunochemical analyses reveal the presence of several antigenic components.

Clinical trial, based on a series of 250 intravenous infusions in 120 patients, constitutes the substance of this report.

It has been demonstrated that:

a) The pyrexia and hypotensive effect, formerly exhibited by intravenous Varidase®, have been markedly reduced.

b) Clinical and EKG exams made before, during and after infusions have revealed no cardiotoxic effect in contrast to the findings of Kellner and Robertson in rabbits.

c) A comparison between patients who developed an active fibrinolytic system in the blood and those who did not, demonstrated that man tolerated high circulating levels of fibrinolytic enzyme without untoward clinical symptoms. This finding is of interest in connection with the hypothesis that some clinical manifestations of the hypersensitivity response (antigen-antibody union) are mediated through activation of the blood protease system.

A method for determining, pre-injection, the anticipated dose of streptokinase required to produce systemic fibrinolysis in the individual patient has proved to be valid by clinical trial. Statistical analysis of these data on 103 patients indicates that systemic fibrinolysis can probably be induced with streptokinase in 85 per cent of the population.

These studies constitute essential pharmacological data on which to base planned therapeutic trials in patients with intravascular thrombosis.

Relation of Exchangeable Sodium, Potassium and Chloride to Total Body Content in Man. GILBERT B. FORBES and ANNE LEWIS, Rochester, N. Y. (Introduced by Lawrence E. Young).

Extensive use is being made of isotopic dilution techniques for the estimation of total body H_2O, Na, K, and Cl in man. A sufficient number of newborn infants have been analyzed by direct chemical means to permit comparisons with this technique. In the adult, however, evaluation of the technique has been largely inferential since data on carcass analysis are so meager. We have recently had the opportunity of analyzing two adult male carcasses for H_2O, fat, Na, K, and Cl; the results of these analyses will be compared with isotopic dilution data published from our laboratory and from the literature. Data on the newborn will also be included.

In the newborn there is a fairly good correspondence between results of isotopic dilution studies and carcass analysis for H_2O, Na, K, and Cl. The adult carcasses analyzed in our laboratory contained, on the average, 530 ml. H_2O, 62 mEq. Na, 52 mEq. K, and 38 mEq. Cl per kilogram on a fresh weight basis, and 690 ml. H_2O, 80 mEq. Na, 67 mEq. K, and 45 mEq. Cl per kilogram of fat-free weight. When one compares these values with those obtained by isotopic dilution it would appear that, while there is good agreement for water, only about two-thirds of total body Na and 85 to 90 per cent of K and Cl are exchangeable.

On the basis of these data, it would appear that the isotopic dilution techniques, though valid for the young infant, may well underestimate total body Na, K, and Cl in the adult and by a significant amount.

The Role of the Basic Fraction of Gamma Globulin in the Flocculation Reactions. EDWARD C. FRANKLIN and HENRY G. KUNKEL,* New York, N. Y.

Much evidence exists indicating that the thymol turbidity, cephalin flocculation and zinc turbidity tests reflect in part changes in the serum γ-globulin. It has been noted that γ-globulin isolated from hepatitis serum reacts more strongly with thymol reagent than γ-globulin from normal serum. Therefore an attempt was made to detect qualitative variations in these proteins, and to determine if all fractions of γ-globulin react similarly with the flocculating reagents. Cohn fraction II-γ-globulin and γ-globulin from normal sera and sera of patients with cirrhosis and hepatitis were separated by starch zone electrophoresis into 8–10 sub-fractions with different mean mobilities. Flocculation tests were performed with each fraction. A small amount of lipid was added in the thymol turbidity test. In the thymol turbidity and cephalin flocculation tests the basic fractions of the γ-globulin migrating more slowly than the peak reacted most vigorously, while the more acidic γ-globulin was inactive. In the zinc turbidity test the fractions making up the peak were most active.

To confirm this apparent effect of the isoelectric point and charge of the γ-globulin, a number of proteins was investigated. Basic proteins such as lysozyme, trypsin and ribonuclease were highly active in the thymol turbidity and cephalin flocculation reactions. Acidic proteins such as β-lactoglobin, ovalbumin, hemoglobin and serum albumin, and α- and β-globulin reacted weakly or not at all.

Groups of normal and pathological sera were separated simultaneously by starch zone electrophoresis, and the distribution of γ-globulin was determined. Sera with positive thymol turbidity and cephalin flocculation tests usually showed an increase in the basic portion of the γ-globulin. Certain exception were encountered, and further evidence was obtained confirming the importance in these reactions of other factors, such as β-lipoproteins and albumin, in addition to the basic γ-globulins.

Errors in Measurements with Serum Proteins Labelled with I_{131}. J. J. FRANKS and E. B. REEVE, Denver, Colo. (Introduced by J. F. Mueller).

Measurements of plasma volume with radio-iodinated serum albumin of high specific activity gave unexpectedly low results in comparison with simultaneous measurements made with T1824. Experiment showed that the labelled albumin is adsorbed to the glass walls of syringes, pipettes and volumetric containers. The quantity lost by adsorption depends on many factors but particularly the area of the adsorbing surface, the solvent, and the concentration of dissolved proteins. For errors in measurements to arise, a significant proportion of the total protein in a container or pipette must be lost. Fifty per cent of the contained protein of physiological saline solutions of 0.3 μ protein per cc., 15 per cent of solutions of 3 μ per cc., and 4 per cent of solutions containing 30 μ per cc. may be lost to the 20 cm.² inner surface of a small test tube. From distilled water solutions even greater quantities are lost. We have not found a solvent suitable for intravenous injection that prevents this adsorption, nor a surface that fails to adsorb. Radio-iodinated rabbit serum albumin and human serum gamma globulin also adsorb to glass. These observations cast doubt on the more recent measurements of plasma volume made with radio-iodinated human albumin of high specific activity, and perhaps also on other measurements made of proteins in very high dilution. Significant loss of radio-iodinated proteins to the walls of syringes, pipettes and volumetric containers can be prevented by addition of sufficient carrier protein. Without such treatment errors of the order of 5 to 20 per cent in the measurement of plasma volume readily arise.

Bidirectional Exchange of Permeable Substances Across the Capillaries of the Human Forearm. EDWARD D. FREIS,* HAROLD W. SCHNAPER, RENATO D. KOVACH, FRANK A. PORFIDO, and LAWRENCE S. LILIENFIELD, Washington, D. C.

Using methods previously described (J. Applied Physiol., 1953, 5, 526; Circulation, 1955, 12, 772.) the forearm transcapillary exchange of 9 labelled substances was studied in 48 subjects.

The concentration of permeable substance expected if there were no transcapillary loss (CPE) was calculated from its relation to the concentration of the impermeable tracer (CI) using the proportion

$$\frac{\text{CPE sample}}{\text{CI sample}} = \frac{\text{CP injectate}}{\text{CI injectate}}.$$

The values obtained from frequent venous samples of CPE and actual concentration (CPA) were plotted against time.

1. *Extent of transcapillary losses.* During the early period the net transcapillary losses averaged as follows: heavy water 96 per cent, thiocyanate 55, glutathione 41, and inulin 25 per cent, suggesting a relationship between permeability and molecular size. However, the relationship was not strict since the losses averaged for sodium 53, sulfate 44, methionine 63 and PAH 54 per cent.

2. *Turnover.* In the early period the curve of CPA lay below the curve CPE but later crossed over and rose above it. The area enclosed prior to the point of crossing, or "equilibrium time" (ET), is proportional to the total net loss. The area enclosed to the right of ET represents the net return. The time where area to the right equals half the area to the left of ET is the "half return time" or HRT.

a) HRT's for sodium, sulfate, thiocyanate and PAH were in the range of 1.2 to 5 times the net loss times. HRT heavy water was prolonged reflecting its free diffusion into a large space. HRT inulin also was prolonged possibly because large molecular size inhibited capillary

reentry. Methionine (freely permeable) exhibited negligible return suggesting incorporation into cells.

b) ET always varied directly with MCT T-1824 signifying that turnover of permeable substances is blood flow dependent.

The Application of I-131 Labeled Albumin to the Study of Acute Changes in Protein Metabolism in Man. KENNETH FREMONT-SMITH and FRANK L. IBER, Washington, D. C. (Introduced by Stanley M. Levenson).

Studies of protein metabolism with I-131 labeled albumin (HISA) have been widely performed during a steady state. The comparison of data from different steady states has been very difficult, however, because of the poor reproducibility among various lots of HISA. Our data were obtained by abruptly altering the steady state halfway through the course of a HISA turnover determination. The semilogarithmic I-131 disappearance curve tends gradually to flatten as the steady state is prolonged. Therefore changes in the steady state which accentuate the decline of the curve are significant, whereas changes which further flatten the curve are not readily interpretable. The cumulative urinary excretion of I-131 provides a direct measure of the rate of albumin degradation. Such information cannot be so clearly obtained with isotopes of sulfur, carbon or nitrogen because of the possibility of reincorporation.

Triiodothyronine. After the control half of the I-131 disappearance curve had been established in four euthyroid individuals on nitrogen balance study, triiodothyronine (1 mg. daily) was administered for either four or seven days. In each case the cumulative urinary I-131 excretion curve became abruptly steeper. This increased rate of albumin degradation persisted for 3 to 7 days after stopping triiodothyronine, coinciding approximately with the period of negative nitrogen balance and accounting for 4 per cent to 7 per cent of the increased urinary nitrogen.

Diet. The effect of an abrupt isocaloric increase in dietary protein was similarly studied in five subjects in good nutritional status. The sudden increase in nitrogen intake from 0.5 to 3.0 gm. per Kg. was associated in four with a slight but definite increase in the albumin degradation rate which persisted even after nitrogen equilibrium was restored.

It is concluded that direct estimation of albumin degradation during acute changes in steady state is feasible with I-131 albumin.

The Efficiency of Ventilation During Voluntary Hyperpnea of Patients with Chronic Obstructive Emphysema. H. W. FRITTS, JR., J. FILLER, A. P. FISHMAN, and A. COURNAND, New York, N. Y. (Introduced by Dickinson W. Richards).

In order to determine the effect of pulmonary disease on the efficiency of ventilation during voluntary hyperpnea, the energy cost of breathing and the work done on the lungs and air were measured in six control subjects and in three patients with chronic obstructive emphysema. The energy cost was estimated by using a modification of the method of Liljestrand (Tr. A. Am. Physicians, 1954, 67, 162.). The mechanical work was obtained by measuring the areas of pressure-volume diagrams. Since esophageal pressure was used in the construction of these diagrams, a separate study was carried out to investigate the fidelity with which pressure fluctuations in the esophagus reflect those which occur in the pleural space. In eight patients with pneumothorax, simultaneous measurements of esophageal and intra-pleural pressure agreed well ($\pm$ 10 per cent) during quiet breathing, but less well ($\pm$ 25 per cent) at large tidal volumes. Despite this disparity, values of work obtained using esophageal pressure agreed within 20 per cent with those obtained using pressure recorded directly from the pleural space. For a particular volume of ventilation, the patients with emphysema expended more energy and performed more work than did the normal subjects. However, no difference in efficiency could be demonstrated, the values varying between 1 and 8 per cent in both the control and patient-groups. In these experiments the work done on the chest wall and diaphragm was not measured. The effect of this component on the calculation of efficiency is currently being analyzed.

The Relation of Spleen Size to the Body Hematocrit/Venous Hematocrit Ratio. HUGH FUDENBERG and JOHN P. MAHONEY, Boston, Mass. (Introduced by Joseph M. Hayman).

Clinical observations indicate that transfusions in the presence of a large spleen often fail to produce a significant increase in the venous hematocrit level. Although Chaplin *et al.* have shown that the body hematocrit/venous hematocrit ratio (BH/VH) is remarkably constant over a wide range in venous hematocrit (.91), the effect of splenomegaly upon this ratio was not determined. However, the high erythrocyte concentration in splenic blood (Gibson *et al.*) suggests that marked change in size of spleen can significantly alter the BH/VH.

To investigate this hypothesis, simultaneous red cell and plasma volumes were determined in four groups of nine patients each, with hematocrits ranging from 17 to 77 per cent: Group 1: individuals with non-palpable spleens; Group 2: previously splenectomized individuals; Group 3: moderate splenomegaly; Group 4: marked splenomegaly. Several disease entities were included in each group. Hexavalent anionic Cr^{51} and trivalent cationic Cr^{51} were used for simultaneous measurement of red cell mass and plasma volume, respectively, using a modification of the method of Gray and Frank.

The BH/VH values obtained in groups 3 (.98) and 4 (1.04) were significantly greater ($p > .01$) than in group 1 (.889). BH/VH was slightly but not significantly less in group 2 than in the control group 1 ($p < .05$).

These observations demonstrate 1) a significant alteration of the BH/VH in the presence of well-defined splenomegaly, probably due to the "pooling" of relatively high hematocrit blood in the large splenic reservoir; 2) that the venous hematocrit is not a reliable index of total

red cell mass in conditions associated with moderate to marked splenomegaly; and 3) in the presence of splenomegaly, both plasma and red cell volumes must be determined for accurate estimation of total blood volumes.

Aldosterone and Human Arterial Hypertension. JACQUES GENEST, GUY LEMIEUX, ANDRÉ DAVIGNON, ERICH KOIW, WOJCIECH NOWACZYNSKI, and PAUL STEYERMARK, Montreal, Canada. (Introduced by J. S. L. Browne).

Purified aldosterone fractions were obtained from 24-hour urine aliquots after 1) immediate chloroform extraction at pH 1, 2) re-extraction after 24 hours' incubation with animal B-glucuronidase at pH 4.5, 3) alkali and water washings, 4) two successive paper chromatographic separations of the crude neutral extract (propylene glycol/toluene and toluene 90-acetate 10/50 per cent aqueous methanol systems). Each bio-assay experiment for determination of aldosterone activity included one control group of adrenalectomized rats, two receiving standard solutions of aldosterone and two receiving dilutions of the urinary aldosterone fraction equivalent to 40 minutes and 20 minutes output. Five normal adult males, six patients with malignant hypertension and seven patients with severe essential hypertension were studied. Diet was unrestricted prior to, or during, the urine collection. No patient presented any sign of cardiac failure. The difference between the normal subjects and the 2 hypertensive groups becomes evident and significant in tests using extracts corresponding to 40 minutes of urinary output. Whereas the normal aldosterone excretion is by our method less than 1.4 micrograms per day, the mean aldosterone excretion in the severe essential hypertension group was found to be 8.1 micrograms per day (fiducial limits at $p = 0.05$: 4–16.5) and in the malignant group 6.3 micrograms per day (fiducial limits: 2.96–13.84). Statistical analysis of the mean difference shows a "t" value of 4.85 ($p < 0.001$) for the urinary Na/K ratio index and of 3.27 ($p < 0.01$) for the Na excretion. This hyperaldosteronism possibly explains the abnormalities of sodium metabolism in hypertensive patients and suggests that human hypertension could be caused by a state of mild and chronic hyperaldosteronism.

Plasma Lipoprotein Metabolism in Normal Individuals and in Children with the Nephrotic Syndrome. DAVID GITLIN * and DAVID CORNWELL, Boston, Mass.

In this study, it was found that the hyperlipemia and hypercholesterolemia characteristic of the nephrotic syndrome are due to an elevation of lower density β-lipoproteins, primarily the S_f 15 to 400 class. The concentrations of α-lipoprotein and higher density β-lipoprotein, S_f 3 to 8, are either normal or somewhat decreased.

The peptide moieties of human α- and β-lipoproteins were labelled with I^{131} and the metabolism of these proteins studied in normal children and adults and in children with the nephrotic syndrome. The half-life of α-lipoprotein was about 4 days in normals, but in children with the nephrotic syndrome, its catabolism was accelerated.

The metabolism of β-lipoprotein was complex. In normal individuals, the lower density β-lipoproteins were rapidly converted to those of higher density and catabolized as such; the latter had a half-life of about 3 days. This conversion did not occur *in vitro* nor was the conversion of a higher density β-lipoprotein (S_f 3 to 8) to one of lower density (S_f 15 to the chylomicra) observed either in normal or nephrotic individuals. In the nephrotic child, the fractional rate of conversion of lower density β-lipoproteins (S_f 15 to 400) to those of higher density was greatly decreased; hyperlipemia in these children was attributable, at least in part, to this conversion failure. The catabolism of higher density β-lipoprotein appeared normal or a bit accelerated in nephrosis, while the low density β-lipoproteins isolated from nephrotic plasma were converted and catabolized normally in healthy individuals.

It would seem, therefore, that: 1) a system for the unidirectional conversion of low density β-lipoproteins to those of higher density exists *in vivo* and is normally operative, 2) there is a partial failure of this conversion system in the nephrotic syndrome, and 3) the metabolism of the lipoproteins explains their plasma concentrations in nephrotic children.

Simultaneous Catheterization of Left and Right Heart. HARRY GOLDBERG, RALPH SMITH, JANET DICKENS, GEORGE RABER, and ASHER WALDOW, Philadelphia, Pa. (Introduced by Joseph DiPalma).

Twenty-five patients with pure aortic stenosis and twenty-eight with pure mitral stenosis were studied by simultaneous left and right heart catheterization. Right heart catheterization was performed in the usual way. Left heart catheterization was performed by the transthoracic approach, inserting a needle into the left atrium. A polyethylene catheter was passed through the needle and advanced into aorta. Pressure gradients and flows were thus obtained simultaneously. Valvular flows, areas, and ventricular works were calculated by a modification of the Gorlin formulae. In aortic stenosis the flow was reduced averaging 163 cc. per sec. The left ventricular-aortic systolic pressure gradients were marked averaging 60 mm. Hg. The valve areas were considerably reduced (0.2 to 1.1 $cm.^2$). An increase in the total left ventricular work was observed averaging 5.2 Kgm. per min. In mitral stenosis the valve flow was diminished averaging 103 cc. per diastolic sec. The left atrial pressures were elevated (10 to 39 mm. Hg). These correlated well with pulmonary capillary pressures. Left atrial–left ventricular filling pressure gradients were consistently observed averaging 13 mm. Hg. The effective right ventricular work was increased averaging 1.2 Kgm. per min. The valve areas were decreased averaging 1.0 cm^2. The pressure gradients were shown to vary directly as the flow when the orifice size remained constant in both mitral and aortic stenosis. As the valve area reduced the flow showed a concomitant reduction. Ventricular work ap-

peared to vary directly with valve area and with valve flow. Combined heart catheterization is not only valuable in assessing the dynamics of valvular disease but also in evaluating surgical techniques. This is illustrated with data obtained in patients before and after mitral and aortic commissurotomy.

Anticoagulant Appearing in Plasma Thromboplastin Component (PTC) Deficiency. ROBERT GOLDSTEIN, MATHEW GELFAND, MARTIN SANDERS, and ROBERT ROSEN, Boston, Mass. (Introduced by Benjamin Alexander).

Resistance to transfusion therapy developing in a patient with PTC deficiency was investigated. The patient, originally considered a hemophiliac, had frequently received blood, plasma and Fraction I prior to the development of resistance. His nephew, our studies revealed, was a classical case of PTC deficiency.

Our initial studies revealed an elevated coagulation time and impaired prothrombin consumption, not corrected *in vitro* by plasma or serum from normal or hemophilic subjects. Whereas his serum did not support thromboplastin generation, his platelets and $BaSO_4$ absorbed plasma behaved normally. His plasma corrected the clotting of hemophilic blood but not that of another PTC deficient individual. Prothrombin, proconvertin, Ac-globulin and fibrinogen were normal.

Twenty months later, refractoriness to blood and plasma had disappeared. He then experienced a life-threatening gastrointestinal hemorrhage and was effectively transfused. Within forty-eight hours he became more refractory to blood than previously. An anticoagulant was now demonstrable in his serum and $BaSO_4$ adsorbed plasma, as determined by their inhibition of thromboplastin generation from entirely normal constituents in the thromboplastin generation test. Inhibition was potentiated by preincubation with normal $BaSO_4$ adsorbed plasma but not normal serum. Inhibitory activity was not evident against plasma thromboplastic activity, once generated, or against tissue thromboplastins. Ac-globulin and antihemophilic factor were not affected by incubation with the inhibitor. It was stable at room temperature for at least three weeks, at 55° C for twenty minutes, and was separated in the 33 to 50 per cent saturated $(NH_4)_2SO_4$ fraction of $BaSO_4$ adsorbed plasma. No antigen-antibody precipitin reaction could be demonstrated. On withholding transfusion, this refractoriness gradually disappeared over four months.

Refractoriness may develop in PTC deficiency subsequent to transfusion therapy due to the appearance of an agent which inhibits the development of plasma thromboplastin.

Adrenal Influences on the Stomach: Peptic Ulcer in Addison's Disease During Adrenal Steroid Therapy. SEYMOUR J. GRAY,* COLIN G. RAMSEY, and GEORGE W. THORN,* Boston, Mass.

The relationship between the adrenal gland and the stomach has been demonstrated by increased gastric acid and pepsin secretion during adrenal stimulation. The adrenal gland has been implicated in the pathogenesis of peptic ulcer by the development or reactivation of ulcer during the administration of ACTH or adrenal steroids. Further evidence of an adrenal-gastric relationship is suggested by correlative studies of gastric and adrenal function in the resting state and following adrenal stimulation.

The present study was undertaken to evaluate further the adrenal influences upon the stomach in states of adrenal hypo and hyperfunction and following bilateral adrenalectomy. The urinary uropepsin excretion was used as an index of gastric secretory activity. Adrenal function was studied concomitantly by measurement of the urinary steroids.

Twelve patients with Addison's disease not receiving glucocorticoid therapy demonstrated a markedly diminished uropepsin excretion (mean 500 units/24 hours) paralleled by a diminution of urinary adrenal steroid output. With replacement glucocorticoid therapy the uropepsin excretion returned to normal levels or above depending upon the replacement dosage.

In eleven patients with adrenal or pituitary hyperactivity there was a significantly elevated uropepsin excretion (mean 7,000 units/24 hours), and a similar increase in adrenal steroid excretion.

Following bilateral adrenalectomy there was a close correlation between the dosage of glucocorticoid replacement therapy and uropepsin excretion. As the dosage was reduced a parallel fall in uropepsin output was observed.

Peptic ulcer is exceedingly rare in untreated Addison's disease. However, gastric or duodenal ulcers developed during the course of these studies in seven Addisonian patients receiving prolonged cortisone replacement therapy. Elevated uropepsin levels were noted in these patients.

The significance of the adrenal gland in peptic ulcer disease, and the evaluation of gastric function during prolonged glucocorticoid therapy will be discussed.

Alkali in the Treatment of Painful Crises in Patients with Sickle Cell Anemia. MORTIMER S. GREENBERG and EDWARD H. KASS,* Boston, Mass.

Painful crises in patients with sickle cell anemia are considered to be a consequence of the intravascular sickling of red cells. Deoxygenation of red cells containing S-hemoglobin leads to sickling with increase in viscosity of capillary and venous blood and ultimate formation of thrombi.

The degree of sickling of red cells *in vitro* at standardized gas tensions (PO_2, 60 mm.; PCO_2, 76 mm. Hg) was observed to be increased when patients were undergoing painful crises either spontaneously or after the administration of adrenocortical hormones. This increased susceptibility to sickling, conveniently measured by changes in the viscosity of whole blood, appeared prior to a crisis and subsided following it. *In vitro,* small decreases in pH between 7.5 and 7.0 produced marked

increases in the degree of sickling of red cells (whole blood viscosity) at standardized gas tensions, presumably as a result of the Bohr effect on the oxygenation of S-hemoglobin.

Therefore, the effects of artificially altering the pH of the blood *in vivo* were investigated. Acidosis, induced twice in an asymptomatic patient with sickle cell disease by the combined administration of ammonium chloride and Diamox®, brought about an increase in susceptibility to sickling of the patient's red cells *in vitro;* this was associated with the occurrence of a typical painful crisis and signs of increased hemolysis in each instance.

Conversely, the intravenous administration of large amounts (1.5 to 3.5 mEq. per Kg. per hr.) of sodium bicarbonate to 4 patients with sickle cell disease during painful crises that had developed spontaneously resulted in a transient decrease in the susceptibility to sickling of their red cells *in vitro.* In 3 of the 4 patients this was followed within an hour by relief of pain.

Although the genetic defect in hemoglobin synthesis is not subject to direct control, manipulation of a non-hemoglobin factor, blood pH, so alters the reactivity of S-hemoglobin that its clinical manifestations may be temporarily modified.

Role of Potassium Gradient in Neuromuscular Function in Normal Subjects and Periodic Paralysis. DAVID GROB,* AKE LILJESTRAND, and RICHARD J. JOHNS, Baltimore, Md.

Changes in neuromuscular function following alteration in plasma K^+ concentration and movement indicate that the resting muscle membrane potential varies with the concentration gradient of K^+ (intracellular to extracellular).

In eight normal subjects, peroral glucose produced greater reduction in venous than arterial K^+, indicating entry of K^+ into muscle. Insulin or adrenaline produced greater reduction in arterial than venous K^+, indicating loss of potassium from muscle and entry into another site. Moderate hypokalemia resulted in slight reduction in the depolarizing action of intra-arterially injected acetylcholine, compatible with hyperpolarization of the muscle membrane due to increased K^+ gradient. Hyperkalemia following oral KCl increased the depolarizing action of acetylcholine and neostigmine, compatible with hypopolarization due to decreased K^+ gradient. There was little or no weakness or reduction in muscle action potential response to nerve stimulation at the levels of hypo- or hyperkalemia attained.

In three patients with familial periodic paralysis glucose produced more marked and more prolonged reduction in plasma K^+, particularly venous, indicating greater uptake of K^+ by muscle than in normals. Insulin or adrenaline produced similar changes, in contrast to loss of K^+ from muscle in normals. Following glucose, insulin, or adrenaline there was also marked reduction in strength, muscle action potential response to nerve stimulation, depolarizing action of acetylcholine and other depolarizing agents, and electrocardiographic T waves. These changes were much greater than occurred in normal subjects with comparable hypokalemia, probably due to larger uptake of K^+ by muscle, resulting in greater K^+ gradient and hyperpolarization, and lowered excitability.

The movement of K^+ into muscle was enhanced during rest, while muscle activity resulted in loss of K^+. Both processes were more pronounced in periodic paralysis than in normals. In both groups cortisone diminished the movement of K^+ into muscle, and in the former it prevented the induction of paralysis.

The Action of Human Plasma on the Isolated Frog Heart: Observations on Subjects With and Without Essential Hypertension. STEPHEN HAJDU and EDWARD LEONARD, Bethesda, Md. (Introduced by Robert P. Grant).

The tension developed by frog cardiac muscle upon stimulation becomes greater with increasing stimulation frequency over a certain range (Bowditch staircase), so that an optimal frequency can be found below which tension is submaximal. Certain steroids, notably cardiac glycosides, desoxycorticosterone, and progesterone, cause a decrease of this optimal frequency, an effect which forms the basis for a bio-assay to determine small concentrations of such substances. Plasma from a number of human subjects has an action similar to that of the above steroids, which was quantitated in the present study by comparing each sample to the effect of a known concentration of strophanthidin. Three experimental groups were chosen: (1) fourteen normal controls, (2) eighteen patients with severe essential hypertension, (3) twenty-three patients without essential hypertension but with a variety of other diseases, including ten patients with hypertension secondary to renal disease or toxemia of pregnancy. Plasmas were assayed for the presence of strophanthidin-like activity, which became manifest after five minutes of contact with the heart, and which disappeared after the plasma was washed out of the heart muscle bath. The results were expressed in strophanthidin equivalents, in micrograms per milliliter. For nine of the fourteen normals the value was zero, the mean for the group was 0.1, and the highest was 0.7 microgram per milliliter. Sixteen of the 23 plasmas in the group without essential hypertension showed no activity, the mean was 0.1, and only one sample was over 1 microgram per milliliter. For the essential hypertension group the mean was 1.8, with only two values below 1.3 micrograms per milliliter. Thus a much higher activity is generally found in the patients with essential hypertension. Although the action is similar to that of certain steroids, the changes in plasma responsible for the results reported have not yet been determined.

Further Studies on the Plasma Protein-Thyroid Hormone Complex in Various Thyroid States in Man. MILTON W. HAMOLSKY, Boston, Mass. (Introduced by A. Stone Freedberg).

We previously presented to this Society infusion and *in vitro* studies indicating a qualitative difference in the

thyroid hormone-plasma protein complex in diffuse toxic goiter *vs.* the euthyroid state in man. This report deals with clinical and experimental factors affecting *in vitro* "uptake" of I-131-l-thyroxine (I-131-THY) and I-131-l-triiodothyronine (I-131-TRI) by (1) rat diaphragm and (2) human erythrocytes (r.b.c.).

(1) *Rat diaphragm:* "Uptake" of I-131-THY and I-131-TRI was greater from plasmas of patients with diffuse toxic goiter than from plasmas of (a) euthyroids, (b) patients with nodular toxic goiter, (c) euthyroid plasmas enriched by stable l-THY or l-TRI to hyperthyroid levels. "Uptakes" were doubled by 10° rise in bath temperatures (5° C to 40° C). Characteristic dilution curves of "uptakes," different in hyperthyroid *vs.* euthyroid plasmas, were obtained. "Uptakes" from heparinized plasma > citrated plasmas > oxalated plasmas. "Uptakes" from sera > plasmas. Prior heating of plasma to 65 to 70° C was required to affect "uptake" (increase).

(2) *R.B.C.:* "Uptakes" from hyperthyroid plasmas > euthyroid plasmas > myxedematous plasmas. In criss-cross runs (r.b.c. of 1 donor in plasma of another), "uptake" determined by thyroid status of *plasma* donor. There was striking reduction of "uptakes" in pregnancy (6th week to 2nd week post-partum). "Uptakes" in patients receiving Dicumarol® increased progressively; in criss-cross experiments, this alteration was found in both plasma and r.b.c. Addition of various plasma fractions did not alter "uptakes." Addition of T.S.H. and A.C.T.H. increased "uptakes." A single saline washing of r.b.c. increased "uptakes." Adsorption of plasmas by $BaSO_4$ (irregularly) and kaolin (consistently) increased "uptakes."

The alterations in "uptakes" under these various conditions indicate the importance of interrelationships between plasma proteins, thyroid hormone components, and tissue factors in determining tissue "uptake" of thyroid hormone. These results confirm our previous observations and are consistent with the concept of a pathogenetic role in hyperthyroidism of qualitative abnormalities in plasma-thyroid hormone-tissue interrelationships.

Pyridoxine Responsive Anemia in the Adult Human. JOHN W. HARRIS,* JAMES M. PRICE, RICHARD M. WHITTINGTON, RUSSELL WEISMAN, JR., and DANIEL L. HORRIGAN, Cleveland, O.

Hematologic remission was induced by parenteral pyridoxine in an adult male with a hypochromic anemia refractory to the usual hematopoietic agents; abnormalities in tryptophan and iron metabolism were also corrected.

In 1947, the patient was found to have a hypochromic anemia (4.5 gm. per 100 ml.) unresponsive to iron, liver extract, yeast, folic and ascorbic acids. In 1948, an apparently spontaneous remission occurred and persisted until 1953 when anemia (hemoglobin 5 gm. per 100 ml.) recurred; oral crude liver extract, citrovorum factor, cyanocobalamin, thiamin, riboflavin, niacin, cortisone, intravenous iron and fresh plasma were ineffective. Decreased erythrocyte autosurvival time was demonstrated. By 1955 he had received 113 transfusions.

Except for malaise, nocturia and pedal edema, symptoms during the entire course were attributable to the anemia; no glossal, neural or dermal involvement was detected. Diet provided an estimated 2,900 Cal and 3 mg. pyridoxine daily.

Pyridoxine hydrochloride 200 mg. I.M. qd × 5 produced prompt subjective improvement; reticulocytes rose to 50.8 per cent on the seventh day followed by elevations in hematocrit to 49 per cent and hemoglobin to 13 gm. per 100 ml. Eleven weeks later the hematocrit had fallen to 27 per cent and hemoglobin to 7.8 gm. per 100 ml. No reticulocyte response followed pyridoxine 1 mg. I.M. qd × 8; but after 10 mg. I.M. qd × 7 a peak of 17.7 per cent was reached and a rapid rise to 52 per cent hematocrit and 14.1 gm. hemoglobin per 100 ml. followed.

Oral loading tests with 4 gm. of l-tryptophan were done before pyridoxine therapy, during the 1-mg. dose and after response to the 10-mg. dose. Measurements of urinary excretion of kynurenin, kynurenic acid, acetylkynurenin, xanthurenic acid, anthranilic acid glycuronide, ortho-amino-hypuric acid and N-methyl-pyridone-5-carboxymid demonstrated abnormalities of tryptophan metabolism that were partly reverted toward normal by the 1-mg. dose and normalized by the 10-mg. pyridoxine dose. Serum iron level of 169 μg. per 100 ml. and iron binding protein saturation of 92 per cent decreased to 67.3 μg. per 100 ml. and 31 per cent after pyridoxine.

Four Chamber Catheterization for Quantitation of Valvular Deformities with Particular Reference to Mitral Stenosis. HANS H. HECHT* and RAMON L. LANGE, Salt Lake City, Utah.

If right heart catheterization is combined with left atrial puncture and catheterization of left atrium and left ventricle, precise information on the physiologic effects of valve deformities other than aortic lesions may be correlated preoperatively with other clinical and hemodynamic observations. Aortic valve disease may be quantitated if left ventricular pulses are compared with central pulse contours obtained from the aorta by retrograde arterial catheterization. Four chamber catheterization and central pulse determinations were carried out on twenty subjects suspected of predominant mitral valvular disease. No adverse reactions were encountered. Though differences in oxygenation were occasionally present during unsteady states, mean "PC" and mean left atrial pressures and pressure contours showed no significant difference. In patients with mitral stenosis, the left ventricular-left atrial gradient varied from less than 5 to more than 25 mm. Hg. Correlating this with other hemodynamic data a physiologic classification is possible which relates the salient mechanical obstruction, as revealed by the mitral valve gradient, with cardiac output, pulmonary artery pressures, vascular resistances and left ventricular pressures. High gradients may be associated with normal

flow and normal pressures, with diminished output and normal pressures, or with diminished flow and elevated pulmonary artery pressures. Obligatory indications for surgery may now be outlined and complicating myocardial factors can be clearly detected by the discrepancies between valve gradient and flow data. Similar considerations apply to aortic valvular disease using an aortic valve gradient and slope values of the anacrotic pulse limbs. Atrial pressure patterns reminiscent of "infundibular stenosis" but apparently caused by movement of the atrioventricular ring were frequently noted, and alterations caused by significant mitral regurgitation were confirmed. The combined techniques allow a reasonably accurate assessment of the consequences of mechanical distortions of all four cardiac valves.

The Mode of Action of Acetyl Strophanthidin on the Failing Human Heart. HARPER K. HELLEMS, TIMOTHY J. REGAN, FREDERICK N. TALMERS, RAYMOND C. CHRISTENSEN, and TASKASHI WADA, Detroit, Mich. (Introduced by Gordon B. Myers).

Since isolated cardiac muscle studies have indicated that ionic content is an important determinant of contraction, the myocardial electrolyte alterations and associated metabolic changes induced by the digitalis analogue, acetyl strophanthidin (1.1 mgm.) have been studied in 7 patients with low output left heart failure. Simultaneous samples from the brachial artery and catheterized coronary sinus were obtained to determine the myocardial arteriovenous difference of Na, K, O_2, CO_2, glucose, lactate, pyruvate, pH and hematocrit in the control period and at frequent intervals after drug administration.

The left ventricular stroke work for the group increased from a control of 61 GmM to a maximum of 82 GmM at 15 minutes, with some increase evident as early as 5 minutes. Control potassium A–V difference of $+ .05 \pm .28$ mEq. per L. (K_A 4.11 mEq. per L.; $K_{C.S.}$ 4.06 mEq. per L.) was not significant. After drug infusion, there was myocardial K loss, attaining a maximum negative A–V difference of $-0.70 \pm .42$ mEq. per L. (K_A 4.40 mEq. per L., $K_{C.S.}$ 5.10 mEq. per L.) ($p < .001$) at 4 minutes. Control values were usually restored by 20 minutes.

Control sodium A–V difference of -1.0 ± 2 mEq. per L. (Na_A 141 mEq. per L., $Na_{C.S.}$ 142 mEq. per L.) was not significant. The maximum A–V difference of $+1 \pm 6$ mEq. per L. (Na_A 141 mEq. per L., $Na_{C.S.}$ 140 mEq. per L.) occurred at 4 minutes. The failure to find significant myocardial uptake of Na after strophanthidin contrasts with our previous findings in the normal dog, in which a decline in stroke work was associated with a similar potassium loss, but a greater uptake of sodium. Thus, presumably the internal ionic content is increased in the normal dog heart and decreased in the failing human heart after strophanthidin.

Myocardial substrate extraction was normal in failure patients, but after strophanthidin there was myocardial release of glucose, occurring shortly after K loss. There was no change in A–V difference of O_2, CO_2, pH, or hematocrit. Characteristic EKG repolarization changes were usually present.

These findings are consistent with the thesis that the positive inotropic effect of digitalis may be dependent upon the presumably diminished internal ionic content effected by the movement of K from the cell.

The Metabolism of Reserpine in Man. LEON HELLMAN * and PAUL NUMEROF, New York, N. Y., and New Brunswick, N. J.

The synthesis of reserpine labeled with radiocarbon in the carboxyl group of the trimethoxybenzoic acid (TMB) side-chain has permitted a detailed examination of the metabolic fate of this important drug in human subjects.

1. Following oral administration of one milligram, 70 per cent of the radioactivity was excreted in feces, 5 per cent in urine, and only traces in CO_2. Unaltered reserpine and TMB derived from *in vivo* breakdown of reserpine were identified by paper chromatography and accounted for virtually all the excreted radioactivity. The fecal activity was chiefly reserpine while that in urine was TMB.

2. An intravenous dose of one milligram was excreted in feces and urine in a manner quantitatively and qualitatively similar to that observed following oral administration. This finding suggests that the oral dose had been absorbed and subsequently excreted into the intestine. The excretion of reserpine into the intestine following both routes of administration is of especial interest in view of the reported effect of reserpine on intestinal serotonin levels.

3. The concentration of unaltered reserpine in blood two hours after the oral dose was 0.0006γ per ml. The intravenous reserpine disappeared rapidly from the circulation, presumably into a tissue depot, and reached a blood level at two hours similar to that observed after the oral dose. TMB constituted a large fraction of blood radioactivity one hour after intravenous administration indicating rapid breakdown of reserpine in tissue since *in vitro* incubation of the labeled drug with blood did not produce chemical change.

4. Studies in the mouse demonstrate a striking species difference with the major fraction of administered reserpine rapidly appearing in the urine as TMB. Similar observations have been made in non-psychotic and psychotic subjects receiving therapeutic doses of reserpine.

The Effect of Oral Ethanol on the Hepatic Glutamic Pyruvic Transaminase Activity in the Rat. KEITH S. HENLEY, HUGH S. WIGGINS, and H. MARVIN POLLARD, Ann Arbor, Mich. (Introduced by F. D. Johnston).

The direct action of alcohol on the liver has been questioned and, therefore, its effect in rats was investigated, using the hepatic glutamic pyruvic transaminase (GPT) activity as a parameter.

Groups of 10 male albino rats, weighing about 280 gms.,

were pairfed. Each group received either water or 20 per cent alcohol as the sole source of fluid for periods of one, four and eight weeks. Minimal daily nutritional requirements were met by appropriate dietary supplements. The liver and brain were removed and placed in liquid air after exsanguination under Nembutal® anesthesia. All organs were stored at $-10°$ C until assayed. GPT activity was estimated in brain and liver homogenates by estimating the amount of pyruvate formed in the transamination reaction with lactic acid dehydrogenase. Results, in liver, were expressed as micromols of pyruvate per mg. of dry weight of tissue per hour.

No significant differences in hepatic GPT activity were noticed at one week (4.21 ± 1.2; 4.12 ± 1.5) or at four weeks (5.64 ± 0.95; 4.22 ± 1.4). At eight weeks, the hepatic GPT activity was much greater in the rats receiving water than in those receiving alcohol (8.46 ± 1.8, and 2.72 ± 1.1, respectively). Again, at eight weeks, the transaminase activity in the rats receiving alcohol was significantly lower than in any other group, while that of the corresponding water-fed group was much higher than any other group. On the other hand, at eight weeks, there was no significant difference in the transaminase activity of the brains of the two groups (0.14 and 0.13 micromol per mg. of wet weight/hour).

These changes indicate a biochemically demonstrable effect of alcohol on the liver of rats.

Factors Determining Fecal Electrolyte Excretion. PHILIP H. HENNEMAN and ELEANOR F. DEMPSEY, Boston, Mass. (Introduced by Fuller Albright).

Thirty-five patients on different diets during complete balance studies have been investigated to clarify the physiological factors controlling fecal electrolyte excretion.

As noted previously fecal nitrogen was independent of intake. Fecal magnesium also did not correlate with intake but did correlate roughly with fecal nitrogen, particularly in the same patient. Since fecal nitrogen is largely bacterial in origin bacteria may also account for most of the fecal magnesium. Fecal calcium in general varied with the intake and was decreased by vitamin D. Fecal phosphorus correlated poorly with the level of intake but correlated well with fecal calcium plus magnesium. This suggests that nearly all dietary phosphorus is absorbed except that amount bound to unabsorbed calcium and magnesium. Potassium was well absorbed and sodium almost completely absorbed; the fecal sodium and potassium were unrelated to the level of intake.

Average daily fecal excretions were: calcium 12.7 mEq.; phosphorus 242 mg.; magnesium 10.1 mEq.; sodium 3.11 mEq.; and potassium 8.98 mEq. Comparison of the average sum of the fecal cations and the average water content suggests that most non-diarrheal stools are isotonic and that the level of the fecal sodium and potassium is related to the water content of the stools.

Studies on the Diurnal Pattern and Ratio of Urinary 17-Hydroxycorticosteroid and 17-Ketosteroid Excretion in Patients with Rheumatoid Arthritis and Non-Rheumatic Chronic Diseases. S. RICHARDSON HILL, JR., HOWARD L. HOLLEY, WILLARD R. STARNES, and LESTER L. HIBBETT, Birmingham, Ala. (Introduced by Tinsley R. Harrison).

Because of the availability of more precise technics for measuring adrenal cortical secretory activity and because of the accumulating evidence for a fairly consistent pattern of steroid excretion in normal subjects, this study of adrenal cortical function in patients with rheumatoid arthritis was undertaken.

Six groups of male subjects were studied: (1) Nineteen ambulatory normal students; (2) ten ambulatory, older, normal subjects; (3) seven hospitalized normal subjects; (4) four ambulatory patients with active rheumatoid arthritis; (5) eight hospitalized patients with active rheumatoid arthritis; and (6) five hospitalized patients of comparable age with non-rheumatic chronic diseases. Urine specimens were collected from 7:00 a.m. to 1:00 p.m., and 1:00 p.m. to 7:00 p.m., and one specimen from 7:00 p.m. to 7:00 a.m., for one or more days on all subjects for determination of the total 17-hydroxycorticosteroid and 17-ketosteroid content.

Adrenal cortical function is within normal limits in all subjects studied. There is no effect of hospitalization alone on the total twenty-four hour urinary output of 17-hydroxycorticoids or of 17-ketosteroids. The characteristic morning increase in steroid output observed in ambulatory subjects is less striking in hospitalized patients, resulting in a somewhat flattened diurnal excretory pattern. The total twenty-four hour urinary output of 17-hydroxycorticosteroids is lower in patients with rheumatoid arthritis and in patients with non-rheumatic chronic diseases than in the three groups of normal subjects. Patients with non-rheumatic chronic diseases have a decreased urinary 17-ketosteroid output ($p < .05$); whereas patients with rheumatoid arthritis have a normal twenty-four hour 17-ketosteroid excretion. This results in a lowered 17-hydroxycorticoid, 17-ketosteroid ratio in patients with rheumatoid arthritis. Thus, while overall adrenal cortical function is normal in patients with rheumatoid arthritis, the secretory pattern differs from that seen in normal subjects and in patients with non-rheumatic chronic diseases.

The Secretion of Pepsin by the Human Stomach. BASIL I. HIRSCHOWITZ, HUGH S. WIGGINS, JOHN LONDON, and H. MARVIN POLLARD, Ann Arbor, Mich. (Introduced by Paul S. Barker).

In continuously-aspirated gastric juice, simultaneous measurement shows a significant relation between the major electrolytes ($[H^+]$, $[Na^+]$, $[K^+]$, $[Cl^-]$) and pepsin, in both basal and stimulated states, strongly suggesting that these components are elaborated by the different cells of the gastric tubule, acting as a functional unit.

In the present report, confining our attention to pepsin, we have found that pepsin production is actively stim-

ulated by any stimulant which will stimulate the increased secretion of HCl, *viz.*, caffeine, alcohol, histamine, hypoglycemia, and oral water or saline. Since the secretion of H^+ ions implies the local release of OH^-, the effects of alkalinizing the blood were studied.

Hyperventilation for 15 to 30 minutes, during both stimulated and unstimulated states, caused an invariable, immediate steep increase (not prevented by atropinization) of pepsin concentrations to levels exceeding, in some cases, those found under any other condition. Simultaneously, a small drop in $[H^+]$ and a rise in $[Na^+]$ occurred, while $[K^+]$, $[Cl^-]$, and volume remained constant.

To differentiate between the secretory effects of lowered CO_2 and elevated pH, produced by hyperventilation, 4N $NaHCO_3$ was rapidly infused intravenously, resulting in effects on pepsin (but not on electrolytes), similar to those of hyperventilation, indicating the importance of pH elevation, rather than CO_2 changes, in mediating this effect. The rise of $[H^+]$, though, is probably the result of CO_2 changes, rather than pH elevation.

These observations imply that the secretion of pepsin may be partly regulated by the local mucosal changes in pH, incident upon the elaboration of H^+ ions and the concomitant release of OH^-. This non-cholinergic mechanism is seen as one additional link in the maintenance of functional unity by the gastric tubule.

Sodium "Space" Studies: The Effect of Certain Hormones. C. J. Hlad, Jr., E. R. Huffman, N. Whipple, and H. Elrick, Denver, Colo. (Introduced by Gilbert S. Gordan).

A simple technique for estimating the volume of distribution of sodium (sodium "space") has been developed to study the action of certain hormones. The procedure consists of the administration of long-lived Na^{22} (10 to 15 μc) by constant intravenous infusion and the radioassay of 12 consecutive 10-min. plasma samples (1 ml.). At the end of the first hour (control), the hormone under study is added to the infusion fluid. In this manner acute changes in sodium "space" could be measured. Eighty-five males, normal with respect to electrolyte metabolism, and ranging in age from 20 to 60, were studied. The mean Na^{22} "space" for the entire group was 23.0 ± 3.2 per cent body weight with no significant difference between the various age groups.

In 15 subjects 1 mg. of 9α-fluorohydrocortisone resulted in a consistent increase (average 27 per cent) in Na^{22} "space." Hydrocortisone had a less consistent effect. Sodium "space" increased in only 5 of the 10 subjects studied and remained unchanged in the others.

Glucagon (10 subjects), insulin (10 subjects), glucose (20 subjects) and isotonic saline (7 subjects) had no definite effect on the volume of distribution of Na^{22}.

Electrophoretic Separation of Anticoagulants from Human Plasma. R. R. Holburn, I. R. Schwartz, R. T. Carroll, and A. Weiss, Philadelphia, Penna. (Introduced by L. M. Tocantins).

Human defibrinated plasma was separated into eleven fractions by a modification of starch electrophoresis. Narrow cotton gauze strips (13 × 1.5 cm.) on a siliconized glass plate formed the bridge between the buffer compartments (Barbital buffer, pH 8.6, 0.05M) encased in an airtight chamber. Each run was made at 4° C and required eleven hours (7 hours for equilibration and 4 hours for plasma separation) using a regulated power supply at 300 V. The pH drop at anode or cathode buffer chambers did not exceed 0.1 unit. The fluid was eluted manually from each strip into silicone-coated test tubes. The eluates were tested for coagulant or anticoagulant activity in a two-stage system (purified prothrombin, dilute thromboplastin, dilute serum, imidazole buffer, the fraction, and calcium). Aliquots of each mixture were added to purified fibrinogen to measure the amount of thrombin formed. One anticoagulant fraction was found by mobility calculations to be in the β_2-globulin position, and to have properties similar to the lipid antithromboplastin obtainable from blood by methanol extraction. The other anticoagulant found close to the albumin fraction was an antithrombin. The fractions with anticoagulant activity were concentrated by dialysis at 4° C against 25 per cent Polyvinylpyrrolidone and their identity confirmed by paper electrophoresis. Fractions were separated from normal, Hemophilia A and Hemophilia B plasmas. The anticoagulant activity of the β_2-globulin fraction was markedly increased in the Hemophilia A plasma. The activity of similar fraction of Hemophilia B plasma was no greater than that of normal. The anticoagulant activity of the β_2-globulin fraction of Hemophilia A plasma can be extracted by ether and is reduced or destroyed by contact with glass particles, paper and asbestos fibers.

The Effects of Serotonin and Antiserotonins in Hypertensive Man. William Hollander and Alan L. Michelson, Boston, Mass. (Introduced by Robert W. Wilkins).

The effects of intravenous serotonin (5-hydroxytryptamine) were studied in 30 hypertensive subjects. In doses of 0.3 to 1.5 mg. it usually increased arterial pressure by 10 to 40 mm. Hg and pulse rate by 10 to 20 beats per minute for 1 to 5 minutes with or without producing an antecedent decrease in blood pressure. Occasionally it caused only a reduction in pressure with an increase in pulse for 20 to 80 seconds. The blood pressure effects of serotonin were not prevented by prior administration of hexamethonium, Regitine®, atropine, Benadryl®, or Neo-Antergan®. However, they were affected by Woolley's benzyl analog of serotonin (BAS, or 1-benzyl-2,5-dimethylserotonin HCl) in intravenous doses greater than 45 mg., which often blocked the pressor but not the depressor effects of serotonin.

In 18 subjects intravenous serotonin (1 mg.) usually produced a 30 per cent decrease in renal plasma flow (PAH clearance) for 15 to 45 minutes. It likewise usually decreased urine flow but for a longer period and without necessarily decreasing glomerular filtration rate (inulin clearance). It reduced sodium excretion in a variable fashion. BAS in intravenous doses of over 25 mg. frequently produced renal effects similar to those of

serotonin itself. In smaller doses BAS usually had no direct effect on renal function but it inconsistently blocked the renal effects of intravenous serotonin.

Of several antiserotonins tried, only BAS seemed practical as an antihypertensive agent. Given orally to 25 hypertensive patients in courses of 1 to 4 weeks (alternating with placebos) for a total of 1 to 6 months it caused a moderate decrease in blood pressure (average, 20/15 mm. Hg). It also caused sedation, bradycardia, increased intestinal motility, and occasionally nasal stuffiness. Since its effects resemble those of reserpine which has been found to alter serotonin activity, it is postulated that BAS may act in a fashion similar to reserpine.

Lymphatic Flow in Human Subjects as Indicated by the Disappearance of I^{131} Labelled Albumin from the Subcutaneous Tissues. WILLIAM HOLLANDER, PAUL REILLY, and BELTON A. BURROWS,* Boston, Mass.

Albumin labelled with I^{131} was injected subcutaneously in doses of 20 to 30 microcuries into the extremities of 48 subjects with and without edema. The disappearance rate, as determined by external monitoring for 3 to 6 days, was found to be a logarithmic function of time. Conversely, radioactivity in the plasma obtained from a remote vein was found to increase rapidly for 18 to 36 hours and slowly thereafter. Of the total radioactivity in the plasma more than 97 per cent was contained in the protein fraction precipitated by 10 per cent trichloracetic acid. Less than 5 per cent of the injected labelled albumin was degraded daily as judged by the appearance of radioactivity in the thyroid gland and urine.

In subjects with extensive edema of an upper extremity following radical axillary lymph node resection the disappearance rate of the injected I^{131} labelled albumin from the affected extremity was from 4 to 12 times less rapid than from the opposite normal extremity. By contrast, the disappearance rate from a limb with thrombophlebitic edema was significantly more rapid than from the opposite normal extremity.

The disappearance rate of I^{131} labelled albumin from the lower extremities in 15 normal subjects at bed rest as measured by a 50 per cent decrease in radioactivity at the site of injection was 25.6 ± 8.5 hours. This rate was markedly increased in 10 cirrhotic and nephrotic patients with hypoproteinemic edema (half time disappearance 9.6 ± 3.2 hours) and in 12 cardiac patients with elevated venous pressure and edema (half time disappearance 12.1 ± 3.8 hours). In 5 of the 12 cardiac patients restudied after effective treatment the disappearance rate had decreased significantly to within the normal range.

The results indicate that lymphatic flow is significantly decreased in lymphedema, but markedly increased in thrombophlebitic, cardiac, and hypoproteinemic edema.

The Renal Concentrating Defect in Potassium Depleted Rats. W. HOLLANDER, JR., R. W. WINTERS, T. F. WILLIAMS, M. HOLLIDAY, J. OLIVER, and L. G. WELT,* Chapel Hill, N. C. and Summit, N. J.

The effect of graded potassium depletion on the renal concentrating mechanism was studied in male Sprague-Dawley rats with initial weights of 300 to 400 grams. Potassium depletion was produced by feeding an electrolyte-free diet supplemented with sodium, bicarbonate and phosphate. Control rats received the same basal diet but supplemented with potassium and chloride as well as with sodium and phosphate.

The ability to form a concentrated urine was tested in the following manner: Each rat received 50 milliunits of Pitressin®-in-oil subcutaneously. Beginning several hours later, urine was collected under oil for twelve hours. The osmotically effective concentration of total solutes of the urine was then determined by the freezing point method. On one occasion, water deprivation was substituted for Pitressin®.

The results indicate that: 1) the maximum concentration of the urine of controls (approximately 2,500 mOsm. per L.) was highly reproducible; 2) potassium depletion was uniformly accompanied by a diminished ability to form a concentrated urine; 3) the defect in concentrating ability was demonstrated in association with comparatively slight potassium deficiency (muscle potassium reduced by only 10 per cent); 4) with increasing degrees of potassium depletion, there was a progressive decrease in the maximum urinary concentration achieved, but despite depletion of four weeks' duration and a 30 per cent reduction in muscle potassium, the maximum concentration of the urine was still almost 70 per cent of normal; 5) the defect appeared to relate to the total solute concentration that the kidney could achieve rather than to the concentration of any particular constituent.

Detailed studies of renal pathology including microdissection of nephrons are in progress.

Isolation from Crude Liver of a Substance Effective in Therapy of a Nutritional Hypochromic Anemia. DANIEL L. HORRIGAN, Cleveland, O. (Introduced by Harold S. Ginsberg).

Two patients with anemia, apparently resulting from a deficiency of a previously unrecognized hematopoietic substance, failed to respond to therapy with iron, folacin, Leucovorin, and vitamin B_{12} during periods of observation of two and eight years, respectively. Prompt and optimal response was obtained in both patients following oral administration of Liquid Extract of Liver, U.S.P. (Valentine). The anemia in these patients was consistently hypochromic and varied between normocytic and microcytic. Poikillocytosis, anisocytosis, and anisochromia of the erythrocytes were marked. Bone marrow showed erythroid hyperplasia with maturation arrest at an erythroblastic level. The serum iron in both patients was high, and saturation of the serum iron binding capacity was markedly elevated.

Isolation of the active substance from the crude liver extract has been attempted in a series of studies in one of these patients. To date, it has been determined that hematopoietic activity is present in a 70 per cent ethanol-soluble, ethyl acetate-extractable fraction of the crude

liver extract. Furthermore, the active substance in this fraction can be adsorbed on anion exchange resin (Dowex-1) from which it is recovered in a well localized effluent fraction. Activity is measured by the clinical response of the patient, which includes prompt reticulocyte response, rapid hemoglobin regeneration, correction of the erythrocytic morphologic abnormalities and increased feeling of well-being.

The active substance has not yet been isolated in pure form. However, studies so far completed would indicate that it is active in oral doses of less than 1.0 mg. daily and that it is not identified with derivatives of known substances essential for erythropoiesis in human beings.

Abnormalities of Iodine Metabolism in Euthyroid Relatives of Patients with Graves' Disease. SIDNEY H. INGBAR,* NORBERT FREINKEL, JAMES T. DOWLING, and LINDY F. KUMAGAI, Boston, Mass.

Consanguineous occurrence of Graves' disease has led to the suggestion that hereditary constitutional factors may be important in the pathogenesis of this disorder. It therefore appeared possible that demonstrable abnormality might be present in clinically normal relatives of thyrotoxic patients.

Previous work has demonstrated an augmented rate of degradation of thyroxine in patients with Graves' disease, despite restoration of the eumetabolic or induction of the myxedematous state. Thyroxine turnover was therefore determined in 12 clinically euthyroid relatives of 5 thyrotoxic patients. Thyroxine half-time was significantly diminished ($p < 0.005$), from a normal of 6.8 ± 0.6 days (mean $\pm$ s.d.) to 5.7 ± 1.1 days. In 5 of the 12, thyroxine half-time was 5.0 days or less. Although radioactive iodine uptakes averaged 61 per cent in this group, basal metabolic rates and protein bound iodine values were normal. In contrast to what may be achieved in patients with active Graves' disease, suppression of increased uptakes was produced by 2 to 4 grains of desiccated thyroid daily. Neither the nature of circulating radioiodinated compounds following a tracer dose of I^{131}, nor the partition of added radiothyroxine between albumin and the specific thyroxine-binding protein of plasma demonstrated any abnormality.

Thyroidal radioiodine uptakes were performed in an additional 44 euthyroid relatives of 19 patients with Graves' disease. For the entire group of 56, 24-hour uptakes (44 ± 8 per cent) were significantly greater ($p < 0.001$) than normal (31 ± 8 per cent). Uptakes exceeding 50 per cent were noted in 22 per cent of the group.

Thus, two distinct abnormalities in the metabolism of iodine were found in certain eumetabolic relatives of patients with Graves' disease: (1) an augmented rate of peripheral degradation of thyroxine, and (2) an increased thyroidal avidity for iodine. Efforts to extend these observations and to elucidate their significance are in progress.

Pulmonary Lesions in Rabbits Following Intravenous Injections of Thromboplastin. SCOTT R. INKLEY, LOUIS GILLESPIE, JR., and SIMON KOLETSKY, Cleveland, O. (Introduced by Walter H. Pritchard).

Because histories of previous obstetrical difficulties in several patients with primary pulmonary hypertension have suggested a possible association between the two, a study was designed to determine if release of thromboplastin into the blood stream could produce pulmonary vascular changes.

Multiple intravenous injections of rabbit thromboplastin were given to twenty-nine female rabbits over a four-month period at varying intervals and in varying dosages. Of twenty-nine animals studied, nine were control animals to which heat inactivated thromboplastin was administered. All but one of the control animals received twenty-seven injections and one received only seven injections because of death due to sepsis.

Twenty animals were given active thromboplastin in varying dosages. There were thirteen immediate deaths following the injection of thromboplastin and in each instance the deaths were associated with the administration of doses larger than 0.1 cc. Seven animals were carried on doses of 0.1 cc. for a total of twenty-seven injections over a period of four months. All but two of the twenty animals in the active group showed significant lesions in the pulmonary bed consisting of intravascular thrombi with organization and reendothelialization, intimal hyperplasia and fibrosis, and apparent medial thickening. There were no significant changes in the control animals.

The possible relationship between the defibrinogenation of blood occurring in the post-partum female as a result of thromboplastin rich amniotic fluid entering the maternal circulation and the subsequent development of pulmonary hypertension of unknown etiology is considered.

Demonstration of the Specific Enzymatic Defect in Galactosemia. KURT J. ISSELBACHER, ELIZABETH A. ANDERSON, and HERMAN M. KALCKAR, Bethesda, Md. (Introduced by Howard A. Eder).

Galactosemia constitutes a congenital abnormality in galactose metabolism. When galactose is ingested by patients with this disease, elevated blood galactose levels and galactosuria result, followed by mental retardation, cataracts and hepatosplenomegaly. These symptoms usually regress or disappear when galactose is eliminated from the diet.

Normally, galactose is metabolized in the body by first being phosphorylated to galactose-1-phosphate (Gal-1-P) by galactokinase and ATP. Gal-1-P is then converted to glucose-1-phosphate (G-1-P) by two reactions which involve a nucleotide, uridine diphosphate glucose (UDPG), and two specific enzymes: (I) Gal-1-P + UDPG $\rightleftarrows$ UDPGal + G-1-P (P-Gal transferase); (II) UDPGal $\rightleftarrows$ UDPG (Galacto-waldenase).

It has recently been shown by a group of British investigators that when erythrocytes of galactosemic subjects are incubated with galactose *in vitro*, an accumula-

tion of Gal-1-P occurs. Normal red cells, under similar conditions, do not demonstrate this accumulation. These observations were confirmed and extended by us and suggested that the defect in galactosemia might be due to a block in step (I) or less likely in step (II).

Therefore, normal and galactosemic red cells were examined for the presence of the enzymes catalyzing these reactions. Hemolysates were incubated with Gal-1-P and UDPG, or with UDPGal, and the reactions measured by specific enzymatic techniques. P-Gal transferase activity was present in normal cells to the extent of 0.75 μM per ml. per hr., but the enzyme was found to be completely absent in the erythrocytes of ten galactosemic subjects. In contrast, galacto-waldenase was present in equal amounts in normal and galactosemic cells. No inhibitors were found to account for the block in reaction (I). Furthermore, the absence of P-Gal transferase could not simply be explained by the lack of galactose intake by the galactosemic subjects, since the enzyme does not disappear from the red cells of normal individuals on galactose-free diets up to 18 months.

These data strongly suggest that galactosemia represents the congenital absence of a specific enzyme, P-Gal transferase. The disease can now be diagnosed by simple spectrophotometric means without resorting to the potentially hazardous galactose tolerance test.

Protection from Viral Infections by Inhibitors in Human Nasal Secretion. GEORGE GEE JACKSON and ARTHUR V. BOAND, Chicago, Ill. (Introduced by Harry F. Dowling).

Susceptibility to common (viral) infections of the respiratory tract, especially the common cold, appears related to factors other than specific humoral antibodies. Polysaccharides present in a wide variety of biologic materials previously have been shown to inhibit the hemagglutination produced by viruses. We have studied human nasal secretions both normal and post-infectious for the presence of non-specific hemagglutination inhibitors using *Influenza* virus as the indicator system. A heat-stable, trypsin-labile (Francis) alpha like inhibitor of serum, and heat-labile inhibitor(s) were present. The latter differed from the beta inhibitor of serum (Chu) in that a considerable constituent was destroyed by salivary R.D.E. (receptor destroying enzyme) and by active influenza virus. The inhibitors were destroyed by sodium periodate and are presumed to be polysaccharides. Chemical and electrophoretic analysis of some nasal secretions will be presented.

Nasal secretion from persons in good health inhibited hemagglutination in dilutions of 1:800 to 1:6,400, and the titer of secretion from persons with respiratory infections ranged from 1:200 to 1:25,600. Three biologic systems have been studied for evidence of inhibitor activity as follows: (1) Nasal secretions effectively inhibited infection of embryonated eggs with *Inflenza A (PR8)* and *B(Lee)* viruses. The active component was the heat-labile, trypsin-stable inhibitor. (2) Bacterial lysis produced by certain staphylococcal bacteriophages was modified by human nasal secretions; and (3) Symptoms among human volunteers challenged with an agent that produced a common-cold were delayed and ameliorated by nasal secretions containing inhibitors. Concentrated heat-stable serum inhibitor had no effect.

A Comparative Study of the Dextran and Radioiodinated Albumin Spaces in Normal and Edematous Subjects. JOHN R. JAENIKE, BERNARD F. SCHREINER, JR., and CHRISTINE WATERHOUSE,* Rochester, N. Y.

Demonstration of the applicability of high molecular weight Dextran to plasma volume studies has stimulated an investigation of the relative volumes of distribution of I^{131} tagged albumin and Dextran (N.R.C. Fraction No. 6, molecular weight 195,000). Simultaneous plasma volume determinations were carried out in ten normal subjects and thirteen patients with congestive heart failure and edema. Eight of the latter were restudied after compensation.

In all groups studied the labeled albumin space was larger than the Dextran space. In patients with congestive heart failure the discrepancy was 9.7 per cent (S.E. $\pm$ 1.2 per cent), and was unchanged after restitution to a compensated state, although plasma volume decreased in all subjects. In normal subjects the difference averaged 6.0 per cent (S.E. $\pm$ 1.0 per cent), a small but apparently significant deviation from the other groups ($P = < .05$).

The data indicate that high molecular weight Dextran is distributed in a space smaller than albumin, and that this space is more representative of the true plasma volume. Although the discrepancy is small, a revision of the present concept of the relationship of venous to total body hematocrit is suggested by these data. Dextran space measurements confirm previous findings by albumin tagging methods of an expanded plasma volume associated with congestive heart failure.

Elongated Macromolecules for Detecting and Characterizing Red Cell Sensitization in Acquired Hemolytic Anemia. JAMES H. JANDL, Boston, Mass. (Introduced by William B. Castle).

A property of red cells from most patients with acquired hemolytic anemia and of red cells sensitized by incomplete antibodies, certain drugs, or metallic cations is their tendency to agglutinate under certain conditions. Since current technics for agglutinating sensitized red cells are frequently hampered by prozones, inadequate sensitivity, the necessity of washing the cells with saline, and a lack of correlation with the clinical effects of sensitization, a need exists for improved methods.

Elongated hydrophilic macromolecules which induce rouleaux and increase the sedimentation rate of normal red cells were observed to cause sensitized cells to form agglutinates and rouleaux that failed to dissociate after dilution with plasma or saline. To utilize this phenomenon clinically the stable synthetic macropolymer polyvinylpyrrolidone (PVP) of 160,000 molecular weight

was selected. Red cell sensitization was demonstrable within several minutes by suspending small volumes of cells or whole blood in a buffered PVP solution and later diluting with saline. Cell clumping persisted only among sensitized cells.

This method was quicker and more sensitive than others available, and was unhampered by prozones or species specificity. The red cells from 3 patients with acquired hemolytic anemia which were inagglutinable by Coombs (antiglobulin) serum were agglutinated by PVP. The agglutinability by Coombs serum of the red cells of 2 other patients was effaced by 3 saline washes whereas strong agglutination was still produced by PVP after 20 washes. A correlation was observed between the *in vivo* survival of sensitized red cells and the permanence of agglutinates of these cells *in vitro* after exposure to PVP. This presumably reflected the effective "stickiness" induced by certain antibodies rather than the amount of antibody present on the cell. Thus the PVP method provides a rapid, sensitive clinical technic for (1) detecting the existence of red cell sensitization, and (2) assessing the physiologic potency of sensitizing agents.

Myxedema, Myotonia and Pseudomyotonia. LEONARD W. JARCHO and FRANK H. TYLER,* Salt Lake City, Utah.

Among the recognized effects of thyroid disease on muscle function is a delay of relaxation of tendon jerks in myxedematous subjects. This phenomenon has been termed "pseudomyotonia." It differs clinically from true myotonia in that the former is not accompanied by the symptom of difficulty in relaxing contracted muscles which characterizes the latter. The appearance of true myotonia in the course of myxedema and its relief by thyroid therapy have been reported, with the implication that this muscle defect is caused by hypothyroidism.

A forty-year-old woman developed myxedema after thyroidectomy for non-toxic nodular goiter. Simultaneously she complained, for the first time in her life, of stiffness and difficulty in relaxing her muscles. Clinically and electromyographically she exhibited the characteristic findings of myotonia, in addition to those of pseudomyotonia. Returned to a euthyroid state by appropriate therapy, her symptoms and clinical signs of myotonia disappeared, but her electromyograms continued to reveal the prolonged electrical firing characteristic of myotonia. Repeated careful family history had produced no evidence of inherited disease. However, on electromyographic examination, several euthyroid relatives proved to exhibit classical myotonia. The occurrence of the trait in the family was consistent with a dominant mode of inheritance. It is suggested that this is a case of Thomsen's myotonia congenita of minimal severity, aggravated by hypothyroidism.

In contrast, a group of patients suffering from myxedema of various causes was investigated electromyographically. All demonstrated pseudomyotonia clinically, but the histories were not typical of myotonia. In spite of the delayed mechanical activity of the muscle, the electrical pattern was entirely normal.

These observations demonstrate that myxedema may aggravate the clinical findings of true myotonia but suggest that true myotonia, in contrast to pseudomyotonia, is not initiated by thyroid deficiency.

Fractionation of Human Gastric Content: The Separation of Intrinsic Factor Activity. PHILLIP C. JOHNSON, VIRGINIA RICHMOND, RANWEL CAPUTTO, and STEWART WOLF,* Oklahoma City, Okla.

An earlier report (Richmond, Caputto, and Wolf) described the fractionation of fasting human gastric content by the technic of ion exchange chromatography using a resin column. After passage through the column, peptic and blood group A activity were not only preserved but were concentrated into separate zones of the effluent. The present report describes the identification of intrinsic factor in the fractionated material. Pooled samples of fasting gastric content from patients with peptic ulcer and healthy subjects were dialyzed and lyophilized prior to introduction into the column. The pH was increased from 3.02 to pH 7 during the run by adding appropriate buffers. The 7 liters of effluent obtained yielded 50 to 75 per cent recovery in terms of dry weight. Forty-four determinations were made on the various fractions from two columns using four patients with classical pernicious anemia as test subjects. The absorption of Co^{60} vitamin B_{12} was determined by both the stool and urine technics. The results of the procedure showed that the first liter of effluent contained the major portion of the protein and carbohydrate in two well defined peaks. Only the fourth liter contained intrinsic factor activity, however, and here the zone of activity was narrowed down to a single 400 cc. fraction. From this fraction as little as 10 mg. of dried material produced absorption of the tagged vitamin B_{12} in subjects in whom no absorption had been demonstrated in control runs. These experiments provide the first step toward the establishment of a normal fractional pattern of human gastric content. Such a pattern would not only allow for the direct recognition of missing biologically active components but, like the fractions of blood serum, might ultimately be useful in differential diagnosis.

The Effect of Liver Disease on Serum Vitamin B_{12} Concentrations. PHILIP N. JONES and ELIZABETH H. MILLS, Chicago, Ill. (Introduced by Richard B. Capps).

In order to explore the relationship of vitamin B_{12} metabolism and liver function, a study was made of both the free and combined serum vitamin B_{12} concentrations in patients with varying degrees of liver damage. Using *Euglena gracilis,* serial serum vitamin B_{12} levels were estimated microbiologically in 36 patients with a variety of liver diseases including cirrhosis, acute hepatitis and hepatic coma.

The patients in liver coma had values between thirty and forty times the normal concentrations. All of the

other patients, except those having biliary cirrhosis, had serum vitamin B_{12} levels three to eight times that of the normal controls. Patients with evidence of acute inflammation and necrosis had a greater proportional rise in the free serum vitamin B_{12} while those with chronic liver damage had a greater increase in the combined fraction. Serial studies showed that serum vitamin B_{12} levels varied in relation to the degree of liver damage as estimated by liver function tests. Assay of 24-hour-urine collections revealed that these patients excrete ten to thirty times the normal amount of vitamin B_{12}, all of which is in the free form.

An attempt to saturate the serum with vitamin B_{12} was made, both *in vivo* and *in vitro,* in order to study the binding capacity of the serum. It was found that in both normal subjects and in the patients with liver disease the serum binding capacity was usually saturated. The excess vitamin B_{12} was recovered in the free fraction in the *in vitro* studies, or in the urine in the *in vivo* studies.

This study indicates that in the presence of both acute and chronic liver disease excessive amounts of vitamin B_{12} are found in the serum and excreted in the urine. The significance of these findings is discussed.

The Relationship of Renal Structural Changes to Renal Function in Lupus Nephritis. ROBERT M. KARK,* VICTOR E. POLLAK, CONRAD L. PIRANI, and ROBERT C. MUEHRCKE, Chicago, Ill.

Serial clinical studies and serial renal biopsies were made in 24 patients with lupus nephritis and in 9 with systemic lupus erythematosus and normal kidneys. Histological analyses assessed overall kidney damage for 61 biopsies and 8 autopsies. Damage to glomerular structures, tubules, interstitium, and vessels was also assessed and graded from zero to 4+. During three years, from 3 to 5 serial biopsies were made in 10 patients, and the development of changes in their renal structure and function will be presented.

Before each biopsy blood creatinine, urea nitrogen nonprotein nitrogen (NPN), urea and endogenous creatinine clearances, 15-minute phenolsulfonphthalein (PSP) excretion, and specific gravity concentration tests were measured. Data from 69 studies revealed creatinine levels above 1.4 mg. per 100 ml. only with severe renal damage (3 to 4+). Creatinine was below 1.4 mg. per 100 ml. in 40 out of 42 instances of lesser damage (0 to 2+). NPN was more frequently elevated above 35 mg. per 100 ml. with 0 to 2+ damage.

Close correlations were observed between degree of renal damage and the kidneys' ability to concentrate urine and excrete PSP. Close correlations also existed between urea clearances and renal damage. Values over 70 per cent were found with normal kidneys or slight damage. Values under 50 per cent were found only with more severe damage (2 to 4+).

Proteinuria correlated with basement membrane or tubular changes, but not with proliferative glomerular changes.

Analyses of structure and function were also made in relation to therapy with steroid hormones. It appears that these substances did not improve or accelerate lupus nephritis.

Increased Erythropoiesis Induced by Androgenic Hormones. B. J. KENNEDY and A. SIGRID GILBERTSEN, Minneapolis, Minn. (Introduced by Ivan Frantz).

Previous preliminary reports by the investigator have described polycythemia occurring during the administration of androgenic hormones to women with metastatic breast cancer. Characterized by constant flushing and plethora, the polycythemia was accentuated by larger doses and prolonged hormone administration. During androgen therapy an increase in hemoglobin of more than 2.0 grams was observed in 16 (48.5 per cent) of 33 patients with breast cancer.

Hematologic studies have been carried out in 26 (43.3 per cent) new patients with metastatic breast cancer who demonstrated erythrocytosis during androgen administration. The average initial hemoglobin was 13.0 grams and hematocrit 38.4 per cent. The average values during erythrocytosis were a hemoglobin of 17.0 grams and 51.5 per cent hematocrit. The highest value was a hemoglobin of 21.6 grams and 63 per cent hematocrit. There were no alterations in the white blood cell or platelet counts.

Examination of bone marrows before therapy and when erythrocytosis occurred revealed a normoblastic hyperplasia without alteration of other cellular components of the marrow. Determination of the red cell mass employing chromium^{51} revealed an increase in red blood cell volume. The red cell survival time was normal. Serum iron values decreased during the development of the erythrocytosis, but erythrocytosis did not occur in patients with iron deficiency.

There was an apparent dissociation between the clinical course of the cancer and the increase in erythroid activity. The latter was not necessarily accompanied by an improvement of the disease, nor could it be correlated with the presence or absence of osseous metastases.

A male patient with anemia and myeloid megakaryocytic hepatosplenomegaly demonstrated an increased erythropoiesis during androgen therapy. The phenomenon was observed in male hypogonadism and in a normal male.

The androgenic hormone is a specific stimulating factor of erythropoiesis, and when administered in massive doses over a prolonged period will increase erythroid activity to a polycythemia level.

Effect of Mild, Steady State Exercise on General and Cerebral Hemodynamics of Patients with Aortic Stenosis. JEROME KLEINERMAN and SALVATORE M. SANCETTA, Cleveland, O. (Introduced by Roy W. Scott).

The effect of mild, steady state exercise (110 to 120 foot pounds per min.) on general and cerebral hemodynamics has been studied in nine patients with pure aortic stenosis (AS). The technics were as previously described in a series of normal control subjects.

There were significant increases in mean minute ventilation (3.76 to 6.21 L. per M^2 per min., $p = < .001$),

mixed oxygen AVD (5.40 to 7.70 v.p.c., $p = < .001$), and cardiac index (2.19 to 2.80 L. per M^2 per min., $p = < .001$). When compared to the normal control subjects there were significant differences in the response of the AVD (normal, 3.93 to 5.53 v.p.c.) (p, normal *vs.* $AS = < .05 > .02$), and the cardiac index (normal, 3.18 to 4.09 L. per M^2 per min.) (p, normal *vs.* $AS = < .02 > .01$). Pulmonary vascular resistance increased, though not significantly (541 to 619 c. g. s., $p = < .1 > .05$). This differed significantly from a decrease in the normal subjects (241 to 190 c. g. s.) (p, normal *vs.* $AS = < .001$), and is related to the state of compensation. ETPR decreased, but not significantly (1860 to 1669 c. g. s., $p = < .2 > .1$), nor was this response significant when compared to the decrease in normal subjects.

Mean CBF decreased significantly by 16 per cent from 60 to 51 cc. per 100 gm. per min. ($p = < .01$). This decrease is not significantly different from a mean 14 per cent decrease in the control subjects. Mean CMR did not change significantly (3.1 to 3.0 cc. per 100 gm. per min.), nor is this significantly different from the change in the control group (3.2 to 3.1).

Cerebral AVO_2D increased significantly (5.3 to 6.3 v. p. c., $p = < .05 > .02$). Arterial pCO_2 decreased significantly *only* when those cases were analyzed in which steady state prevailed through the study ($p = < .01$).

CVR increased significantly (1.5 to 2.0 units) ($p = < .01$). Despite a significant but smaller increase in CVR in the control group (1.8 to 2.0 units, $p = < .05 > .02$), there appears to be a statistically significant difference between the increases occurring in the AS and the control groups (p, normal *vs.* $AS = < .05 > .01$). This greater increase in CVR occurring in the AS patients may explain the syncopal episodes known to occur in patients with aortic stenosis.

Electrical Alternans in Single Ventricular Fibers of the Frog Heart. MORRIS KLEINFELD, EDWARD STEIN, and JOHN MAGIN, New York, N. Y. (Introduced by Charles E. Kossmann).

Electrical alternans was recorded from single ventricular fibers of the frog heart in 14 experiments following the single administration of varying concentrations of L-thyroxin or triiodothyronine (TIT), and after the induction of acute anoxia. The most effective agent for producing the phenomenon was triiodothyronine.

In approximately 88 per cent of the experiments on the *intact* heart electrical alternans was present simultaneously in an external bipolar electrocardiogram and in the ventricular fiber. In the remainder (12 per cent) the alternans was observed only in the bipolar electrocardiogram. In the experiments employing an *isolated* ventricular strip of the frog for the recording of both the intracellular action potential and changes in isometric tension by means of a mechano-electronic transducer tube (RCA 5734), electrical and mechanical alternans were observed simultaneously in two experiments, one with triiodothyronine and one with acute anoxia.

In the intact heart, this phenomenon occurred during normal or slow rates, and during normal or depressed cardiac contractility, but more frequently with the latter. The alternans was of transient character and not infrequently followed a deterioration of electrical activity manifested by varying degrees of A-V block and bizarre ventricular complexes. At these times mechanical activity also showed impairment of function often terminating in asystole. In approximately 30 per cent there was a return to normal electrical and mechanical activity in 10 to 90 minutes after onset.

Although electrical alternans usually involved multiple phases of the action potential, four fairly distinct types were observed. These were alternation in: (1) the rate of depolarization; (2) the rate of repolarization; (3) the magnitude of the membrane action potential, and (4) hyperpolarization.

Although the experiments establish the occurrence of electrical alternans at the cell membrane level, the exact mechanism underlying the various forms is still obscure. Tentatively it is postulated that alternation of the magnitude of the action potential is due to alternate variation in the relative refractoriness of the membrane. Alternation in the rate of depolarization, rate of repolarization and in hyperpolarization of the membrane is attributed to alternate degrees of permeability of the membrane to sodium and potassium ions during excitation and recovery. The exact mechanism by which L-thyroxin and triiodothyronine alter the presumed alternation in membrane permeability to these ions is as yet unexplained.

The Influence of the Metabolic State of Tubercle Bacilli upon the Action of Isoniazid "In Vitro." DIETER KOCH-WESER, Chicago, Ill. (Introduced by Robert H. Ebert).

Even after prolonged isoniazid therapy one can isolate viable isoniazid sensitive tubercle bacilli from tuberculous lymph nodes, bone lesions and caseous pulmonary lesions. That this is not due to lack of penetration has been shown by administering C^{14} labeled isoniazid which is then found in high concentration in the center of such lesions. It could be related to the metabolic state of the organisms.

To test this *in vitro* C^{14} labeled isoniazid was added to liquid Dubos' medium with tubercle bacilli in the logarithmic phase of growth at 37°. It inhibits growth and renders the organisms non-acid-fast within 10 days. Four mg. of these non-acid-fast bacilli, when pasteurized and injected into a guinea pig, fail to produce hypersensitivity to old tuberculin. These bacilli have taken up radioactive drug which cannot be removed by repeated washings in fresh Dubos. Resuspended these bacilli do not release the radioactivity into the fresh medium, demonstrating that the drug is bound irreversibly by actively metabolizing organisms. If labeled isoniazid is added to bacilli which are not growing either at 4° or after 60 days in exhausted medium at 37° the organisms remain acid-fast and still sensitize guinea pigs. These bacilli also retained labeled drug, which, however, after resuspension is almost completely lost into the fresh medium. Metabolically inactive organisms bind the drug reversibly.

The same occurs if labeled isoniazid is added 3 days after streptomycin. It is taken up reversibly not interfering with acid-fastness nor sensitizing ability of the bacilli. Labeled isoniazid added either simultaneously with streptomycin or after PAS is bound irreversibly with loss of acid-fastness and sensitizing ability.

This suggests that isoniazid to be fully effective has to be taken up irreversibly by metabolizing organisms; in poorly oxygenated lesions and after previous therapy that might not be the case.

The Effect of Large Doses of Adrenocortical Hormones on the High Output of Paget's Disease. HIROSHI KUIDA, ELLIOT RAPAPORT, and FLORENCE W. HAYNES, Boston, Mass. (Introduced by Lewis Dexter).

The observation that large doses of adrenocortical hormones produced clinical improvement and suppression of activity, as judged by alkaline phosphotase levels, in osteitis deformans prompted a study of the effects of such therapy upon the increased cardiac output frequently encountered in this disease. The cardiac output was measured by the indicator dilution technique in seven cases of extensive Paget's disease prior to and again 9 to 99 days following the institution of therapy. Pretreatment cardiac indices were uniformly elevated, averaging 5.2 liters per minute per M^2 (range 3.2 to 6.4 liters per minute per M^2). During treatment the cardiac indices consistently fell to normal levels, averaging 3.3 liters per minute per M^2 (range 2.6 to 4.1 liters per minute per M^2). This represents an average reduction from pretherapy levels of 37 per cent (range 13 to 52 per cent). Calculated total peripheral resistance increased from an average of 1,120 to 1,770 dynes sec. $cm.^{-5}$ and derived arteriovenous oxygen differences increased from 28 to 43 cc. per liter. Pretreatment values for total blood volume were 4,230 cc., mean arterial blood pressure 108 mm. Hg, and oxygen consumption 141 cc. per minute per M^2 and remained essentially unaltered (4,312 cc., 111 mm. Hg, and 137 cc. per minute per M^2, respectively) and could not account for changes in cardiac output. No significant changes in body weight or hematocrit occurred. The pretreatment data are compatible with the interpretation of Edholm and Howarth of the existence of arteriovenous shunts in involved bones as the cause of the high output. The mechanisms by which steroid therapy promote the observed effects were not elucidated by this study.

Relationship of Gallop Sounds to Intracardiac Pressures. PETER T. KUO, TRUMAN G. SCHNABEL, JR., WILLIAM S. BLAKEMORE, and STEPHEN B. LANGFELD, Philadelphia, Pa. (Introduced by William A. Jeffers).

Extracardiac recording of events associated with gallop sounds in the past had suggested that these sounds are produced by reflux closure of the auriculoventricular valves. A more precise correlation of the gallop sound with the changing atrial and ventricular pressure levels would help to elucidate the mechanism of production of these sounds.

Pulmonary capillary pulse pressure (wedge pressure) and phonocardiogram were recorded simultaneously in five patients with chronic rheumatic heart disease and mitral insufficiency. In each of the five patients the gallop sound occurred on descending limb of the "V" wave, 0.02 to 0.04 second before the trough of the pressure curve.

Simultaneous recordings of the left atrial and left ventricular pressures through two catheters, were obtained in eight patients with mitral insufficiency and diastolic gallop by left heart catheterization. In five of the eight patients a protodiastolic sound was recorded while the left atrial pressure was 6.3 to 18.4 mm. Hg higher than that of the left ventricle. In three patients with presystolic gallop, the sound was recorded when the left atrial pressure was 4.2 to 6.1 mm. Hg above the left ventricular pressure.

These observations clearly indicate that the gallop sound occurs with progressive ventricular filling. It is unlikely that the mitral valves could close and vibrate while the left atrial pressure is higher than the left ventricular pressure.

Intrarenal Circulatory Dynamics and Glomerular Permeability to Hemoglobin in Dogs. WILLOUGHBY LATHEM and A. D. BENJAMIN, West Haven, Conn. (Introduced by Philip K. Bondy).

The rate of passage of protein molecules through capillary walls appears to be influenced not only by the characteristics of the molecule and the membrane but also by the level of intracapillary hydrostatic pressure and the rate of hydrodynamic flow across the membrane. In the present study the influence of these factors on the glomerular capillary permeability to hemoglobin was investigated in dogs.

Plasma hemoglobin levels were maintained by intravenous infusion at relatively constant levels well above threshold. Infusions of l-norepinephrine were administered intravenously at rates sufficient to reduce renal plasma flow (RPF-C_{pah}), both to reduce and maintain glomerular filtration rate (GFR-creatinine clearance-C_{cr}) and to elevate, reduce, and maintain intraglomerular pressure (as judged by the filtration fraction). The renal clearance of hemoglobin (C_{Hgb}) increased under these various conditions provided GFR was not reduced more than 50 per cent; below this figure, C_{Hgb} was also reduced. However, in all studies, the ratio C_{Hgb}/C_{cr} increased. This was attributable to an increase in hemoglobin concentration in glomerular filtrate rather than to a change in tubular reabsorption since a plot of urinary hemoglobin excretion against the product of GFR and plasma hemoglobin concentration indicated that glomerular permeability to hemoglobin increased relative to that of creatinine. Moreover, in some instances the changes of hemoglobin excretion were greater than Tm_{Hgb}. This change in permeability could not be correlated with alterations in GFR or in intraglomerular pressure. There was, however, a correlation with RPF, suggesting that the rate of blood flow through the glo-

merulus conditioned the rate of diffusion of hemoglobin molecules across the glomerular capillary membrane.

Tissue Metabolism and Ion Transport. ALEXANDER LEAF* and ALAN RENSHAW, Oxford, England.

The Redox Hypothesis to explain active ion transport has a quantitative limit which may be tested experimentally, as indicated by R. E. Davies. When oxygen is the electron acceptor a maximum of 4 hydrogen ions may be produced, or 4 univalent cations transported, per molecule of oxygen consumed.

The equivalence of short-circuit current and active sodium transport through the isolated frog's skin demonstrated by Ussing allows a method of measuring the rate of ion transport electrically. Simultaneous measurements of oxygen consumption were made using a polarographic technique with vibrating platinum electrodes.

The results of 52 periods in which simultaneous measurements of active ion transport and total oxygen consumption were made show a mean value of 6.3 ions of sodium transported per molecule of oxygen consumed. Only seven periods gave ratios below 4; the range was 2.0 to 11.2. The unmodified Redox Hypothesis is thus inadequate to account for observed ion transport rates.

As neurohypophyseal extract increases sodium transport through this preparation, its effect on sodium transport and oxygen consumption was tested. The mean ratio of the increments of sodium ions transported to oxygen molecules consumed following Pitressin® was 20.5 ± 3.1. The highly significant increase of about 20 per cent in oxygen consumption following Pitressin® was confirmed by classical manometric techniques.

Calculations show that operation of the sodium pump probably requires a minimum of 10 to 20 per cent of the total energy produced by the tissue. Active ion transport depends upon oxidative energy supplies and the highest rates of transport observed indicate that two or more ions may be moved per high energy phosphate bond synthesized.

Studies on the Pro-Enzymatic Activity of the First Component of Human Complement (C'1). IRWIN H. LEPOW, OSCAR D. RATNOFF,* and LOUIS PILLEMER, Cleveland, O.

It has often been postulated that antigen-antibody reactions activate a proenzyme in serum. Recently we presented data suggesting that the first component of complement (C'1) may be a proenzyme. Direct evidence has now been obtained by two independent methods that this first component may be the precursor of an esterase.

The first approach required the partial purification of C'1 by a new procedure based on maintaining C'1 at high ionic strength and at pH 5.5. This fraction lost its C'1 activity when ionic strength and pH were adjusted to those of serum. Simultaneously, it acquired the capacity both to hydrolyze p-toluenesulfonyl-l-arginine methyl ester and to inactivate the fourth component of complement. This enzymatic activity was not due to plasmin, thrombin, cholinesterase or acid or alkaline phosphatase.

The second approach took advantage of the adsorption of C'1 by aggregates of pneumococcal specific soluble substance, type III, and homologous rabbit antiserum. The adsorption was performed under conditions minimizing the adsorption or destruction of other components. A fraction was eluted from the antigen-antibody-C'1 complex that represented less than 0.05 per cent of the total serum nitrogen. This "eluate factor" also hydrolyzed p-toluenesulfonyl-l-arginine methyl ester and inactivated the fourth component of complement. However, it did not exhibit any hemolytic complement component activities.

Both Levine and Becker have reported that diisopropyl fluorophosphate, an esterase inhibitor, inhibited immune hemolysis by guinea pig complement. We have found that diisopropyl fluorophosphate also inhibited the inactivation of the fourth component of complement by the esterase derived from C'1.

These observations provide evidence that antigen-antibody reactions activate a proenzyme in serum. They also offer an explanation for the inactivation of complement in complement "fixation" reactions. The role of the esterase in immunity and hypersensitivity remains to be investigated.

The Effect of Intravenous Calcium Infusion on Electrolyte Excretion in Man. MARVIN F. LEVITT, MARK H. HALPERN, AVRON Y. SWEET, and DONALD GRIBETZ, New York, N. Y. (Introduced by Alexander B. Gutman).

Patients with protracted hypercalcemia frequently develop polyuria and salt depletion generally ascribed to chronic tubular damage and nephrocalcinosis. To clarify the role of hypercalcemia *per se* on salt excretion, the effects of rapid intravenous infusion of calcium were studied.

Renal clearances, plasma electrolyte composition, and the rate of electrolyte excretion were measured in 8 normal subjects before, during, and after a two-hour infusion of calcium lactate or gluconate (0.1 mg. Ca per min. per Kg.) sufficient to raise serum calcium levels to 12 to 14 mg. per cent. The calcium infusions induced a prompt sodium and chloride diuresis, the rates of sodium and chloride excretion increasing from a mean of 242 and 259 microEq. per min. to 829 and 701 microEq. per min., respectively. Coincidentally, the rate of calcium excretion rose from a mean of 7 to 55 microEq. per min. Immediately following cessation of the infusion, the plasma calcium fell to normal but the rate of calcium and salt excretion approached control values more slowly. The rate of phosphorus excretion tended to rise gradually, reaching maximum values one hour after the calcium infusion was discontinued.

During the infusion, 4 of the subjects showed a transient increase in filtration rate of 10 per cent. The increased rate of electrolyte excretion persisted after the filtration rates returned to normal in these subjects and occurred with equal consistency in those subjects in whom no change in filtration rate could be detected.

Preliminary experiments revealed that when the plasma calcium concentration and the rate of excretion of free calcium are rapidly reduced by a chelating agent, the rates of sodium and chloride excretion fall.

These data suggest that a change in the filtered load of calcium effects a prompt and reciprocal change in the rate of tubular absorption of sodium and chloride.

The Analysis of Cr^{51} Erythrocyte Survival Curves. ALLYN B. LEY and KLAUS MAYER, New York, N. Y. (Introduced by Rulon W. Rawson).

In the course of studies on erythrocyte survival by the chromate-51 technique, significant differences have been observed in the shape of the curves expressing the disappearance of circulating radioactivity amongst subjects in various hematologic states. These graphic differences have been subjected to mathematical analysis, and a simplified nomographic approach has been developed which permits a quantitative differentiation between erythrocyte destruction due to random loss on the one hand and that due to erythrocytic ageing factors on the other. Furthermore, in patients in hematologic equilibrium tentatively valid estimations of rates of red cell production can be deduced from this approach regardless of the relative contributions of the random or ageing factors.

In normal subjects the loss of circulating radioactivity appears to be predominantly related to erythrocyte ageing. In patients with the anemia associated with neoplastic disease, the loss, while usually considerably more rapid than normal, also appears to be predominantly related to ageing. This confirms evidence suggested by previous studies employing transfusions of normal blood into such recipients. In contrast, in patients with congenital spherocytosis or with classical acquired hemolytic anemia the loss appears to be practically entirely at random. It is inferred, therefore, that the mechanism of increased red cell destruction in the anemia associated with neoplastic disease is fundamentally different from that in the classical types of hemolytic anemia.

Paradoxical Electrolyte Effects of Delta 1-9 Fluorohydrocortisone in Hypocorticoid Subjects. M. C. LI, D. M. BERGENSTAL, and R. H. PARROTT, Bethesda, Md. (Introduced by Seymour S. Kety).

Metabolic balance studies were made on one Addisonian, three eucorticoid, and three adrenalectomized subjects receiving delta 1-9 fluorohydrocortisone, Dl-FF, a new potent synthetic adrenal-type steroid. It was observed that Dl-FF possessed profound protein catabolic and eosinopenic activities about twice as great as fluorohydrocortisone and five times as great as delta 1 hydrocortisone. The initial sodium retention was marked, equally potent to fluorohydrocortisone, however, transient. Sodium diuresis occurred in 6 to 9 days with eventual sodium balance. Potassium diuretic effect was prominent throughout and was about twice as potent as that of fluorohydrocortisone.

Dl-FF 0.5 mgm. to 1 mgm. by mouth daily in divided doses was adequate to maintain adrenalectomized or Addisonian patients on an essentially eucorticoid status. Plasma corticoid and urinary steroid values were essentially zero. Potassium supplementation was necessary to prevent hypokalemia. The Addisonian subject maintained on Dl-FF 1 mgm. with intake of sodium of 130 mEq. and potassium 60 mEq. daily had a potassium loss of about 16 mEq. per day; a sodium gain of 180 mEq. in the first two days became balanced starting the third day. When sodium intake was reduced to 13 mEq. per day, urinary sodium and potassium excretion fell rapidly and was essentially balanced in 6 days. Comparative studies were made with cortisone acetate 37.5 mgm. per day as maintenance. There was marked sodium loss in the urine with development of hyponatremia, hypotension, and dehydration by the sixth day. Comparable results were obtained in an adrenalectomized patient.

The sodium retaining, diuretic and consequential balancing ability of Dl-FF may be explained by an increase of renal glomerular filtration rate and plasma flow. The paradoxical and persistent sodium retaining activity, however, in conjunction with loss of potassium diuretic effect during the period of restricted sodium intake, may be due to an increase of tubular reabsorption of these two electrolytes by Dl-FF.

The Hematocrit of the Dog Kidney: Differences in Red Blood Cell and Plasma Transit Times. LAWRENCE S. LILIENFIELD, FRANK A. PORFIDO, and JOHN C. ROSE, Washington, D. C. (Introduced by Harold Jeghers).

Studies by Pappenheimer and co-workers have suggested that red cell shunting occurs in dog kidneys. If such shunting exists, it might be reflected in differences between red cell and plasma transit times through the kidney. In an attempt to investigate this further, the following experiment was performed in nine mongrel dogs:

Under pentobarbital anesthesia, the left kidney was exposed and the vessels dissected free. A large polyethylene catheter was inserted into the renal vein toward the kidney, and connected to another catheter placed in a femoral vein. The renal vein was ligated distally. In effect, an elongated, exteriorized renal vein was made available. A ureteral catheter monitored urine flow, and in all experiments, urine was actively excreted.

One-half ml. of a well-mixed suspension of Cr^{51} tagged dog red cells and I^{131} tagged albumin was injected rapidly into the renal artery. Prior to injection, the renal vein catheter was disconnected from the femoral vein and renal vein blood permitted to flow freely. Total kidney blood flow was collected continuously for one minute, in two-second interval samples.

Cr^{51} activity of washed red cells and I^{131} activity of plasma in each sample was plotted logarithmically against time. Mean circulation times (MCT) of red cells and plasma were then determined. In each case the MCT for red cells was found to be faster than that of the plasma

(Ratio = .82 ± .040). However the washout downslopes for both indicators were identical.

These data indicate that red cells lead plasma or are shunted through those vascular pathways in the kidney that are of the shortest length. In the longer pathways from renal artery to renal vein, red cells and plasma move together. The calculated total circulating hematocrit in the dog kidney averaged .88 ± .033 per cent of the large vessel hematocrit.

Measurement of the Threshold to Visceral Pain Induced by Distension of the Large Intestine. MARTIN LIPKIN and MARVIN H. SLEISENGER, New York, N. Y. (Introduced by Thomas P. Almy).

Previous studies of visceral sensation have demonstrated that the application of pressure to the wall of the intestine may evoke pain. In order to evaluate visceral pain in quantitative terms, the following experiments were performed. A latex balloon, attached to a firm plastic catheter, was passed into the sigmoid colon. The catheter was attached to a large cylindrical water vessel and the entire system filled with water. By means of a valve, sudden pressure was then applied to the wall of the intestine, and the time to onset of pain (suprapubic; cramping or sharp) noted. The applied pressure was constant during the experimental determination. Balloon distension measurements at various pressures were made.

In the first series of experiments the level of water pressure was varied, and the relationship between applied pressure and time to onset of pain was studied in 11 subjects. It was found that as the intensity of the stimulus decreased the duration of time to onset of pain increased. The shortest time interval to onset of pain was 2 seconds, with a corresponding pressure of 58 cm. of water. The lowest pressure at which pain was perceived was 52 cm. of water, occurring at 23 seconds. Thus the intensity-duration relationships for visceral pain appear to be similar to those for cutaneous pain.

In the second series of experiments the time to onset of pain was noted after sudden application of 100 cm. water pressure. In 23 subjects the mean time to onset of pain was 5.3 seconds (S.D. ± 2.5). Two subjects experienced no pain at this level of pressure.

The reproducibility of these measurements in the same individual, the effect of the state of contractility of the intestine, and the action of drugs upon visceral pain threshold are now being evaluated.

The Influence of Vaccination Upon Intestinal Infection of Family Contacts of Poliomyelitis Patients. MARTHA J. LIPSON, FREDERICK C. ROBBINS,* and WILNA A. WOODS, Cleveland, O.

This study was undertaken to determine whether immunization with formaldehyde killed poliomyelitis vaccine influences the likelihood of intestinal infection with poliomyelitis virus.

To obtain information pertinent to this question the family unit was selected for study. Stools and sera were obtained from as many members of each family as possible. The stools were tested for the presence of virus and the sera for neutralizing antibody.

Three groups of families were studied:

1) 24 families from Indiana (1954) in which a case of poliomyelitis occurred and in which there was one or more child 5 to 9 years of age. Nine of these children received 3 injections of vaccine.
2) 17 similar families from the Cleveland area in 1955 with one or more vaccinated child. These children received a single dose of vaccine.
3) 16 families from Cleveland in which there was a vaccinated child but the index case proved not to be poliomyelitis.

Materials were collected as soon as possible after recognition of the index case.

The results of virus isolation revealed virus in the intestinal contents of 3 of 8 vaccinated contacts in the Indiana group and 10 of 17 in those from Cleveland, or a total of 13 (52 per cent) of 25 for the entire study. The control group consisted of 31 unvaccinated children of the same age (5 to 9 years) and 21 (67 per cent) of these were excreting virus. Thus vaccination had no marked effect upon the number of intestinal carriers of virus among family contacts.

Somewhat higher antibody levels were found in the vaccinated children than in the controls. The antibody pattern in the vaccinates closely paralleled that in the adult contacts. However, the rate of virus excretion in the latter was only 14 per cent. This raises the question of whether serum antibody relates to immunity to intestinal infection.

Glucose Metabolism in Human Subjects with Neoplastic Diseases. PAUL A. MARKS and JONATHAN BISHOP, New York, N. Y. (Introduced by Alfred Gellhorn).

Disturbances in host tissue protein metabolism associated with tumor growth have been described. However, alterations in glucose metabolism associated with neoplasia in human subjects have not been defined.

A study of glucose metabolism in selected patients (adequate dietary intake, ambulatory, no weight change for six months, no family history of diabetes mellitus) with leukemia, lymphoma and clinically localized carcinoma was performed employing a technique of intravenous glucose tolerance test (G.T.T.) which permitted calculation of the fractional rate of blood glucose disappearance ($K_{G.D.}$). Observations based on 86 G.T.T. in 33 patients and 17 normal individuals revealed that the subjects with malignancy had a significantly lower $K_{G.D.}$ (mean = 2.24 ± 0.71 per cent per min.) than the control group (mean = 4.19 ± 1.31 per cent per min.). This finding contrasts with the recognized high rate of glycolysis that characterizes metabolism of tumor tissue.

No significant difference existed between the two

groups in fasting blood sugar (control, mean = 80 ± 9 mg. per cent; patient, mean = 88 ± 12 mg. per cent) or urinary glucose excretion during the G.T.T. The decreased $K_{G.D.}$ of patients with malignancy was not associated with alterations in the estimated volume distribution of glucose or in sodium space (Na^{24}). The neoplastic group had as great a decrease, during the G.T.T., in serum phosphate (controls, mean = 0.67 ± 0.19 mg. per cent; patients, mean = 0.61 ± 0.24 mg. per cent) and in serum potassium (controls, mean = 0.41 ± 0.28 mEq. per L.; patients, mean = 0.59 ± 0.32 mEq. per L.) as the control group.

Administration of insulin (0.1 μ per kg. body weight) was associated with a constant fractional rate of decrease in blood sugar in both groups. Of 14 patients with decreased $K_{G.D.}$, 6 were relatively insulin resistant.

Serial observations in four patients suggest that associated with effective chemotherapy or extirpation of tumor (while on constant diet and despite negative nitrogen balance as determined in two patients) the defect in glucose metabolism reverts toward normal.

The Use of Microbial Enumeration Techniques in the Evaluation of Antibacterial Agents in the Treatment of Experimental Staphylococcal Infections of Mice. ROBERT M. MCCUNE, P. A. PETER DINEEN, and JOHN C. BATTEN, New York, N. Y. (Introduced by Edwin D. Kilbourne).

Staphylococcal infections present one of the most difficult problems in the treatment of infectious diseases today. In the past, laboratory approaches to these problems have primarily been concerned with *in vitro* observations, or animal studies limited to histopathologic or survival time experiments. It is felt that the dynamics of staphylococcal infections can be more sensitively reflected by quantitative changes in total populations of staphylococci within various organs of the infected host.

Potentially fatal infections were induced in mice with known numbers of penicillin-resistant staphylococci. Subsequently, serial studies were made to determine the fate of staphylococcal populations of the lungs, spleens and kidneys of mice during the natural course of infection and in infections modified by various methods of therapy.

Host-staphylococcal relationships were different in various organs of untreated animals. Pronounced multiplication was seen in the kidney, where large abscesses formed and ultimately led to destruction of the renal tissue and death of the animal. There was a slight increase in the lung populations during the early phase of infection. A falling bacterial census was seen in the spleens. No abscess formation or necrosis of tissue was seen in the lungs or spleens.

Significant differences were seen in the response of staphylococcal populations to various drugs used singly and in combination in the different organs.

The drugs studied in these experiments were penicillin, streptomycin, erythromycin, streptonivicin and the tetracycline derivatives.

There was clear evidence that penicillin antagonized the effectiveness of other drugs in this penicillin-resistant staphylococcal infection.

The phenomenon of microbial persistence was seen in all treatment groups, whereby drug susceptible microorganisms survived in animal tissues despite twenty-eight days of therapy.

The influence of therapy was also studied when the infection was allowed to progress until lesions were established.

On Intracellular Edema and Renal Adjustments in Severe Chronic Malnutrition. JACK METCOFF,* SILVESTRE FRENK, GUSTAVO GORDILLO, FEDERICO GOMEZ, RAFAEL RAMOS GALVAN, and IRENA ANTONOWICZ, Boston, Mass. and Mexico, D.F.

Variously known as Kwashiorkor (Africa), Malnutrition (India, Mexico), Shibi-Gachaki (Japan), Mehlnahrschaden (Germany), severe *chronic malnutrition* of infants originates from inadequate food intake and recurrent episodes of diarrhea with eventual dehydration or edema. The mortality, estimated at 30 to 60 per cent reflects disturbances in water and salt metabolism not amenable to the type of parenteral fluid therapy which is effective in *acute* diarrheal dehydration of *well-nourished* infants. This world-wide problem prompted investigations of water and electrolyte metabolism in chronically malnourished Mexican children. Serial muscle and skin biopsies (14 infants, 4 of whom died) with balance measurements (8), inulin and PAH clearances (6), and response to infused NaCl loads (3) were studied.

In muscle, intracellular water was increased whether the extracellular (chloride) phase was expanded or contracted. Intracellular potassium content ($(K)_{icw}$) was not significantly reduced although intracellular concentration was (90 to 120 mM per L.). Markedly increased $(Na)_{icw}$ correlated best with augmented intracellular water and was independent of $(K)_{icw}$. In two fatal episodes, although the potassium content of muscle did not change significantly, extensive intracellular accumulation of water and sodium was observed. Recovery, in another patient, demonstrated the reversible nature of similar changes.

Skin showed a large chloride "excess."

As previously noted, apparent retentions of certain ions in balance measurements were inconsistent with tissue composition data.

In dehydrated infants plasma was hypotonic (250 to 274 mosm per L.), inulin clearance reduced, and copious hypotonic urine was excreted. Isotonic (0.15 m) or hypertonic (0.92 m) NaCl infusions caused 50 to 60 per cent expansion of ecw without changing C_{in}. Hypertonic saline effectively expanded ecw, by shift of endogenous water from intracellular to extracellular fluid, with striking clinical improvement. "Osmoreceptor" sensitivity and tubular responsiveness to endogenous antidiuretic hormone activity were demonstrated.

Severe chronic malnutrition apparently becomes fatal when sodium and water accumulate intracellularly, and

reduced glomerular filtration with increased tubular water rejection fail to defend osmolarity of the body fluids.

The Effect of Serotonin on Respiration in Asthmatic and Non-Asthmatic Subjects. ALAN L. MICHELSON and WILLIAM HOLLANDER, Boston, Mass. (Introduced by Francis C. Lowell).

Serotonin hydrochloride in doses ranging from 0.5 mg. to 1.5 mg. was given intravenously to more than 50 normal, hypertensive and asthmatic patients. The respiratory response to the injected material was recorded by means of closed system spirometry. Forty-five patients responded in a characteristic manner to doses varying from 0.5 to 1.5 mg. given in one dose intravenously. Five patients failed to respond to this dosage. The majority of patients responded with a period of marked hyperventilation associated with elevation of the respiratory midposition. A smaller percentage of patients responded with a period of apnea followed by hyperventilation. Both responses were transitory. Asthmatic patients responded in the same manner as normals or hypertensives. Various agents including Hexamethonium, Regitine®, Atropine, Neo-Antergan®, Chlorpromazine and the benzyl analogue of serotonin were studied in an effort to block the respiratory response.

Serotonin hydrochloride in concentration up to 10 mg. per cc. was given by aerosol repeatedly to 10 normal and 20 asthmatic subjects. Kymographic tracings of expiration were obtained before and following the nebulization of serotonin. Normal subjects failed to show any change in speed of expiration or in the total volume expired. Asthmatic patients consistently showed both a decrease in total volume expired and in the speed of expiration. This effect was blocked partially or completely by prior intravenous injection of Neo-Antergan® or Benadryl®.

Normals, patients with hypertension, and patients with bronchial asthma responded in the same manner to intravenous serotonin. However, when serotonin is given by aerosol only patients with bronchial asthma respond with a decrease in vital capacity and a decrease in speed of expiration.

The relation between the two types of response will be discussed as will the possible reasons for the observed differences in non-asthmatic and asthmatic subjects.

The Plasma Disappearance, Excretion and Tissue Distribution of Intravenous Cobalt^{60} Vitamin B_{12} in Normal Subjects and Patients with Chronic Myelogenous Leukemia. AARON MILLER, HOWARD F. CORBUS, and JOHN F. SULLIVAN, Boston, Mass. (Introduced by Bernard M. Jacobson).

The serum in chronic myelogenous leukemia shows an increased concentration and *in vitro* binding of vitamin B_{12}. Four micrograms of Cobalt^{60}-labelled vitamin B_{12} were injected intravenously into normal subjects and leukemic patients. Its plasma disappearance, tissue distribution and urinary and fecal excretion were determined. A rapid decline in plasma radio-activity occurred in normal subjects: 7 to 12 per cent of the dose remained at two hours and 4 to 10 per cent at twenty-four hours. Urine and stools contained negligible amounts of radio-activity. In seven out of eight patients with myelogenous leukemia, plasma radio-activity disappeared more slowly: 50 to 63 per cent of the dose remained at two hours and 38 to 44 per cent at twenty-four hours. After twenty-four hours, the disappearance rate decreased (half-time of five days). One patient in remission from chronic myelogenous leukemia, two with myeloid metaplasia and one with chronic lymphocytic leukemia had normal rates of disappearance.

External monitoring showed an increase in liver radio-activity throughout the period of observation (7 to 22 days) not associated with a comparable fall in plasma radio-activity. The leukemic patients showed a smaller rise in hepatic radio-activity than did the normals. The radio-activity in the organs of two myelogenous leukemic patients was determined post-mortem. The liver contained 39 to 42 per cent of the administered dose, the spleen 8 to 11 per cent and the other viscera less than 1 per cent. The concentration of radio-activity in liver (counts per gram) was seven times greater than in spleen and other viscera. Red and white cells from both groups contained no radio-activity.

The increased *in vivo* plasma binding of vitamin B_{12} in myelogenous leukemia without preferential concentration in leukemic tissue suggests that the turnover of vitamin B_{12} in leukemic tissue is not increased. The decreased plasma disappearance may prove to be of diagnostic value in differentiating chronic myelogenous leukemia from leukemoid states.

The Relationships Between Components of the Rapid Expiratory Flow and Volume Curves to Pulmonary Viscous Resistance. WILLIAM F. MILLER, ROBERT L. JOHNSON, JR., and NANCY WU, Dallas, Tex. (Introduced by Elias Strauss).

The extent to which specific timed segments (timed expiratory capacities, TEC) of the rapid expiratory volume curve constitute estimates of pulmonary viscous resistance was investigated.

Preliminary artificial airway obstruction in normal subjects produced greater percentage decreases of the 0.5 sec. EC than were produced in either the MBC or the 1.0 sec. EC. Total vital capacities (TVC) showed no decrease even with resistances above 120 cm. H_2O per L. per sec.

Using the intraesophageal balloon method, pulmonary resistance was measured in 10 normal subjects and in 25 patients with various disturbances of ventilation. Measurements were made both during quiet breathing and during recording of pneumotachographic flow and integrated fast expiratory volume curves.

There was good correlation between the log

$$\frac{0.5 \text{ sec. EC} \times 100}{\text{TVC}}$$

and mean expiratory resistance ($r = -0.88$) when expiratory resistance was less than 11 cm. H_2O per L. per sec. When resistance exceeded this level, further decline of the 0.5 sec. EC ratio below 30 per cent was limited by a concomitant decrease of the TVC.

Simultaneous recording of transpulmonary pressure and fast flow or volume curves provide a basis for differentiating mechanisms of increased pulmonary resistance.

Patients with chronic pulmonary emphysema exhibited relatively high initial flow rates with abrupt decreases at low transpulmonic pressures, whereas patients with bronchial asthma showed lower initial flow rates without evidence of early collapse of inelastic airways.

Thus, the TEC provides a reliable estimate of pulmonary viscous resistance and, further, when obtained by a method permitting visualization of transpulmonic pressure with rapid expiratory flow or volume curves, a means of defining the nature of the increased resistance is obtained.

The Disappearance of T-1824 from the Circulation in Heart Failure. W. R. MILNOR and H. I. CRARY, Baltimore, Md. (Introduced by E. C. Andrus).

It has been suggested that the blue dye T-1824 escapes from the circulation more rapidly than usual, and therefore gives falsely high measurements of plasma volume, in the presence of congestive heart failure. To test this hypothesis, the initial dilution curve and the subsequent disappearance of T-1824 from the plasma after a single intravenous injection were measured in 17 patients with chronic heart disease. Eleven patients were studied during an acute episode of congestive heart failure, with marked peripheral edema, and in 5 of these patients the studies were repeated after compensation had been restored.

In all patients studied the plasma disappearance rates after the 6th minute post injection were not significantly different from those previously observed in normal subjects. The peak concentration of the initial dilution curve was lowered in the patients with failure, but not more than could be accounted for by the increase in intrathoracic blood volume which also occurred in these patients.

Plasma volume, calculated by extrapolation to zero time from observed concentrations at 20 to 60 minutes, was lower when cardiac compensation was restored than it had been during failure, in the 5 cases where such comparisons were made, but there was no significant change in the disappearance rates.

These results suggest that the measurement of plasma volume by T-1824 dilution is no less valid in patients with congestive heart failure than in other subjects. The data do not, however, exclude the possibility of a relatively rapid loss of T-1824 from the intravascular compartment during the first few re-circulations of the dye, provided it did not continue beyond the 4th minute post-injection.

In Vivo Measurement of Splenic Circulation: A Rapid Method for the Demonstration of Splenic Red Cell Sequestration. ARNO G. MOTULSKY, FREDERICK CASSERD, and ELOISE GIBLETT, Seattle, Wash. (Introduced by Wm. M. M. Kirby).

Delayed equilibration of tagged erythrocytes in the peripheral blood has been previously observed in certain instances of marked splenomegaly. Such mixing curves can only be identified when large volumes of red cells are sequestered within the spleen. In order to detect slow splenic mixing when the mass of sequestered cells was not very large, direct measurements of splenic circulation were made.

Radioactive assay of whole spleens removed at intervals after injection of tagged cells indicated that rats with hemolytic anemia due to methylcellulose splenomegaly had prolonged splenic mixing.

In man, similar measurements were made by placing scintillation counters over surface projections of the spleen and liver. The accumulation of activity over these areas after injection of Cr^{51} tagged erythrocytes was simultaneously recorded for approximately one hour. Mixing in normal and nonsequestrating spleens such as in hereditary nonspherocytic hemolytic disease and polycythemia vera was completed within a few minutes. Splenic and hepatic mixing curves were essentially similar. The most prolonged splenic mixing curves with normal liver tracings were observed in the spherocytic anemias where equilibration was delayed as long as 60 minutes. In some cases of autoimmune hemolytic disease, Thalassemia, leukemia and paroxysmal nocturnal hemoglobinuria prolonged splenic mixing curves could be demonstrated.

These studies indicate that slow mixing of tagged cells may be caused by congenital (hereditary spherocytosis) or acquired (globulin coated) cellular defects or by enlargement of the splenic pulp and packing of the pulp with normal cells. Prolonged mixing in patients and experimental animals could be related to shortened red cell life and increased splenic deposition of Cr^{51}. Splenectomy results will be shown.

This method promises to be of value for the study of the human splenic circulation and in the evaluation of patients for splenectomy.

Regulation of the Secretion of Aldosterone in Man. ALEX F. MULLER, ANNE M. RIONDEL, ELIZABETH L. MANNING, and RENÉ S. MACH, Geneva, Switzerland (Introduced by Walter Bauer).

The present work was undertaken to study in man the effects of hydration, dehydration and ACTH on the urinary excretion of aldosterone. The experiments were carried out under controlled metabolic conditions. Aldosterone was determined according to the method of Neher and Wettstein.

Excessive hydration due to Pitressin® Tannate and water decreased immediately the excretion of aldosterone, thus causing the well known renal sodium loss; in reverse, the rebound water diuresis after stopping Pitressin® stimulated aldosterone secretion, which resulted

secondarily in sodium retention. Also, during 8 hours of acute water-loading without Pitressin® in a normal subject on a low sodium diet, the aldosterone excretion decreased significantly. Dehydration, however, by fluid loss after excessive perspiration was followed by an increased aldosterone output. Also, acute dehydration after water deprivation in a patient with diabetes insipidus increased the production of aldosterone considerably; subsequent hydration diminished it.

These studies demonstrate, on the one hand, that the variations in aldosterone secretion proceed rapidly, and on the other hand they may be interpreted as giving evidence that it is the state of cellular hydration which regulates the secretion of aldosterone.

Moreover there seems to exist a definite influence of ACTH on the production of aldosterone. The already elevated aldosterone excretion, which followed the stopping of Pitressin® administration, was greatly accentuated by ACTH. In a hypophysectomized patient aldosterone excretion increased on the first day of ACTH therapy, but decreased gradually the subsequent days in spite of continuous ACTH administration. The pattern of increased aldosterone excretion in several normal subjects on ACTH was variable. The response was either immediate or delayed, but always brief and transitory. Two mechanisms of pituitary action can be postulated, either a direct stimulation of the adrenal gland by ACTH, or, what seems also possible, an indirect regulation of the secretion of aldosterone, conditioned by the effects of the corticosteroids on water distribution.

Potassium to Sodium Ratio as an Index of Aldosterone Output. P. J. MULROW, A. H. LIEBERMAN, B. B. JOHNSON, and J. A. LUETSCHER, JR.,* San Francisco, Calif.

Aldosterone output of normal man is affected by changes in sodium intake. Elimination of sodium from the diet is followed by a five-fold increase in aldosterone excretion. When sodium intake is maintained above 450 mEq. per day, aldosterone is not detectable in urine. An inverse relationship between sodium and aldosterone in urine can be demonstrated. This relationship is altered when potassium intake is modified.

Potassium depletion significantly decreases aldosterone in urine of men on normal sodium intake. Potassium loading is followed by an increase in aldosterone output, which is smaller than that observed during sodium deprivation.

When sodium is withdrawn from the diet of normal men depleted of potassium, aldosterone increases as sodium in urine falls, but this increase is less than that observed on a diet deficient solely in sodium. Repletion of potassium is followed by further increase in aldosterone output. When ammonium chloride is administered after depletion of both potassium and sodium, aldosterone output increases, and potassium is excreted in preference to sodium.

Aldosterone output is not consistently related to the volume or electrolyte concentration of extracellular fluid, but the role of intracellular composition is still to be defined. There is a direct correlation of aldosterone in urine with urine potassium to sodium ratio in normal men and patients with normal renal function. In such subjects, in the absence of exogenous hormone, this ratio can be used to estimate endogenous aldosterone output.

Production of Diuresis in Hyponatremic Edematous States with Alcohol. H. V. MURDAUGH, JR., Durham, N. C. (Introduced by Julian M. Ruffin).

The finding of concentrated urines in certain edematous patients with evidence of hemodilution, coupled with the demonstration by previous investigators (Leaf) of antidiuretic activity of the plasma during water loading in dogs with induced hyponatremia, prompted inquiry into the possible role of antidiuretic hormone (A.D.H.) in hyponatremic edematous states. Since alcohol reportedly inhibits the release of A.D.H., it was used in an attempt to induce diuresis. It was found that alcohol could increase urine output during transient postoperative oliguria.

Absolute alcohol (15 to 30 ml. orally in fruit juice or intravenously as a 3 per cent infusion) was then administered to patients with severe postoperative oliguria, water intoxication, postvalvulotomy hyponatremic syndrome, and one patient in refractory congestive failure, all of whom had concentrated urines in the face of edema, hyponatremia, and hemodilution evidenced by decreased plasma osmolarity. The response in these subjects was a diuresis with a 3- to 14-fold increase in 24-hour urine output and a return of the plasma osmolarity and sodium concentration to normal. The increases that occurred in plasma osmolarity ranged from 16 to 63 mosm. per L., and the increases in serum sodium concentration ranged from 15 to 41 mEq. per L. Dilute urines during diuresis, the return of the plasma to a normal sodium concentration in each patient without sodium administration, and electrolyte excretion studies in two of the patients indicate that the diuresis obtained was a true water diuresis.

The patient with congestive failure had been refractory to mercurial diuretics and demonstrated a return of responsiveness to Mercuhydrin® after his plasma electrolytes had returned to normal.

The diuresis in response to alcohol in these patients presents a therapeutic tool in a difficult clinical problem and suggests an altered response in certain hyponatremic states with release of A.D.H. in the presence of a decreased plasma osmolarity.

Teratogenic and Toxic Effects of Antimetabolites in the Rat: With Special Reference to 6-Diazo-5-Oxo-1-Norleucine (DON). M. LOIS MURPHY and DAVID A. KARNOFSKY,* New York, N. Y.

Because of the relationship between neoplastic and embryonic tissues, chemicals inhibiting the growth of cancer were studied for their effects on the rat fetus. Drugs were injected intraperitoneally into the pregnant rat at various stages of gestation. Observations were made on teratogenic effects in surviving fetuses and on the ratio of the fetal to maternal LD_{50}.

The embryo implants around the 8th day, organo-

genesis is well-advanced by the 14th day. Embryos were relatively resistant to drugs prior to implantation, and appeared most sensitive to the lethal and teratogenic effects between implantation and the 13th day of gestation.

Radiation and nitrogen mustard are toxic and teratogenic. The ratio of fetal to maternal toxicity is in the range of 1 to 5 and this relationship persists during the major portion of pregnancy. Six amino nicotinamide, a nicotinic acid antagonist, is highly teratogenic at 11 days, but doses lethal to the fetus also cause considerable maternal toxicity.

Antimetabolites which interfere with purine metabolism have exhibited selective injury to the fetus. The 4-amino analogue of folic acid, aminopterin, is maximally active during the 9th to 12th days of gestation and the ratio of fetal to maternal toxicity is approximately 1 to 15. This sensitivity rapidly diminished to the maternal level after the 13th day. The most active agents are o-diazoacetyl-l-serine (azaserine) and 6-diazo-5-oxo-l-norleucine (DON). Azaserine is teratogenic and the fetal-maternal toxicity ratio during the critical period (9 to 12 days) is 1 to 40. DON is more selective; during the same period the fetal LD_{50} is 0.1 mg. per kg. as compared to a maternal LD_{50} of approximately 50 mg. per kg.; a ratio of 1 to 500. Teratogenic effects are rare in the surviving embryos.

The possible mechanisms of action of compounds exhibiting antineoplastic activity and teratogenic and toxic effects on the fetus will be discussed.

The Circulatory Effects of Protoveratrines on Hypertensive Patients With and Without Congestive Heart Failure. GORDON S. MYERS, JAMES H. CURRENS, ALLAN L. FRIEDLICH, and J. GORDON SCANNELL, Boston, Mass. (Introduced by Edward F. Bland).

Protoveratrine is a mixture of two purified alkaloids, protoveratrine A and B, prepared from veratrum album. During cardiac catheterization study 12 hypertensive patients without congestive heart failure received a single intravenous dose of protoveratrine. Maximal fall in systemic blood pressure, slowing of the heart rate and reduction of cardiac index and total systemic resistance usually occurred fifteen minutes after injection of the drug. There was little change in stroke output despite the bradycardia. Lowering of the calculated cardiac index resulted from decrease in oxygen consumption and from a rise in arteriovenous oxygen difference in the majority of cases. Nine additional patients given protoveratrine A or B intravenously showed entirely similar responses. Oral administration of protoveratrine to four patients produced the same effects, the maximal changes in pulse, blood pressure and cardiac index occurring between 2 and 3 hours after ingestion. Previous atropinization did not abolish the characteristic circulatory changes produced by protoveratrine, including a significant decrease in heart rate.

Intravenous protoveratrine given to 9 hypertensive patients with left ventricular failure resulted in striking reduction in pulmonary artery and pulmonary wedge pressures. In contrast to patients free of congestive failure, there was usually a rise in stroke output at the time of maximal fall of blood pressure, pulse rate, cardiac index and total systemic resistance. The improved stroke output of the left ventricle, despite a lower filling pressure, may be the result of the decrease in left ventricular work, with or without a digitalis-like effect of protoveratrine on the failing myocardium. The data are in accord with our clinical experience of the favorable effects of protoveratrine on hypertensive patients with left heart failure.

Immune-Adherence With Poliovirus Hominis. ROBERT A. NELSON, JR.* and JANICE TAVERNE, London, England.

Initial experiments with poliomyelitis virus indicate that virus particles which are sensitized with antibody (Ab) and complement (C') will react in immune-adherence (I-A) with human erythrocytes. I-A is measured by the clumping of erythrocytes after incubation with virus, Ab, and C' for about 60 min. at 37° C.

Assays of I-A have been used to titrate Ab in serum from persons infected with virus and immunized with "inactivated" virus. From limited trials, it appears that the Ab reactive in I-A parallels virus-neutralizing Ab rather than complement-fixing Ab. A provisional technique has been employed for rapid qualitative and quantitative tests for Ab in human and in animal sera.

Estimations of the number of antigenically active viral particles may be made by I-A. From such measurements it is possible to determine the influence on antigenic potency of various methods for "inactivating" live virus. The advantage in economy of time and materials is considerable when compared with the practice of measuring antigenicity by titration of Ab after injection of "inactivated" virus into experimental animals.

While the nature of the linkage of sensitized virus to erythrocytes remains obscure, it is clear that I-A is not concerned with the "receptor sites" on the erythrocyte surface which adsorb spontaneously certain other viruses. Moreover, the clumping of erythrocytes in I-A is brought about by linkages which are distinct from those involved in the well-known agglutination of animal erythrocytes by certain viruses or other soluble antigens.

An Abnormality of Cardiac Myosin Associated with Chronic Congestive Heart Failure in the Dog. ROBERT E. OLSON,* ERIC ELLENBOGEN, HOWARD STERN, and MARIA M. L. LIANG, Pittsburgh, Pa.

Chronic congestive heart failure characterized by edema, ascites, weakness, reduced exercise tolerance, cardio- and hepatomegaly, and elevated end-diastolic filling pressures has been produced in dogs by surgical avulsion of the tricuspid valve and stenosis of the pulmonary artery. Studies of the myocardial metabolism of these animals by cardiac catheterization have shown no defect in oxidative metabolism, and determinations of the high energy phosphate compounds in these myocardia

have revealed no apparent defect in oxidative phosphorylation in hearts of dogs in low-output failure produced in this manner.

In order to study the contractile proteins of normal dogs and dogs in congestive heart failure under physiological conditions, the chests of these animals were opened under Nembutal® anesthesia with maintenance of respiration by intermittent oxygen under positive pressure. The animals were sacrificed by rapid excision of the beating heart, and the whole heart was immediately chilled in iced deionized water. The myosin was extracted and purified by a modified procedure of Szent-Gyorgyi as previously described (Federation Proc., 1955, **14**, 207). Physical-chemical characterization of the respective myosins from normal and failing heart was accomplished by determination of the sedimentation constant and coefficient of boundary spreading in the analytical ultracentrifuge, solubility, partial specific volume, intrinsic viscosity, and ATP-ase activity. From these constants, the molecular weight of normal cardiac myosin was estimated to be about 250,000; whereas, that of myosin isolated from the failing heart appeared to range from 500,000 to 750,000. The data are consistent with the view that an abnormal stable aggregate of cardiac myosin is formed in cardiac failure.

The possibility that these changes in the molecular configuration of myosin in cardiac failure are related to the changes in contractility observed in the failing heart is under intensive study.

Effect of Hydrocortisone and Prednisone on Patients with "Controlled" Congestive Heart Failure. Preliminary Report. ROBERT G. PAGE and A. R. LAVENDER, Chicago, Ill. (Introduced by Wright Adams).

Because of recent reports which have appeared in the literature, it seemed worthwhile to follow the effects of adrenal cortical steroids and their derivatives in patients with congestive heart failure. Several patients have been studied. This report deals with the effects of hydrocortisone and prednisone on water and electrolyte balance in one patient who has been followed over a long period of time.

Our patients are placed on a fixed dietary intake for the entire study and aliquots are analyzed for their sodium, potassium, chloride, nitrogen, and water content at regular intervals. Urine and feces are saved and similarly analyzed. Fecal specimens are pooled for 4-day periods. Blood is drawn at the beginning of these periods and analyzed for serum pH, CO_2, Na, K, Cl, and H_2O content.

In the first experimental period, 160 mgm. of hydrocortisone was given daily for sixteen days. During this time, the patient's weight was constant and the serum electrolytes did not change appreciably. There was a marked retention of sodium and, based on the chloride space, a shift of water from the intra- to the extracellular space. Potassium balance showed no change when corrected for nitrogen. When hydrocortisone was discontinued, there was a marked diuresis of water and sodium most of which occurred on the 3rd, 4th, and 5th recovery days.

Prednisone was given in a dose of 60 mgm. per day for a similar period. This caused a sodium and water diuresis and an increase in intracellular potassium. On discontinuing the drug, the sodium excretion dropped sharply and the weight leveled off. As far as we know these effects of prednisone have not been described.

The response to these drugs emphasizes the possibility of an intrinsic hormonal imbalance in patients with congestive heart failure. Extrinsic hormonal therapy can alter this in a way which may benefit such patients.

The Influence of Intravenous Infusions of Sodium Chloride Solutions on the Renal Excretion of Sodium in Patients with Cirrhosis of the Liver. SOLOMON PAPPER and LAWRENCE SAXON, Boston, Mass. (Introduced by Maurice B. Strauss).

The natriuretic response to acute sodium loading administered as isotonic, hypertonic and hypotonic saline was studied in seven patients with cirrhosis of the liver. Ascites was marked in two patients, moderate in two and minimal in three. The two with marked ascites were gaining weight at the time of study whereas the other five maintained a constant weight.

While taking a diet containing 10 to 20 mEq. of sodium chloride daily, each subject was studied as follows. After a control period of 60 to 100 minutes 338 mEq. of sodium chloride was given intravenously over a 90-minute interval. The salt was given as an 850 mEq. per L. solution on one occasion, 154 mEq. per L. solution on another, and 112 mEq. per L. solution on a third occasion. Observations were continued for 2½ hours after the infusion was completed.

The data confirm unpublished observations of Strauss, Birchard, and Saxon that expansion of extracellular volume with a hypotonic saline infusion often results in increased sodium excretion in edematous patients with cirrhosis of the liver. We have previously reported that in normals there is no significant difference in the natriuretic response evoked by sodium salts infused in various concentrations. The present observations indicate that this also applies to patients with cirrhosis of the liver. Furthermore, the two patients with moderate ascites and two of the three with minimal ascites excreted the salt loads at a rate comparable to the normal, suggesting that, despite the presence of ascites, these "decompensated" patients were able to excrete sodium salts rapidly. Thus salt loads in excess of the abnormal amount already present in edema and ascitic fluid may be handled quite well.

Carbon Dioxide-Induced Dyspnea in a Patient with Complete Respiratory Paralysis. JOHN L. PATTERSON, JR.,* P. FRANKLIN MULLINAX, JR., THOMAS H. BAIN, and JOHN J. KRUEGER, Richmond, Va.

Current theories of the pathogenesis of the symptom "dyspnea" are concerned with: decreased ratio of ventilatory reserve to maximum breathing capacity; increased

work of breathing; dyssynergy of respiratory muscles; increased tone of intercostal muscles; and increased stimulation of the respiratory center (G. W. Wright).

The opportunity of eliminating the factors postulated in all save the last of these theories presented itself in patient R. G. This patient, an intelligent 20-year-old female, suffered complete loss of intercostal and diaphragmatic muscle function from acute anterior poliomyelitis four years previously, without development of bulbar signs. There was no return of muscle function except for minimal sternomastoid and upper trapezius action. Aside from glossopharyngeal breathing, her ventilatory capacity was close to zero.

While in the Drinker respirator, the patient was given air through a mouthpiece, then was shifted to 7 per cent CO_2 in air at a time unknown to her and the observer. Symptoms were timed by blink of the eyes.

In two experiments at normal tidal and minute volumes, disturbing breathlessness was experienced after 1.5 and 4.5 minutes of CO_2 inhalation. The sensation was described as "getting real tired inside my chest"; "felt like it was not going deep enough and . . . if I could only take deep breaths it would go away." In another study, the patient was hyperventilated to a low alveolar pCO_2 (22 mm. Hg). Fifteen minutes of CO_2 breathing raised the alveolar pCO_2 to high normal (44 mm. Hg) but produced no symptoms whatever.

These results suggest that elevation of blood carbon dioxide tension, representing a strong stimulus to the respiratory center, produced the sensation of breathlessness. The findings are consistent with the theory that the respiratory center itself acts as the sensory receptor for "dyspnea," and are incompatible with other current theories.

Studies on the Glutathione Reductase of Leukemic Leukocytes. JACK PENSKY and AUSTIN S. WEISBERGER,* Cleveland, O.

Abnormalities of sulfhydryl metabolism have frequently been implicated in leukocytic disorders. Particle-free extracts of leukemic leukocytes were therefore studied for the presence of enzymes affecting sulfhydryl compounds. Glutathione reductase, an enzyme which reduces oxidized glutathione in the presence of reduced triphosphopyridine nucleotide (TPNH), was demonstrated in these extracts. This enzyme has not previously been demonstrated in leukocytes. In view of the apparent importance of cystine and related compounds in leukocyte metabolism, the effect of these compounds on glutathione reductase was studied.

In the presence of *chemically* produced TPNH, the reduction of oxidized glutathione is accelerated by M/1,000 L-cystine and several related compounds. Some structural requirements for this accelerating effect have been determined. These include a disulfide group and amino groups *alpha* to the sulfur atoms. Thus acceleration occurs with M/1,000 D-cystine, DL-homocystine or *bis*-dithioethanolamine but not with L-cysteine, reduced glutathione, djenkolic acid or *bis*-dithiopropionic acid. A similar accelerating effect by these compounds is obtained with other pyridine nucleotide enzymes of leukocytes, such as lactic dehydrogenase and malic dehydrogenase in the presence of chemically produced diphosphopyridine nucleotide (DPNH). The accelerating effect can be blocked by M/1,000 DL-selenocystine, although this compound does not affect these enzymes directly.

No accelerating effect is produced by these compounds in the presence of *enzymatically* produced TPNH or DPNH. The effect may therefore be due to binding by L-cystine of inhibitory metals or other impurities. Thus, a similar acceleration of glutathione reductase can be obtained in the presence of 4×10^{-5} *M* versene. However, versene does not accelerate DPNH oxidation with leukocytic lactic dehydrogenase. The observed difference in reaction rate between chemically and enzymatically produced TPNH and DPNH may be an important factor in assays involving other pyridine nucleotide enzymes.

On the Significance of Arterial Pressure Pulse Distortion and Hemodynamics. LYSLE H. PETERSON * and P. H. GERST, Philadelphia, Penna.

Systolic pressure in the femoral artery may exceed that in the aortic root by more than 50 per cent. The explanation of this and other distortions of pressure waves in the arterial system is of considerable importance in understanding hemodynamics. Many workers consider that the arterial system functions as a resonating compression chamber ("Windkessel") and that the "central" pressure pulse becomes distorted, during its transmission, by becoming fused with pressure waves reflected from the periphery. The genesis of the Standing-wave hypothesis was an extension of these concepts. There are, however, alternative explanations for the observed distortions involving fundamentally different views of the properties of the arterial system as well factual and theoretical objections to the reflected wave concept. To test such possibilities specific volume pulses have been introduced at various sites within intact, living dogs' arterial systems. Such volume pulses induce pressure pulses whose contour and transmission depend upon the properties of the system. These pressure pulses, when induced within the aortic root and followed peripherally, underwent distortions which were apparently not due to reflected waves. Indeed, pulses, induced within the periphery and followed proximally, became rapidly attenuated and did not reach the aortic root. Since these pulses include many wave frequencies these data imply that: 1. reflected waves do not play a significant role in pulse deformation, 2. the arterial system functions as a conducting ("transmission") line with damping rather than as a resonating system; hence, different wave frequencies would travel at different velocities. Since the central pulse is composed of various harmonics, they would become reoriented and attenuated within the pulse during its transmission, thus deforming the pulse in the manner observed. In our opinion, these results should initiate the exodus of the Standing-wave hypothesis.

The Variability of Extracellular Fluid Space (Sucrose) in Man During a 24-Hour Period. RICHARD E. PETERSON, JAMES J. O'TOOLE, and WALTER M. KIRKENDALL, Iowa City, Ia. (Introduced by James W. Culbertson).

In fifty studies of extracellular fluid space (EFS) with six-hour sucrose infusions considerable fluctuation in that space was noted. Being interested in following EFS at intervals over long periods of time, we felt it important to determine the range and frequency of variation in this space over 24 hours.

Measurements of EFS in the supine position by a standard dilution technique of calibrated-rate sucrose infusions were made in 10 fasting persons without known fluid or electrolyte abnormalities. Despite rigidly controlled sucrose infusion rates (variation less than ± 0.5 per cent) the plasma sucrose level varied within ± 15 per cent of the mean. Drinking water was available as desired. Urine was collected by indwelling catheter and urine flow was maintained at an average of 1 to 2.5 ml. per min. Urine sucrose recovery ranged from 86 to 103 per cent. Estimates of EFS were made at hourly intervals and were corrected for sucrose recovery. Standard error of duplicate measurements show an analytical error for sucrose not exceeding ± 2 per cent.

Fluctuations in EFS from 15 per cent to 60 per cent were observed to occur, often in brief periods. Time of day, fluid ingestion, and body weight changes were unrelated to EFS changes. In 4 studies cardiac output, glomerular filtration rate, and renal blood flow were measured also and no correlation was observed with changes in EFS. During each study fluctuations in renal clearance of sucrose were observed. Such changes in renal excretion accentuate or mask changes in EFS by preventing the equilibration of sucrose between plasma and extravascular fluid.

Many wide fluctuations in EFS were independent of renal sucrose clearance changes and must represent physiologic variations in the space. Such large shifts of fluid imply that striking variations in gastrointestinal tract water, in cellular hydration, or in other body fluid stores may occur during a "steady" state.

An Abnormality in Thyroxine-Binding in Nephrosis. LILLIAN RECANT,* St. Louis, Mo.

Low levels of protein bound iodine have been observed in patients with the nephrotic syndrome. This has been accounted for *in part* by excessive urinary loss of thyroxine associated with the proteinuria. However, other factors must be considered such as alterations in the binding capacity of the nephrotic serum proteins for thyroxine.

This paper is concerned with the study of thyroxine-binding capacity of serum and urinary proteins in 15 nephrotic subjects. Radio-iodine labelled thyroxine (50 micrograms per cent) was incubated with serum and urine *in vitro* and subjected to paper electrophoresis. Radio-thyroxine was localized by the use of a scintillation counter. In the nephrotics a distinctly abnormal binding pattern was noted in that 70 to 100 per cent of the thyroxine was bound in the alpha-2 globulin area. In contrast, both normal and untreated myxedematous subjects bound most of the thyroxine in the albumin fraction. The abnormal binding in the nephrotic does not appear to be due to hypo-albuminemia since normal patterns are observed in the hypo-albuminemias of liver disease and sprue and the abnormal nephrotic pattern is not altered by the *in vitro* addition of crystalline human albumin. Further, nephrotic urinary binding patterns resemble the serum patterns despite the greater proportion of albumin to other protein in the urine.

In serial studies of nephrotic patients before, during and after steroid administration, reversal of these patterns towards normal occurred within 48 hours of onset of therapy. Similar reversals occurred in spontaneous diuresis. These changes were apparent before quantitative change in serum proteins could be measured. The physiological significance of the abnormal binding patterns in nephrotics and the relationship to steroids are under intensive investigation.

The Absorption of Water and Sodium from the Small Bowel of Patients with Nontropical Sprue. RICHARD J. REITEMEIER, JOHN A. HIGGINS, PHILIP R. LEE, and JOHN F. SCHOLER, Rochester, Minn. (Introduced by Eric E. Wollaeger).

The rate of absorption of isotopically labelled water and sodium from the small bowel has been determined during fasting in 14 patients with nontropical sprue. The results obtained have been compared with those derived from similar observations made previously in healthy persons.

The rate of absorption was determined by integration of the rate of appearance of the labelled substance in the arterial blood during its absorption, with its rate of disappearance from arterial blood after its rapid intravenous injection. In the initial tests a mean rate of arterial disappearance, previously determined in healthy persons, was used in the integration. In later tests the accuracy of the method was improved by use of dual isotopes, THO and D_{20}, Na^{22} and Na^{24}, which allowed simultaneous determination of the rates of appearance and disappearance of each pair. One of each pair of isotopes was placed in the small bowel, and at the same time the other was given intravenously. The concentrations of the four isotopes were then determined in samples of blood drawn in quick succession through an indwelling arterial needle.

When the patients were tested during a relapse of their disease, the rate of absorption of both water and sodium was abnormally slow. During remissions the rates approached, or became equal to, those of healthy persons.

Administration of cortisone for 2 to 4 weeks to two of the patients did not significantly alter the retarded rates of absorption of sodium or water, although the general condition of the patients was improved thereby.

Inhibition of Aldosterone Secretion by Amphenone in Man. ALBERT E. RENOLD, JEAN CRABBE, ERIC J. ROSS, LUIS HERNANDO-AVENDANO, DON H. NELSON, and GEORGE W. THORN,* Boston, Mass.

Amphenone (1,2-bis-(p-aminophenyl)-2-methylpropan-one-1) has been previously shown to alter the secretion of adrenal cortical hormones (Hertz et al., Rec. Progress in Hormone Research *XI*–1955). The inhibitory effect of the compound on the secretion of glucocorticoids has been clearly demonstrated in animals and now in man. A study of amphenone in fourteen patients has shown that sodium diuresis occurs during the period of administration in patients maintained on a restricted intake of sodium. The sodium diuresis was maintained for 24 hours after discontinuation of amphenone administration and was then followed by a striking "rebound" retention of sodium. In an adrenalectomized patient no sodium diuresis or "rebound" retention occurred. Urinary aldosterone was measured in five patients by a modification of the chemical method of Neher and Wettstein. A marked decrease in urinary aldosterone levels during amphenone therapy was followed by a "rebound" increase 48 hours following withdrawal. The effect of amphenone on aldosterone secretion was also measured in a hypertensive patient with metastatic adrenal cortical carcinoma. Control levels were found to be markedly elevated (200 microgm. per day) and a profound decrease followed amphenone administration. These and other studies suggest that the effect of amphenone is primarily concerned with the inhibition of biosynthesis or release of aldosterone by the adrenal cortex.

Studies on the Persistence of Staphylococcal Bacteremia in Rabbits. DAVID E. ROGERS, New York, N. Y. (Introduced by Walsh McDermott).

Previous studies in this laboratory have shown that pathogenic staphylococci remain viable within the cytoplasm of living leukocytes. Recent observations indicate that the intracellular residence of staphylococci may play an important role in the persistence of staphylococcal bacteremia in rabbits.

When staphylococci were injected into the veins of rabbits, persistent bacteremia invariably followed the phase of rapid blood stream clearance. Differential blood cultures obtained from hepatic and peripheral veins revealed that the protracted bacteremia was not due to saturation of known removal mechanisms.

The present studies indicate that injected staphylococci are rapidly phagocyted by circulating polymorphonuclear leukocytes *in vivo*. Some staphylococci in the injected microbial population survive within the cytoplasm of granulocytes which remain in the circulating blood. This intracellular location appears to protect staphylococci from removal in the reticuloendothelial system.

Animals rendered granulocytopenic with nitrogen mustard cleared greater numbers of injected staphylococci from the circulation than animals with normal numbers of circulating leukocytes. When some of the injected staphylococci were incorporated within homologous polymorphonuclear leukocytes *in vitro* before administration to rabbits, such "intracellular" staphylococci were not removed from the blood stream as effectively as unphagocyted microorganisms.

Equal numbers of staphylococci and pneumococci were also injected simultaneously into the veins of rabbits. Pneumococci did not survive within circulating leukocytes, and these microorganisms were completely removed from the circulation during a 90-minute period. In contrast, virtually all of the viable staphylococci remaining in the circulation at 20 minutes were contained within granulocytes, and staphylococcal bacteremia persisted for many hours.

These studies suggest that the phagocytosis of living staphylococci, and the subsequent transport of the microorganisms within the cytoplasm of leukocytes, may account for the initial persistence of staphylococcal bacteremia in rabbits. This phenomenon may have important bearing on the persistence of bacteremia due to certain other microorganisms.

Circulatory Changes Due to Hypothermia in Anesthetized Man. JOHN C. ROSE, THOMAS F. MCDERMOTT, LAWRENCE S. LILIENFIELD, FRANK A. PORFIDO, and ROBERT T. KELLEY, Washington, D. C. (Introduced by Laurence H. Kyle).

The increasing use of hypothermia as an adjunct to general anesthesia demands that its hemodynamic effects in *man* be completely understood.

Eight patients without evident cardiovascular disease, undergoing thoracic or abdominal surgery for malignancy, were cooled to between 30.5 and 32° C (rectal telethermometer) by packing in ice. Following the induction of anesthesia, but before cooling, first circulation indicator-dilution curves were obtained by sampling femoral arterial blood after peripheral vein injection of radioiodinated albumin. Direct pressure pulse contours were recorded from the femoral artery. After cooling, before surgery, these procedures were repeated. The period of cooling (average 2 hours) was kept as free as possible of other influences such as drugs and fluids. Shivering did not occur in any patient.

In all eight cases, cardiac output fell (mean 25 per cent); mean circulation time was prolonged (mean 53 per cent), and heart rate decreased (mean 29 per cent). Hematocrits were increased (mean 7.5 per cent), and, in four patients studied, plasma volumes were reduced. Central blood volume (Hamilton) changes were variable.

Femoral arterial pressure pulse contours consistently showed prolonged systole with a slowly rising anacrotic limb. Mean arterial pressure rose in 5 cases and fell in 3 (range + 72 per cent to − 25 per cent). Total peripheral resistance alterations were inconstant, increasing in 6, decreasing in 2 (range + 158 per cent to − 23 per cent). Cardiac work was increased at low temperatures in 3, decreased in 5 (range + 36 per cent to − 62 per cent). Evidently, in this temperature range, homeostatic vaso-

pressor reflexes are responsible for variable circulatory adjustments.

The results, thus far, are not wholly in agreement with data obtained in animals. The discrepancy may be partly explained by the inability to completely control the effects of premedication and anesthetic agents. However, the data indicate that the clinical employment of this degree of hypothermia is not attended by uniform hemodynamic effects.

The Influence of Alcohol Ingestion upon Maintained Water Diuresis. JACK D. ROSENBAUM,* SOLOMON PAPPER, and HENRY W. COHEN, Boston, Mass.

Previous studies have demonstrated that when a large water load is maintained in the seated normal subject the initial high rate of urine flow usually declines moderately. The declining rate of flow is not associated with a fall in endogenous creatinine clearance but has been related to the concomitant decrease in excretion of solute, particularly sodium. Further observations indicate that under these conditions there is a small decrease in osmolar clearance with a much greater fall in free-water clearance and a tendency for urine osmolarity to rise slightly. The possibility that small amounts of antidiuretic hormone (ADH) are released in such experiments has not been excluded.

Since alcohol administration inhibits ADH production, the effect of ingestion of whiskey during water diuresis was examined in seven essentially healthy men. A water load of 20 ml. per kilo was established and maintained in each subject. When urine flow had declined each drank approximately 60 ml. of alcohol in the form of whiskey. In four subjects there was no augmentation of flow or fall in urine osmolarity (corrected for alcohol content). In the other three subjects the imbibition of alcohol was followed by a slight rise in flow to levels below the initial maximal rates with a fall in corrected urine osmolarity and without a rise in osmolar clearance (corrected for alcohol). It is inferred that the characteristic decline in diuresis is not dependent upon ADH action. However, despite maintenance of a large water load, inapparent small amounts of ADH may be secreted so that it is difficult to determine whether a state of complete "physiologic diabetes insipidus" exists.

Plasma Albumin Deficiency: The Cause of Hyperlipemia and Hypercholesteremia in Experimental Nephrosis. RAY H. ROSENMAN, SANFORD O. BYERS, and MEYER FRIEDMAN,* San Francisco, Calif.

The hyperlipemia and hypercholesteremia occurring in experimental nephrotic rats have been found to be due to the loss of plasma albumin also occurring in this syndrome.

This has been demonstrated in three different experiments. (1) When the *external* loss of urine was prevented by ureteral ligation or ureteral-vena caval anastomosis in rats injected with nephrosis-producing anti-kidney serum (AKS), no significant hyperlipemia and hypercholesteremia occurred despite development of the usual renal changes induced by AKS injection. (2) Artificial maintenance of normal plasma albumin levels by continuous intravenous infusion of plasma albumin was found to prevent the rise of plasma lipid and cholesterol in rats injected concomitantly with AKS, and to rapidly lower elevated plasma lipid and cholesterol of rats injected previously with AKS. The subsequent disappearance of the infused albumin from the circulation was associated with a rise of plasma lipid and cholesterol to levels of the control nephrotic rats. (3) Artificial reduction of the plasma albumin (plasmaphoresis) prior to injection of AKS resulted in augmentation of the hyperlipemia and hypercholesteremia, as compared to the controls.

In additional experiments the anti-hyperlipemic and anti-hypercholesteremic properties of administered albumin were found to be markedly enhanced by concomitant administration of heparin.

The possible relationship of plasma albumin deficiency to interference with lipolysis and the latter's hypercholesteremic effects will be discussed.

Decreased Permeability of Capillaries to Protein in Chronic Congestive Heart Failure. RICHARD S. ROSS and W. GORDON WALKER, Baltimore, Md. (Introduced by Gilbert H. Mudge).

Plasma disappearance curves of I^{131} labelled human serum albumin have been constructed from observations extending over a fourteen-day period following single intravenous injections into human subjects. A "mean transcapillary exchange rate" can be obtained by assuming that the fall in concentration with time during the first few days results from both the transcapillary equilibration of the labelled material with the extravascular protein pool and the metabolic degradation of the protein. In eleven normal individuals the "mean transcapillary exchange rate" is more closely correlated with the mass of circulating protein ($r = +.7$, $p < .001$) and the plasma volume ($r = +.6$, $p < .02$) than with the serum protein concentration. Capillary permeability to protein increases with a rise in the plasma volume and the total mass of circulating protein. Thus, changing permeability serves to maintain the plasma volume within relatively narrow limits despite wide variation in the total mass of exchangeable protein. In the group of normal subjects, an eight-fold variation in total exchangeable protein is accompanied by only a three-fold variation in plasma volume. In sharp contrast are the findings in the group of seven patients with congestive failure. In this group the relationship between "mean transcapillary exchange rate" and plasma volume is reversed ($r = -.6$), the larger the plasma volume the smaller the exchange rate. A significant decrease in the fraction of the total protein which is extravascular ($p < .01$) seems to be a consequence of this decrease in permeability. Thus it appears that the decreased permeability of the capillaries to protein maintains the expanded plasma volume of congestive heart failure.

The Metabolic Role of Vitamin B_{12} in Pernicious Anemia. R. WAYNE RUNDLES * and SPENCER S. BREWER, JR., Durham, N. C.

The function of vitamin B_{12}, and related enzymes, has been studied by observing the effects of metabolites related to the biosynthesis of nucleic acid when given to patients with pernicious anemia in relapse.

Orotic acid, a non-methylated pyrimidine precursor, was given to 3 patients. One with post-gastrectomy disease, initial RBC 2,000,000, was given 3 gm. daily by mouth. A reticulocyte peak of 30 per cent occurred on the 13th day. A second individual with an initial RBC of 1,480,000 treated similarly had a reticulocyte peak of 13.2 per cent on the 13th day. A second peak occurred after the orotic acid was increased to 6 gm. per day for 1 week. A satisfactory clinical and hematologic remission followed in both cases. A third patient had a slight reticulocytosis when given 0.5 gm. of orotic acid per day intravenously. A greater, but still suboptimal, response was then produced by the administration of 6 gm. daily by mouth.

Two patients were maintained on orotic acid for 5 and 8 months, respectively, when they had a slow return of macrocytic anemia. There was no evidence of neurologic disease, marrow hypocellularity, *etc.* The addition of methionine at this time was of no benefit, and there was little or no response to ureidosuccinic, uridylic and cytidilic acids.

In a patient with nutritional macrocytic anemia, orotic acid produced no hematologic response. Thymidine alone gave a slight effect. With inosine in addition, there was a sharp but unsustained reticulocytosis.

The above observations indicate that the major consequence of vitamin B_{12} deficiency in the human is a defect in general pyrimidine synthesis, or incorporation, rather than in one-carbon compound metabolism, or pyrimidine nucleoside methylation, as commonly thought.

Sulfation Factor, a Serum Component Mediating the Action of Growth Hormone in Stimulating Incorporation of Sulfate into Cartilage. WILLIAM D. SALMON, JR. and WILLIAM H. DAUGHADAY,* St. Louis, Mo.

The importance of the pituitary growth hormone in regulating the synthesis of chondroitin sulfate by cartilage has been established in rats. The action of growth hormone on this synthetic process has been investigated by incubating isolated rat cartilage with S^{35} sulfate. The uptake of S^{35} by costal, nasal, and xiphoid cartilage measured after 24 hours of incubation was halved by hypophysectomy. Twelve hours after injection of 0.5 mg. growth hormone into hypophysectomized (hypox) rats, uptake of S^{35} by costal cartilage was restored to normal. Addition of growth hormone to the incubation medium in the presence or absence of hypox rat plasma did not increase S^{35} uptake by hypox cartilage.

Incubation of hypox cartilage with normal rat serum, however, more than doubled the sulfate uptake as compared to cartilage incubated in hypox rat serum. The component of normal serum which increases uptake of sulfate by cartilage has been called *sulfation factor.* A progressive increase in the rate of S^{35} uptake was observed during 18 hours when hypox cartilage was incubated with factor-containing serum. Stimulation of uptake occurred even when normal serum was diluted 1 to 70 with phosphosaline buffer. *Sulfation factor* activity appeared in the plasma of hypox rats 6 hours after the injection of growth hormone and was greatest 12 hours after injection.

The properties of *sulfation factor* and its relation to known hormones are incompletely known. The effects observed cannot be attributed to changes in serum inorganic sulfate. Considerable activity remained after extensive dialysis. L-Thyroxine was inactive *in vitro.* Insulin increased S^{35} uptake by hypox cartilage but not by cartilage from hypox rats given growth hormone. The insulin effect is distinct from that due to *sulfation factor* because even with 1.0 unit per ml. insulin the stimulation of S^{35} uptake was much less than with *sulfation factor.*

Fatigue of the Sweat Glands in Heat Stroke. IRVING L. SCHWARTZ and SHINJI ITOH, New York, N. Y. (Introduced by Vincent P. Dole).

In normal humans thermal regulation is achieved by a delicate balance between chemical heat production and physical heat loss.

When environmental temperature is below body temperature and body heat production is normal, the major heat loss occurs through radiation, conduction and convection from the skin. These mechanisms are inoperative when environmental temperature equals body temperature and act adversely when environmental temperature is higher than body temperature. Under the latter circumstances the only significant mechanism for heat dissipation is evaporation of sweat from the skin surface.

Recent studies have shown that, during prolonged exposure to heat and following intradermal injection of cholinergic drugs, the rate of sweat production decreases because of failure of the secretory mechanism of the glands.

The time required for functional restoration of the fatigued glands was measured by collecting sweat following each of two consecutive intracutaneous injections of Mecholyl (2 mg.) given at successively lengthening time intervals into the same skin site. In normal unacclimatized subjects the time required for full recovery of glandular function was found to be 6 hours. Acclimatization reduced the recovery time by approximately one-half, provided that the acclimatizing procedure did not produce clinical heat retention.

In those instances in which heat retention was induced experimentally, and in patients with heat stroke, the response to the initial injection of Mecholyl was subnormal by 50 to 100 fold and the time required for full recovery of glandular function exceeded 24 hours.

It is concluded that fatigue of the sweat glands and retarded restoration of secretory function are prime fac-

tors in the induction and progression of the clinical syndrome of heat stroke.

The Effect of Diet on Potassium Deficiency and Acid-Base Balance. ROBERT SCHWARTZ and JOHN CRAIG, Boston, Mass. (Introduced by Charles A. Janeway).

Clinically potassium deficiency unrelated to alkalosis has been observed in renal disease, diarrheal states, and diabetes mellitus. In animal studies with potassium deficient diets, the original Darrow hypothesis related change in muscle cellular potassium and sodium to change in plasma bicarbonate. More recently, a hydrogen-potassium exchange (cell K^+ replaced by H^+) has been invoked to explain the metabolic alkalosis of potassium deficiency. Since in the above experiments, a potentially alkalinizing diet was administered, observations were made of the effects of a non-alkaline ash diet upon the development of potassium deficiency.

Three groups of 125-gm. male rats were pair fed a diet containing a standardized salt mixture ($CaCl_2$, $CaHPO_4$, $MgSO_4$, and trace minerals) for two weeks. The experimental group (6 animals) received the above without sodium or potassium. Another group (9 animals) received a $NaHCO_3$ supplement (300 mM per Kg. diet), but no K. The control group (9 animals) received $NaHCO_3$ and $KHCO_3$ 150 mM per Kg. diet each. The Na-K deficient group ceased growth immediately and became polyuric within four days. Serum analysis indicated a metabolic acidosis (decreased pH and CO_2 content) and hypokaliemia. Muscle showed decreased cellular potassium with increased sodium, although no exogenous sodium or potassium was available in this group. The potassium deficient, sodium bicarbonate loaded group gained weight slowly and had the typical hypokaliemic, hypochloremic metabolic alkalosis with diminished muscle potassium as previously described. Metabolic alkalosis characterized K deficiency only if the diet contained $NaHCO_3$; without dietary $NaHCO_3$ metabolic acidosis occurred. In the latter instance no relationship between extracellular pH, pCO_2, or HCO_3 and intracellular K was evident, although the extent of cellular cation deficit was identical in both groups. The data do not preclude the exchange of cellular K^+, H^+, and Na^+; however, with Na and K deficiency it is evident that such a simplified hypothesis alone fails to explain the observations.

Studies on the Mechanism of a Sodium-Losing Syndrome in Two Patients with Mediastinal Tumors. WILLIAM B. SCHWARTZ,* WARREN BENNETT, SIDNEY CURELOP, and FREDERIC C. BARTTER,* Boston, Mass., and Bethesda, Md.

Sodium-losing syndromes associated with pulmonary and central nervous system disorders have been described repeatedly, but no adequate explanation of mechanism has been proposed.

In the present studies, two patients with mediastinal tumors have been observed showing (1) progressive, severe hyponatremia associated with urinary sodium loss, not accompanied by weight loss, despite high sodium intake, (2) sustained hypertonicity of the urine in the face of steadily decreasing tonicity of the serum, (3) normal to high glomerular filtration rate and renal plasma flow and no clinical evidence of renal disease, (4) no clinical or laboratory evidence of impairment in adrenal cortical function, and (5) partial response to desoxycorticosterone or fluorohydrocortisone with sodium retention and rise of serum sodium concentration.

Both subjects responded to water deprivation with return of serum sodium to normal. In one subject, where appropriate studies were possible, it was shown that this was a result of renal sodium conservation. After infusion into this subject of saline slightly hypertonic to his serum, a brisk diuresis of markedly hypotonic urine ensued.

The finding of a hypertonic urine with a normal glomerular filtration rate may be taken as evidence for the continued production of antidiuretic hormone (ADH). It is unlikely that this was due to a lowered threshold for stimulation of the osmoreceptors, since hypertonicity of the urine was observed even when plasma osmolality was reduced to nearly 200 mOsm. In the absence of evidence of volume depletion, it is proposed that some other stimulus to ADH production could best account for the findings in the present patients. Exactly analogous sequelae are known to follow in normal subjects whose fluid volume is expanded by the administration of exogenous antidiuretic hormone and water. It is suggested that in the present subjects the disease process resulted in an inappropriate stimulus to continuous ADH production, that excess of some critical body fluid volume resulted and that this in turn led to sodium-wasting.

Application of a New Quantitative Method for Studying the QRS Complex of the EKG in Cardiac Patients. GEORGE E. SEIDEN, Philadelphia, Pa. (Introduced by Francis C. Wood).

Based on studies of body surface potential distribution in human-shaped models equipped with a variable location current dipole, and on cancellation experiments performed in this laboratory indicating the applicability of these results to cardiac patients, a seven-electrode lead system was devised by Frank, capable of rendering reasonably accurate orthogonal QRS components. For dipole location within a 5 cm. × 5 cm. area at midventricle level (representative for 90 per cent of forty cardiac patients whose dipole locations were determined experimentally), the lead vectors of this system are accurate to plus minus 5 degrees in angle and 20 per cent in length in torso models.

This lead system was employed to study 150 assorted cardiacs and normals, and found to be practical, requiring about 15 minutes per subject. A protractor was used for placement of 5 electrodes on the thorax at dipole level, as determined by a mirror pattern technique. The remaining two electrodes were placed on the base of the right neck posteriorly and on the left leg. Potential differences were processed by Frank's resistive adding network to yield three relatively orthogonal dipole com-

ponents of equal normalization, any two of which could be displayed after suitable amplification as a vector loop projection.

Besides expected theoretic advantages of relative invulnerability to body shape and dipole location parameters, good performance was observed concerning muscle tremor and 60 cps interference, since resultant components were usually larger than those for other currently used systems.

Three dimensional wire loops were made for each case from 2 of the 3 loop projections, and checked for accuracy against the third. The great majority of QRS loops tended to be planar, indicating the feasibility of use of an electronic resolver to present edgewise and broadside oscilloscopic views of loops, resulting in a technique suitable for more quantitative clinical electrocardiography than heretofore possible.

Studies on the Activation of Human Plasminogen (Profibrinolysin). SOL SHERRY * and NORMA ALKJAERSIG, St. Louis, Mo.

Based on the development of new techniques, studies have been conducted on the activation of highly purified human plasminogen (profibrinolysin), the precursor of the naturally occurring proteolytic enzyme of plasma (plasmin or fibrinolysin). Three types of activation have been investigated; a) spontaneous, b) urokinase, and c) streptokinase. Plasmin was assayed by the hydrolysis of synthetic amino acid esters and by casein proteolysis.

Studies on the spontaneous activation of plasminogen were carried out by incubating plasminogen in the presence of 50 per cent glycerol at neutral pH. The glycerol acted as a stabilizing agent for plasminogen and plasmin, and allowed the activation to proceed to completion without loss of active enzyme. Evidence has been obtained that an acid soluble fragment is split from plasminogen during the activation. As a result of these experiments, highly purified plasmin preparations, free of plasminogen, were obtained for an investigation of the physical and biochemical properties of human plasmin.

Urokinase was prepared from normal human urine according to von Kaulla, and was shown to catalytically convert plasminogen to plasmin.

The true kinetics of plasminogen activation by streptokinase could not be studied unless the streptokinase was removed from the activation system prior to the assay of plasmin. When this was accomplished by quantitative precipitation of the plasmin, free of SK, by 1M NaCl at pH 2.0, the catalytic nature of the activation of plasminogen by streptokinase could be precisely described. The plasmin isolated following activation by urokinase or streptokinase had the same properties as spontaneously activated plasmin. Evidence was obtained that streptokinase is capable of interacting with human plasmin to form a reversible complex with somewhat different properties than plasmin. The streptokinase-plasmin complex is more strikingly adsorbed on fibrin than plasmin alone, accounting, in part, for the enhanced fibrinolytic activity of human plasmin in the presence of streptokinase.

The Role of a New Anticoagulant Proteolytic Inhibitor in Blood Coagulation. N. RAPHAEL SHULMAN, Bethesda, Md. (Introduced by Theodore J. Abernethy).

Purification of a proteolytic inhibitor with anticoagulant activity, a protein of molecular weight 17,000, from normal human plasma and urine was recently reported from this laboratory. It acts on a prothrombin derivative which forms during the conversion of prothrombin to thrombin and its anticoagulant effect depends on the *rate* of prothrombin conversion regardless of which conversion factor determines the rate. Trypsin can neutralize the anticoagulant effect by binding the inhibitor.

The inhibitor accounts for less than 5 per cent of total plasma proteolytic inhibition but this amount is effective in coagulation. It can be considered as a buffer in coagulation, tending to maintain fluidity of blood by preventing thrombin formation at slow rates of prothrombin conversion which might occur intravascularly but not interfering with thrombin formation as conversion accelerates extravascularly. It may account in part for the lag-phase seen whenever blood clots and for the paradoxical shortening of clotting time when normal or even hemophilic plasma is diluted.

Tests done on blood of a female patient with a prolonged clotting time and normal values for all known coagulation factors suggest that excessive proteolytic inhibitor anticoagulant may cause a hemorrhagic disorder. Abnormalities observed in the patient's blood could be reproduced in normal blood by addition of the purified inhibitor but not by addition of other anticoagulants such as heparin or lipid antithromboplastin. The patient's anticoagulant was non-dialyzable and stable at 60° as is the purified inhibitor. Trypsin added to the patient's blood corrected the defect completely *in vitro*. Intravenous crystalline trypsin in large amounts shortened the patient's clotting time and improved prothrombin consumption without altering plasma prothrombin, fibrinogen or accelerator concentration. Some patients who have been considered to have a circulating antithromboplastin may represent instances of excessive proteolytic inhibitor anticoagulant. This possibility should be borne in mind in considering approaches to therapy.

Body Composition and Blood Volume in Normal and Disease States. WILLIAM E. SIRI, Berkeley, Calif. (Introduced by John H. Lawrence).

The gross body constituents, fat, water protein and mineral, are important biological variables in both health and disease, partly because of profound alterations associated with many disease states and in part for their interrelation with other factors such as blood volume and metabolic rate. Although such studies in patients have long been hampered by technical obstacles, the difficulties are largely circumvented by a new method for measuring corporal density in which the displacement of helium by the body volume is measured within a closed chamber. Combined with total body water determined with tritium, accurate estimates of body constituents are now secured quickly with little inconvenience to subjects.

With these techniques and P^{32}-labeled red cells, body composition and blood volumes were measured and intercorrelated in a heterogenous group of 300 normal persons and about 150 patients. The normal subjects, who included men and women from 20 to 80 years, and ranging from extreme leanness to frank obesity, provided a measure of the mean values and dispersion in composition and blood volume for normal persons. For this group, red cell volume averaged 36 ml. per Kg. of fat-free weight, with an additional 4 ml. for each Kg. of adipose tissue. Plasma volume however appears better correlated with total body water with a mean of 72.6 ml. per liter. The potential value of body composition to investigation of disease is illustrated in studies of groups of patients with polycythemia and leukemia. In neither disease does red cell volume correlate with body constituents, but a clearer differentiation from normal values is possible on the basis of fat-free weight. Plasma volume in polycythemia is uncorrelated, but in leukemia it is normally correlated with total water. Other groups investigated included adrenalectomy and/or hypophyseal irradiation patients with carcinoma of the breast, some of whom were studied periodically to observe progressive changes in constituents and blood volume. Similar studies were performed on smaller groups with various carcinomata and also with endocrine dysfunction. The latter are of particular interest in that observed alterations in body composition in some metabolic disorders are not in accord with conventional concepts.

Staphylococcal Septicemia: The Importance of Host Factors. J. M. SMITH and A. B. VICKERS, Iowa City, Ia. (Introduced by W. M. Fowler).

The cases of staphylococcal septicemia seen at the State University of Iowa Hospital between the years of 1936 and 1955 have been examined regarding the factors involved in survival after treatment. This study has also brought to light several host factors which are of importance in the initiation of the infection. For instance disease occurs in males more than females in the ratio of more than 2 to 1. The most commonly affected and spared age groups have been defined. Autopsy showed the lungs and kidneys to be most often infected with a difference in the two sexes. Studies have been made of the fatality effect of the various lesions. It was noted that creatinines and ureas were most often abnormal in fatal cases and it has been deduced that kidney malfunction contributes to death. This series showed that a hemolytic staphylococcus aureus was the commonest cause of the disease but that cases were caused by staphylococcus albus and of these cases some were fatal. In this series diabetes in the patient or family were predisposing factors. Cancer and leukemia likewise were predisposing factors. Eighteen per cent of the cases followed a prostatectomy operation. The primary focus of the disease had a bearing on the eventual mortality. Blood cultures may be intermittently positive only. In diagnosis the chest x-ray and intravenous pyelograms and kidney function tests are all helpful. Nasal swabs are also important in cases which have been treated elsewhere previously. Prognosis is unfavorable when there are a large number of staphylococci in the blood or when the maximal white cell count is low. It is favorable when there is maximum temperature of less than 100°. Treatment is most favorable when penicillin is used in large doses. Surgery also has a place in treatment.

Inhibition of Pituitary Activity Following Administration of Derivatives of Pituitary Preparations. MARTIN SONENBERG* and WILLIAM L. MONEY, New York, N. Y.

Derivatives of anterior pituitary preparations, based on functional groups of proteins, were prepared. These were tested in chicks for stimulatory and inhibitory properties on endogenous and exogenous thyrotropin and gonadotropins. Three preparations, involving amino group substitutions, were found to have a marked effect on thyrotropic and gonadotropic activity of the original preparation. These substituted preparations also had the ability to inhibit thyrotropic but not gonadotropic activity.

An acetylated preparation, prepared with acetic anhydride at 2° C, yielded a preparation with 1.64 per cent acetyl groups. Amino groups, determined by the nitrous acid reaction, were almost completely blocked. The administration of such materials in doses approximately 10 times normal amounts of unmodified pituitary preparations, resulted in a complete loss of gonadotropic and thyrotropic activity as measured by gonad and thyroid weights and thyroidal I^{131} uptake. Such preparations apparently inhibited endogenous thyrotropic pituitary activity since the measured end points were significantly less than in untreated controls. Following the simultaneous administration of acetylated preparations and exogenous pituitary material, there was inhibition of thyrotropic but not gonadotropic activity. Control substances including acetic acid, acetylated bovine serum albumin or other pituitary derivatives, with or without unmodified pituitary material, showed no effect on thyrotropic or gonadotropic activity.

Anticoagulant Effect of Excess Platelets. THEODORE H. SPAET, New York, N. Y. (Introduced by Robert G. Bloch).

In the thromboplastin generation test concentrations of platelets above the optimal range display anticoagulant activity. Platelet lipid was prepared from acetone-dried human platelets by chloroform extraction. The residue obtained after evaporation of the chloroform behaved like whole platelets in the thromboplastin generation test in that it promoted thromboplastin generation at optimal concentrations but was anticoagulant when highly concentrated. Neither coagulant nor anticoagulant properties of platelet lipid were affected by prolonged dialysis against saline, adsorption with barium sulfate, or heating to 100° C for 10 minutes.

When added in the early stages of the thromboplastin

generation test, the platelet anticoagulant caused retarded thromboplastin formation; when added later in the test, it interfered with the action of formed thromboplastin. The platelet anticoagulant also inhibited the action of tissue thromboplastin in the 1-stage prothrombin determination, but it failed to affect the action of thrombin on plasma.

The anticoagulant was neutralized by preparations containing a great excess of labile factor. Preparations of concentrated stable accelerator, antihemophilic globulin, plasma thromboplastin component, or calcium were ineffective.

When platelets of patients with thrombocythemia were used in the thromboplastin generation test, they were inhibitory at circulating levels. Dilution of thrombocythemic platelets to normal concentrations produced normal activity.

It is concluded that excess platelets interfere with the action of labile factor in both thromboplastin formation and prothrombin conversion. This anticoagulant effect may be partly responsible for the hemorrhagic manifestations of patients with thrombocythemia.

Studies on Receptor-Effector Mechanisms Influencing Renal Hemodynamics and Water-Sodium Exchanges in Intact, Diabetes Insipidus, Ascitic and Renal-Denervated Dogs. J. STAMLER,* L. DREIFUS, E. MARCUS, S. WONG, E. E. HASBROUCK, J. T. SHERIDAN, K. MAIER, and A. ELLIS, Chicago, Ill.

Previous work in this department led to the conclusion that altered renal sodium-water handling in edema-forming states occurs in response to changes in total organism fluid homeostasis. Function of the kidneys changes under the influence of multiple receptor-effector arcs, nervous and humero-hormonal, arising extrarenally. The present studies were undertaken to investigate possible receptor-effector mechanisms. In one series of experiments, it was demonstrated that intact, diabetes insipidus (DI) and renal denervated unanesthetized dogs exhibited a similar water-sodium diuresis in response to sustained infusion of Ringer's solution (10 cc. per min.). Further, all three Ringer's-"loaded" preparations manifested a similar prompt increment of water-sodium diuresis following rapid injection of an additional 200 cc. of Ringer's solution at various sites in the circulation. Rapid injection of 200 cc. isotonic glucose solution also consistently enhanced H_2O, but not Na, diuresis. Ringer's-loaded ascitic dogs also exhibited a qualitatively normal enhanced diuresis following rapid infusion of 200 cc. of Ringer's or glucose. Thus, mechanisms effectively operate in Ringer's-loaded dogs to distinguish and rapidly respond to water-sodium *vs.* water sudden expansions of plasma-extra-cellular fluid volume; these mechanisms continue to function in diabetes insipidus, renal denervated and ascitic animals. In other experiments, rapid removal of arterial blood (100 cc.) was shown to effect a transient depression of H_2O-Na diuresis in Ringer's-loaded intact dogs. This response was enhanced in DI animals; it was apparently suppressed by renal denervation and by slow bleeding. In a third series of experiments, constriction of the carotid arteries of Ringer's-loaded, intact, unanesthetized dogs was shown to effect a prompt, sustained increase in Na-H_2O diuresis. These findings elucidate certain theoretical problems of renal function in edema-forming states.

Preliminary Report on the Effects of a Plasma Lipid Mobilizing Factor in Man. WILLIAM A. STEIGER, CHRIS J. D. ZARAFONETIS, GLADYS M. MILLER, JOSEPH SEIFTER, and DAVID BAEDER, Philadelphia, Pa. (Introduced by Richard A. Kern).

Seifter and Baeder recently reported the isolation of a naturally occurring plasma fraction from man and several animal species which has potent lipid mobilizing effects. Injections of large doses in animals resulted in hyperphagia and weight loss with depletion of the usual body fat depots in addition to a marked hyperlipemia. Preliminary studies of the effects in man of this lipid mobilizing fraction (LMF) confirms the hyperlipemia which occurs in animals following injection.

Twelve patients received a single intravenous injection of 0.25 to 1.0 mg. per Kg. of LMF. Plasma concentrations of total cholesterol, total fatty acids, and lipid phosphorus were approximately doubled following injection of LMF. Maximum values were obtained 2 to 3 hours after injection, and the elevations persisted for at least 24 hours. Average total cholesterol rose from 231 mg. per cent to 438 mg. per cent at 3 hours, average total fatty acid rose from 306 mg. per cent to 584 mg. per cent, and the average lipid phosphorus rose from 6.90 mg. per cent to 13.28 mg. per cent. There was no change in the lipid phosphorus: cholesterol ratio.

Two patients received LMF daily for 5 and 14 days, respectively. The fasting lipid levels continued to rise daily during the course of treatment, and they remained elevated 2 to 3 times above control values 5 days after injections were discontinued. The maximum values obtained were on the eighth day 2 hours after injection when the total cholesterol was 906 mg. per cent, total fatty acid was 1,058 mg. per cent and lipid phosphorus was 19.55 mg. per cent. Measurements of eosinophil counts, clotting times, thyroid I_{131} uptake, BMR and a properdin-like material will be reported.

Radiothyroxine Turnover Studies in Myxedema, Thyrotoxicosis, and Hypermetabolism without Endocrine Disease. KENNETH STERLING * and ROBERT B. CHODOS, Syracuse, N. Y.

The rate of peripheral degradation of circulating thyroid hormone was studied with I^{131}-labeled l-thyroxine in normal subjects, patients with thyroid disease, and hypermetabolic patients without endocrine disease (leukemia, fever). After intravenous injection of a tracer amount of radiothyroxine the rate of disappearance of plasma radioactivity was determined. The slow exponential component of the disappearance curve was interpreted as the rate of metabolic degradation.

The extrathyroidal organic iodine (EOI) pools calculated by the isotope dilution principle were diminished in myxedema and increased in thyrotoxicosis, as compared with the normals. The turnover rates of extrathyroidal hormone obtained from the half-times of disappearance were slower than normal in myxedema, and accelerated in thyrotoxicosis. The absolute organic iodine degradation rates, being the products of the pools and turnovers, showed even wider divergences between the different groups. The mean values ± standard deviation in micrograms of iodine per day (adjusted to 1.73 m² surface area) were as follows:

Normal	51 ± 9
Myxedema	18 ± 4
Thyrotoxicosis	197 ± 35
Hypermetabolism	100 ± 18

The hypermetabolic subjects without endocrine disease had increases in pools, turnovers, and degradation rates, although less pronounced than in thyrotoxicosis. The elevated values in non-specific hypermetabolism suggested that peripheral tissue metabolism, or some function thereof, has a major role in determining hormone degradation rate.

Administration of large amounts of thyroxine or triiodothyronine or both to myxedematous subjects failed to alter the shape of the disappearance curve. This indicated turnover of a fixed proportion of the EOI pool daily, during short term studies, despite marked elevation of the plasma PBI concentration, enlargement of the EOI pool, and increase of the absolute organic iodine degradation rate.

The Urinary Excretion of the Epinephrines in Anxiety States. HIRSH SULKOWITCH, West Newton, Mass. (Introduced by Mark D. Altschule).

The excretion of urinary catecholamines was studied in patients with anxiety and in a few with pheochromocytoma. The measurements were made by means of simplified chemical techniques for the determination of pressor amine concentrations which have been developed. It has been found that the output of the urinary epinephrines in anxiety may equal or exceed the quantities excreted in patients with pheochromocytoma. The effect of adrenolytic and/or hypotensive agents on the excretion of the pressor amines and their degradation products will be discussed. The relationship between the chemical values and those found by bioassay will be shown.

Nonspecific Adherence of Platelets and Leukocytes to Antibody-Sensitized Red Cells: A Mechanism Producing Thrombocytopenia and Leukopenia During Incompatible Transfusions. SCOTT N. SWISHER,* Rochester, N. Y.

Acute thrombocytopenia and leukopenia following hemolytic transfusion reactions in man are well documented but the frequency, characteristics of the hemolytic process and the mechanisms responsible for the reduction in platelets and leukocytes are unknown. Acute severe thrombocytopenia and neutropenia regularly accompany *in vivo* hemolysis of canine A-positive red cells by canine anti-A. This antibody is an hemolysin which fixes complement and sensitizes erythrocytes for the antiglobulin reaction. Thrombocytopenia is accompanied by the appearance of platelets firmly agglutinated with the incompatible red cells in venous blood; these agglutinates are found in the circulation in diminishing numbers while the thrombocytopenia persists and then disappear as the platelets return. Erythrophagocytes and occasional clumps of leukocytes agglutinated with red cells are found in blood samples obtained immediately after onset of the hemolytic reactions. The agglutinated erythrocyte-platelet masses are rapidly removed from the circulation and thrombocytopenia ensues. Erythrophagocytes are probably similarly removed.

Platelet-erythrocyte agglutination, erythrophagocytosis, and leukocyte-erythrocyte agglutination can be observed *in vitro*. These observations show that erythrocyte-platelet, and erythrocyte-leukocyte agglutination may be a nonspecific attachment of platelets or leukocytes to the surfaces of antibody-sensitized red cells.

Transfusions of incompatible cells to recipients with canine anti-B, C, or D, do not produce thrombocytopenia or leukopenia, even though rapid removal of the incompatible red cells without hemoglobinemia occurs. These antibodies are "saline" agglutinins and do not produce platelet-erythrocyte agglutination or erythrophagocytosis *in vitro*. The capacity of a number of human isoantibodies to produce *in vitro* platelet-erythrocyte agglutination and erythrophagocytosis has been studied.

These findings bear implications for studies of antigenic composition of leukocytes or platelets by the method of coagglutination and for demonstration of platelet or leukocyte antibodies by infusion of plasma into recipients.

Ultrafiltrability of Electrolytes After Death. R. TARAIL and T. E. BENNETT, Buffalo, N. Y. (Introduced by David K. Miller).

Concentrations of potassium, sodium, and chloride were measured in serum of blood (cardiac puncture) from 26 unselected patients shortly after death from miscellaneous causes in a cancer hospital. Serum was separated from cells within three hours except in occasional instances of clotting impairment. Ultrafiltrates of fresh serum were prepared by Lavietes' anaerobic method.

Serum potassium was impressively elevated: The mean was 8.7 millimoles per liter; standard deviation 2.2; range 5.0 to 13.1. Serum sodium was usually depressed or normal: The mean was 132 millimoles per liter; standard deviation 15; range 103 to 161. Serum chloride was almost always depressed or normal: The mean was 88.5 millimoles per liter; standard deviation 14; range 52.8 to 111.

Molar concentrations of serum potassium and sodium were indistinguishable from their concentrations in respective ultrafiltrates. But distribution ratios of potas-

sium and sodium (expressed as molal concentration of ion in water of ultrafiltrate divided by its concentration in serum water) were significantly below 1.00 because of the lower concentration of water in serum: The mean distribution ratio for potassium was 0.96 ± 0.016 † (range 0.91 to 1.08); for sodium 0.96 ± 0.003 † (range 0.93 to 0.98). In contrast, molar concentration of serum chloride was invariably less than ultrafiltrate chloride and distribution ratios (based upon molalities) were 1.02 to 1.13 with a mean of 1.05 ± 0.005.†

It is concluded: (1) No significant deviations in ultrafiltrability of potassium or sodium from our previously reported findings in normal subjects are discernible. (2) Distribution ratios of potassium, sodium, and chloride after death appear to coincide with predictions of a Gibbs-Donnan equilibrium. (3) Striking hyperkalemia, perhaps agonal in great part, is a significant correlate of the events of death in hospital populations akin to that under scrutiny.

Quantitative Study of Gastrointestinal Motor Activity. E. CLINTON TEXTER, JR., and HUBBARD W. SMITH, Chicago, Ill. (Introduced by David P. Earle).

Disordered motor activity of the gastrointestinal tract is a common cause for distress, but ordinary methods may not disclose the nature of the abnormality. Confusion has arisen concerning the relation of disordered motor activity to the production of gastrointestinal symptoms including symptoms of peptic ulcer.

A sensitive system of tandem open-end catheters connected to strain gages was employed to record intralumen pressures from the stomach in 85 normal subjects and ulcer patients. The amplitude and duration of 4,000 waves were analyzed. The data revealed an inverse exponential distribution of wave amplitudes and a double peaked distribution of wave durations.

A quantitative estimate of motor activity was obtained by totaling the duration of all waves and expressing this as a percentage of the total period of the study. No qualitative differences were observed between normals and ulcer patients. There was a significant quantitative increase in motor activity in ulcer patients during ulcer pain as compared with the pain-free period. The changes in stomach consisted primarily of a change in basal pressure while the changes in the duodenum were mainly an increase in short activity with a duration less than 20 seconds having a variable amplitude. The former are equivalent to the type III waves of Templeton and Lawson as applied to motor studies in human beings. The latter includes both types I and II. Although types I and II are differentiated on the basis of amplitude, this differentiation appears to be arbitrary. Waves with a duration longer than 35 seconds appeared to be a fairly distinct group and conform essentially to type III waves. The data also revealed many more waves of short duration than hitherto observed. These could not be classified according to the classification of Templeton and Lawson.

† Standard error of mean.

Hyperreactivity to Adrenalin as a Possible Mechanism in Endotoxin Shock. LEWIS THOMAS * and BENJAMIN W. ZWEIFACH, New York, N. Y.

The reaction of shock produced in rabbits by the lipopolysaccharide endotoxins of gram negative bacteria is accompanied by the following manifestations: generalized peripheral arteriolar constriction (followed terminally by vasodilatation and stasis), hyperglycemia (followed by hypoglycemia), depletion of liver glycogen and fever. Delaunay, Boquet and others have suggested that adrenalin may in some way be implicated in this reaction.

We have observed that the local response to adrenalin becomes drastically altered under the systemic influence of endotoxin. In the rabbit, an intradermal injection of 0.2 cc. of 1 to 10,000 adrenalin produces extensive lesions of hemorrhagic necrosis when given within 1 hour after an intravenous injection of endotoxin. Several highly purified endotoxins of *E. coli, E. typhosa* and *S. marcescens* were used, in doses of 5 to 50 micrograms per kilo. The necrotizing effect of adrenalin appears to be due to its capacity to cause prolonged ischemia in endotoxin-treated rabbits. It is prevented by chlorpromazine, but is not affected by cortisone, heparin or nitrogen mustard.

The adrenalin-potentiating action of endotoxin is also demonstrable as a local effect. When mixtures of endotoxin and adrenalin are injected intradermally, hemorrhagic lesions resembling local Shwartzman reactions occur within a few hours.

In rats, extreme hyperreactivity to adrenalin occurs during a period of 3 hours after intravenous endotoxin. This has been demonstrated by direct observation of the response to topically applied adrenalin in the vessels of the appendiceal mesentery. The sensitivity of arterioles and venules becomes increased more than one thousandfold, and vasoconstriction can be produced by less than 0.0001 microgram of adrenalin. Petechial hemorrhages frequently occur at the site of adrenal application.

It is suggested that the systemic intoxicating effects of endotoxin may be due, at least in part, to altered reactivity to adrenalin. The possible relationship between these observations and mechanisms involved in traumatic shock will be discussed.

Studies on Pantothenic Acid and Potassium. G. H. M. THORNTON, R. E. HODGES, R. I. LUBIN, W. R. WILSON, and KATE DAUM, Iowa City, Ia. (Introduced by William B. Bean).

For the past 4 years we have studied 14 normal subjects and 6 patients with different diseases subsisting on a partially synthetic, purified diet devoid of pantothenic acid but containing a pantothenic acid antagonist. After 5 to 15 days of this regimen a clinical state characterized by lethargy, weakness, burning paresthesias and tetany occurred. Serum potassium reached low levels and electrocardiographic changes consistent with hypokalemia appeared. Metabolic alkalosis, tetany, alteration in glucose tolerance and insulin sensitivity occurred. Histamine

refractory achlorhydria developed at the same time. Ability to excrete water after drinking a large amount declined. There was a reduction in eosinopenia induced by ACTH, suggesting a decrease in activity of the adrenal cortex.

Recent studies which include data on potassium balance will be discussed and the interrelationships of pantothenic acid, coenzyme A and adrenal cortical functions will be presented.

The Effect of Desoxycorticosterone with either High or Low Sodium Diets on the Electrolyte Composition of Arterial Wall. LOUIS TOBIAN,* Minneapolis, Minn.

Desoxycorticosterone given to rats for seven weeks along with a high sodium intake resulted in 9 per cent more water, 10 per cent more sodium, but no more chloride per gram of aorta solids than was found in normal rats on a moderate sodium intake. Moreover desoxycorticosterone with a high sodium intake also resulted in 34 per cent more potassium, 32 per cent more magnesium, and 30 per cent more phosphorus per gram of non-collagen protein than was present in the aorta wall of normal rats eating moderate amounts of sodium. These differences were all significant.

When desoxycorticosterone was administered with a diet very low in sodium, hypertension was completely prevented and all the deviations from normal mentioned above were also prevented. The low sodium diet with desoxycorticosterone keeps the sodium content in the aorta wall from being greater than that of normal rats on a moderate sodium intake, but it is still 14 per cent greater than that of normal rats on a very low sodium intake. This difference was significant.

Desoxycorticosterone plus the very low sodium diet significantly decreased the water content of the aorta wall 10 per cent below levels seen in similarly fed normal rats. The arterial pressure of the rats receiving desoxycorticosterone and a diet very low in sodium actually fell significantly to a level 12 per cent below their pre-treatment pressures. This is in contrast to the rise in arterial pressure which frequently follows desoxycorticosterone and a high sodium intake. It is of some interest that the low sodium diet, which prevents the hypertension following desoxycorticosterone, also largely prevents the electrolyte abnormalities in the arterial wall resulting from desoxycorticosterone.

Pulmonary Distensibility in Mitral Stenosis and Congenital Heart Disease. GERARD M. TURINO, New York, N. Y. (Introduced by Alfred P. Fishman).

To elucidate some of the factors which may contribute to the reduction of pulmonary distensibility in patients with heart disease, pulmonary artery pressure, pulmonary blood flow and compliance have been measured in 14 patients with clinically compensated mitral stenosis and in 4 patients with increased pulmonary blood flow due to left to right congenital shunts without pulmonary hypertension. Lung compliance alone, was measured in 8 normal subjects.

Using intra-esophageal pressure as an index of intrathoracic pressure, compliance (Δ pulmonary volume/Δ intrathoracic pressure) was measured from 1) the pulmonary pressure-volume loop determined at rest during varying frequencies and tidal volume, and 2) from the pressure-volume relationship of a single inspiratory capacity during periods of zero air flow. Pulmonary artery pressure, and mixed venous blood for determination of cardiac output by the direct Fick method, were obtained by cardiac catheterization.

In patients with mitral stenosis and normal pulmonary artery pressure, compliance was normal. In the presence of pulmonary hypertension, compliance was usually, but not consistently reduced. In patients with increased pulmonary blood flow and normal pulmonary artery pressure, compliance was reduced. Reduced compliance in both groups of patients, was associated with an increase in the work of breathing. In normal subjects, vital capacity was closely correlated with lung compliance; in patients, the reduction in lung compliance paralleled the reduction in vital capacity.

In 3 patients studied 3 to 6 months following mitral commissurotomy, there was no change in compliance.

These observations suggest that reduced pulmonary compliance is associated with pulmonary hypertension in patients with mitral stenosis and with increased pulmonary blood flow in congenital heart disease. In mitral stenosis, the factors responsible for the reduction in compliance may not be rapidly reversible.

Effects of Elevated Blood Ammonium Concentration on Respiratory Exchange. PARKER VANAMEE, J. WILLIAM POPPELL, HENRY T. RANDALL, and KATHLEEN E. ROBERTS,* New York, N. Y.

We have observed respiratory alkalosis in patients with hepatic coma associated with elevated blood ammonium. In dogs, respiratory alkalosis has been produced by the infusion of ammonium acetate. These observations suggest that ammonium is a direct respiratory stimulant. In an attempt to clarify the minimal concentration of ammonium in the peripheral blood which is necessary to stimulate respiration the following studies have been pursued. Dogs have been infused with ammonium acetate in various concentrations. Prior to and during the infusion, respiratory minute volume, blood ammonium, carbon dioxide and pH were determined. These studies show that the infusion of ammonium acetate resulting in blood ammonium concentrations of 170 to 400 micrograms per cent, was associated with increased respiratory minute volume of 190 to 300 per cent over the control value, and a respiratory alkalosis. These findings have been correlated with observations made on 58 patients having hepatic decompensation but without any pulmonary disease. The majority of these patients were hyperventilating and presented the chemical picture of respiratory alkalosis. Although many of these patients had blood ammonium levels over 200 micrograms per cent, 24 of them presented this picture in the presence of blood ammonium concentration of 110 to 190 micrograms per cent

(normal values 30 to 100 micrograms per cent). These findings suggest that small elevations of blood ammonium stimulate respiratory exchange, and the finding of respiratory alkalosis in patients in hepatic decompensation, although not diagnostic, is nevertheless suggestive evidence of ammonium toxicity.

Studies on Familial Nephrosis: Evidence for a Unitarian Concept. ROBERT L. VERNIER, MARILYN G. FARQUHAR, JOEL G. BRUNSON, and ROBERT A. GOOD, Minneapolis, Minn. (Introduced by Irvine McQuarrie).

We have observed multiple cases of nephrosis in each of four families. In one family the entire spectrum of nephrotic syndrome occurred among four children involved. Clinically, cases could be described as follows:

Case I; male, symptoms at 4 months, died age 7 years, renal insufficiency.

Case II; male, 5 years, transient "pure" nephrosis.

Case III; female, 3 years, symptoms at 4 months, chronic nephrotic syndrome.

Case IV; female, 3 months, "pure" nephrosis since birth.

Father, mother and half-brother are normal. Mother had "nephritis" at 16 years, and has had "pre-eclampsia" with each pregnancy.

To gain insight into the natural history of nephrosis, and inter-relationships of the clinical stages and pathological states, surgical renal biopsies were obtained from each involved member of the family. These were studied by light and electron microscopy, and correlated with clinical, laboratory, and discrete renal function studies.

Microscopic findings as revealed by light microscopy were: chronic glomerulonephritis (Case I); sub-acute glomerulonephritis (Case III); and normal kidney (Case IV). Assuming a single etiologic basis for these cases, we conclude that familial nephrosis may be a chronic, progressive disease, reflected clinically and pathologically in a variety of patterns.

Electron microscopy revealed a striking lesion of the epithelial cells of the glomerular capillaries present in all the children regardless of the clinical phase or pathology of the disease as observed by light microscopy. The lesion consists of distortion and smudging of epithelial podocytes along the basement membrane.

We interpret these observations as evidence for the hypothesis that various forms of nephrosis are in reality a single disease, the unifying lesion of which may be observed at the sub-microscopic level with the electron microscope. Comparison of these lesions with renal biopsies obtained on 40 cases of diverse renal disease of childhood will be presented.

Oxidative Phosphorylation in the Hepatic Mitochondria of Normal, Diabetic, and Insulin Treated Cats. JOHN W. VESTER, Philadelphia, Pa. (Introduced by William C. Stadie).

The following studies were done in an attempt to determine whether an aberration in oxidation coupled to high energy phosphate formation contributes to the derangements in hepatic metabolism in the insulin-free animal. The cat was selected because of the ready feasibility of pancreatectomy, ensuring complete insulin lack. Mitochondria were prepared from the liver samples by homogenization in 0.25 *M* sucrose and differential centrifugation. Aliquots of mitochondria were allowed to respire in Warburg vessels for measurement of oxygen uptake. Substrate was sodium pyruvate in phosphate buffer, pH 7.4. ADP and glucose-hexokinase served as phosphate acceptor and glucose-6-phosphate formation was measured directly, enzymatically, or by phosphate disappearance.

Seven normal cats gave mean phosphorylation values of 77.6 ± 18.7 micromoles per gram mitochondria per minute; oxygen uptake 31.6 ± 5.5 microatoms per gram per minute; P : O ratio 2.4 ± 0.25. Six animals sacrificed three days after pancreatectomy showed a mean of 19.0 ± 4.8 micromoles phosphate per gram per minute; 16.5 ± 2.5 microatoms oxygen per gram per minute; P : O 1.1 ± 0.14. The differences between the groups are statistically significant.

In a second series, mitochondria were made from liver biopsies taken in the insulin free state and after administration to the pancreatectomized animal of sufficient insulin to a) maintain weight and b) suppress glycosuria as much as possible without producing hypoglycemia. The mean phosphorylation value, of eight studies of insulin-free hepatic biopsy mitochondria, was 35.1 ± 8.8 micromoles per gram per minute; oxygen uptake 26.9 ± 3.9 microatoms per gram per minute; P : O 1.2 ± 0.19. For mitochondria prepared from hepatic biopsies taken from animals maintained on insulin as described above, the mean values of seven observations were 117.6 ± 19.5 micromoles phosphate per gram per minute; 48.7 ± 6.1 microatoms oxygen per gram per minute; P : O 2.3 ± 0.14. The differences between these two groups are statistically significant. The values in the insulin treated state do not differ significantly from the normal.

The Relation of Copper to Ceruloplasmin Activity and Zinc to Malic and Lactic Dehydrogenase Activity in Acute Myocardial Infarction. WARREN E. C. WACKER, S. JAMES ADELSTEIN, DAVID D. ULMER, and BERT L. VALLEE,* Boston, Mass.

The existence of hypercupremia in myocardial infarction has led to an investigation of *serum metalloenzymes* in this disease.

In normal and pregnant individuals, ceruloplasmin, the copper protein of serum, oxidizes a number of substrates, including paraphenylenediamine and benzidine, in proportion to the serum copper concentration. Alcohol, glutamic and lactic dehydrogenases have recently been identified as zinc enzymes in this laboratory; malic dehydrogenase tentatively so. We have found that normal human serum exhibits appreciable malic and lactic dehydrogenase activity, while there is no significant oxidation of ethanol and glutamic acid.

While the serum copper concentration *rises* and becomes maximal between the fifth and eleventh post-

infarction day, the serum zinc concentration is significantly *decreased* in this disease.

The present data indicate an increased rate of paraphenylenediamine oxidation in the sera of patients with acute myocardial infarction, in proportion to the rise in serum copper content. However, the rate of benzidine oxidation of the same sera is elevated above that which can be accounted for by the increase in copper alone. No explanation for this deviation is at hand, although it may be postulated that an additional benzidine oxidase activity, not apparent under normal conditions, accounts for the disproportionation of this rate of oxidation in myocardial infarction.

The enzymatic activity of malic and lactic dehydrogenases is significantly elevated in the sera of patients with acute myocardial infarction. The *increase* in malic dehydrogenase and lactic dehydrogenase and the *decrease* of serum zinc occur within 24 hours following the acute injury becoming maximal on the third post-infarction day.

The apparently paradoxical reciprocal relationship between serum zinc *concentration* and zinc dehydrogenase *activity* can be explained on the basis of biochemical findings.

Lactic dehydrogenase and malic dehydrogenase activities are being employed as routine aids to diagnose myocardial infarction. These methods are simple and rapid. They represent valuable adjuvants in the management of this disease providing pertinent information not available by non-enzymatic techniques.

The Pressor Effect of the Antidiuretic Principle of the Posterior Pituitary in Orthostatic Hypotension. HENRY N. WAGNER, JR., and EUGENE BRAUNWALD, Bethesda, Md. (Introduced by Charles G. Zubrod).

In three patients with diffuse autonomic nervous system disease manifested by orthostatic hypotension, anhidrosis and impotence, the antidiuretic principle of the posterior pituitary produced a marked rise in arterial pressure. In contrast, in normal subjects, posterior pituitary extracts had no significant effect on arterial pressure. In patients with orthostatic hypotension, intravenous infusion of 1 milliunit per minute of purified vasopressin resulted in a 20 to 30 mm. Hg rise in arterial pressure. Arterial pressures greater than 200/130 mm. Hg were produced by a single intravenous injection of 1 unit of purified vasopressin, or by continuous infusion of 10 milliunits per minute. The increase in arterial pressure was related to a rise in total vascular resistance and was not due to a change in the cardiac output. In addition, vasopressin administration resulted in a rise in calculated pulmonary and renal vascular resistances.

In an effort to determine the basis for the striking vascular responsiveness of these patients to vasopressin, ganglionic transmission was blocked with tetraethyl ammonium chloride in three subjects without orthostatic hypotension. Whereas vasopressin previously had no significant effect on arterial pressure, a rise in arterial pressure occurred subsequent to vasopressin administration during ganglionic blockade. The increased sensitivity to the pressor activity of vasopressin in patients with orthostatic hypotension may be related to increased reactivity of vascular smooth muscle and to absence of normal vasodepressor reflexes. This effect was observed with dosages approaching the physiologic range of endogenous antidiuretic hormone secretion.

Administration of various posterior pituitary preparations, gave marked relief of hypotensive symptoms to all patients with orthostatic hypotension. Therapeutically, vasopressin has been of greater value than sympathomimetic drugs in these patients.

Sources of Blood Ammonium After Feeding Protein to Patients with Hepatic Cirrhosis. LESLIE T. WEBSTER, JR., and CHARLES S. DAVIDSON,* Boston, Mass.

Excessive dietary protein or gastrointestinal bleeding may induce the syndrome of impending hepatic coma in susceptible individuals with hepatic cirrhosis or Eck fistula. The increased blood ammonium-nitrogen (NH_4-N) concentrations usually associated with this syndrome implicate a disorder in nitrogen metabolism. These investigations demonstrate sources of blood NH_4-N after feeding protein and amino acids, and during intravenous urea.

Observations were made on patients with cirrhosis and prominent abdominal collateral veins. None had confusion, tremor, hemorrhage, shock, or serum nonprotein nitrogen concentrations over 50 mg. per cent. Fasting NH_4-N concentrations were reproducible on antecubital or abdominal collateral venous blood samples drawn during the day in any given patient. A prompt (15 to 30-minute) rise in abdominal collateral and either no change or a prompt rise in antecubital venous blood NH_4-N concentrations occurred after protein by mouth (20 to 50 gm., 4 patients, 7 occasions), after l-glutamine orally (10 gm., 1 patient), or during urea given intravenously at a constant rate (1.8 to 150 mg. per minute, 2 patients, 3 occasions).

Blood NH_4-N increases after protein or glutamine did not result either from contained free NH_4-N or from liberation of α-amino-nitrogen because no increase occurred following NH_4Cl (1 patient) or following 7 amino acids (exclusive of glutamine) fed singly or in a mixture (2 patients, 3 occasions) containing even more NH_4-N than was analyzed in the protein or the glutamine fed.

The blood NH_4-N increase during intravenous urea probably was due to gastrointestinal bacterial urease activity, because it was prevented by oral neomycin (1 patient). However, neomycin did not prevent the blood NH_4-N rises after protein (2 patients) or after glutamine (1 patient), suggesting that these rises were independent of bacterial action and urea production.

These data suggest that non-bacterial hydrolysis of the amide-nitrogen of glutamine in the small intestine is one source of the blood NH_4-N rise which occurs after protein feeding.

Vasodepressor Syncope: Acute Circulatory Disorganization. A. M. WEISSLER, H. D. MCINTOSH, E. H. ESTES, JR., and J. V. WARREN,* Durham, N. C.

Vasodepressor syncope offers an interesting experimental situation for the study of physiologic mechanisms controlling blood pressure and cardiac output. Typical vasodepressor fainting has been induced by 60° tilt, accompanied usually by oral sodium nitrite, in 30 young male subjects. With the fall in arterial pressure, the cardiac output (dye technique) has been found to change but little beyond the usual postural decrease. At the time when the average mean arterial pressure was 43 mm. Hg, the average cardiac index had decreased 0.4 liter from the upright control value of 2.9 liters. Calculations indicated that the hypotension resulted to a large degree from a decreased peripheral vascular resistance. No correlation was noted between the observed changes in cardiac output and the fall in arterial pressure, or with the level of consciousness. Both unconsciousness and electroencephalographic slow wave activity were well correlated with arterial blood pressure changes.

Failure of the heart to compensate for the sudden diminution of peripheral resistance by the usual increase in cardiac output is striking. It might result from either reflex cardiac depression or inability of the heart to respond because of limited venous return. To study this problem, the effect of G-suit inflation, full time negative pressure breathing, albumin induced plasma volume increases, and rapid return of the subject to the recumbent position have been studied. Such measures, all of which raise effective central venous pressure, usually prevented or aborted the syncopal episode. In some instances, the cardiac output rose to supernormal values indicating the presence of cardiostimulatory, rather than cardioinhibitory, effects.

The major circulatory event of vasodepressor syncope, therefore, would appear to be not only widespread loss of peripheral resistance, but its occurrence in the face of inability of the heart to compensate by an increase in output.

The Effect of Chlorpromazine and Meperidine, Alone and Combined, Upon Respiration in Man. HERBERT WENDEL, CHRISTIAN J. LAMBERTSEN, and JOHN B. LONGENHAGEN, Philadelphia, Pa. (Introduced by Isaac Starr).

Separate and combined effects upon respiration of chlorpromazine hydrochloride (25 mgm./70 kgm. i.m.) and meperidine hydrochloride (100 mgm./70 kgm. i.m.) were studied in six subjects. Effects were measured at a controlled "alveolar" pCO_2 of 46 mm. Hg as changes in respiratory minute volume before and at intervals after drug injection. End-tidal pCO_2 was recorded by an infrared CO_2 analyzer and percentage CO_2 added to inspired air was continuously adjusted to prevent significant deviations from the desired pCO_2 level. By raising pCO_2 the respiratory response to a drug is magnified, thus permitting assessment of small and otherwise unnoticeable respiratory effects. Keeping pCO_2 constant eliminates compensatory influences of pCO_2 changes accompanying ventilatory responses to drugs. Expressed as mean lowering of RMV over a three-hour post-injection period, chlorpromazine alone depressed respiration 8 per cent meperidine alone 43 per cent. One subject was less depressed by the combined drugs than by meperidine alone. In five of the six subjects chlorpromazine with meperidine produced an average depression 50 per cent greater than with meperidine alone. In two of these subjects the combined effect was respectively 4.3 and 2.6 times that of the sum of the separate effects of the two drugs. Three and a half hours after injection, when meperidine depression had decreased to half its maximum, the maximum depression of the combined drugs was reduced by only 14 per cent. This suggests that chlorpromazine usually potentiates the intensity of meperidine depression of respiration either in the form of simple addition or supra-additive synergism, while also prolonging the inhibitory effect of meperidine on respiration.

The Role of Convertin in Experimental In Vivo Thrombosis. STANFORD WESSLER, JONATHAN D. BALLON, and MARVIN GILBERT, Boston, Mass. (Introduced by Herrman L. Blumgart).

In dogs, the systemic infusion of serum induces a temporary hypercoagulable state during which massive thrombosis can be routinely produced in areas of retarded blood flow. The active fraction responsible for hypercoagulability has been recovered from canine serum by barium sulfate adsorption and subsequent elution with citrate. It is rich in convertin (Owren method) and essentially devoid of thromboplastin, ac-globulin, antihemophilic globulin, prothrombin, thrombin and fibrinogen.

To evaluate the role of convertin in thrombus formation by the method employed, barium sulfate serum eluates were prepared from 40 "donor" dogs in which serum convertin had been reduced to various levels by Dicumarol®. Thirty-five "recipient" dogs, in which serum convertin was similarly depressed by Dicumarol®, were infused with eluates from the "donor" animals. The detailed results of the many experimental permutations and combinations of eluates infused into convertin-depleted animals will be presented. Most striking was the production of thrombi with eluates from "donor" dogs with convertin values of less than one per cent. In a series of 14 experiments in which serum convertin of both "donor" and "recipient" animals was less than one per cent, thrombi were produced in 6 instances. Under such conditions, the concentration of convertin at the site of thrombus formation could only have been negligible.

These observations indicate that convertin is not essential for the formation of intravascular clots by the method employed and that there are present in the barium sulfate eluate of heavily dicumarolized canine serum one or more potent and as yet unidentified moieties effective in inducing intravascular coagulation.

Metabolic Effects of an Anabolic Steroid, 17-Alpha-Ethyl-17-Hydroxy-Norandrostenone in Human Subjects. RAYMOND E. WESTON,* MARIAN C. ISAACS, ROBERT ROSENBLUM, DONALD M. GIBBONS, and JACOB GROSSMAN, New York, N. Y.

The therapeutic problems presented by the malnutrition which complicates severe illness are well known. Although nutritional repletion may be accomplished by luxus consumption, particularly of protein-rich foods, anorexia or the adverse metabolic effects of increased intakes, for example, in cardiac patients, make this approach impractical. Therefore, drugs promoting anabolism on moderate food intakes have long been sought. All previously potent anabolic agents have also been strongly androgenic. However, 17-alpha-ethyl-17-hydroxy-norandrostenone (SC 5914) recently has been reported to be strongly anabolic but only weakly androgenic in animals.

The effects of this compound on body weight, blood chemistries, metabolic balances for sodium, chloride, potassium, nitrogen, phosphorus, calcium, and water, and urinary excretions of creatinine, sulfur, and total solutes were studied in one obese young woman and in five patients with congenital or rheumatic heart disease in congestive failure and varying nutritional states. All were on fixed fluid, and low sodium and calcium intakes, providing approximately 11.5 grams of nitrogen and, except for the obese subject who received 980 calories, 1300 to 2100 calories daily.

Within 24 to 48 hours daily intramuscular doses of 25 to 75 mgm. for 6 to 24 days resulted in weight gain and retention of nitrogen (1.5 to 2.5 grams daily), phosphorus (0.1 to 0.3 gram daily), sulfur, and potassium (10 to 30 mEq. daily), which generally persisted until 4 to 7 days after discontinuing the drug. No consistent changes in sodium, chloride, or calcium balances were observed. One cardiac patient, who exhibited increased signs of congestive failure during a typical anabolic response to 25 mgm. of SC 5914, developed a catabolic reaction despite doubling of the dose.

If chronic studies further establish no toxicity and low androgenicity in human subjects, wider clinical applications of this new anabolic agent are indicated.

Distribution of Splanchnic Circulation Times in Cirrhosis. H. O. WHEELER, B. COMBES, A. W. CHILDS, and S. E. BRADLEY,* New York, N. Y.

The distribution of transit times required for the movement of blood from aorta to hepatic veins through the different components of the splanchnic vasculature (derived from a comparative analysis of the arterial and hepatic venous radioactivity curves following intravenous injection of I^{131} labelled human serum albumin—Trans. Assoc. Am. Phys., 1955, **68**, 177) was evaluated in four normal human subjects and in five patients with cirrhosis. The distributions of splanchnic transit times about the means were unimodal and somewhat skewed to the right (toward the longer transit times) in normal subjects (range: 35.7 ± S.D. 16.8 to 49.5 ± 23.4 seconds). In the cirrhotic patients the patterns differed from the normal in several respects. In two, the initial slope of the distribution curve was steeper than normal, suggesting the presence of a significant proportion of shorter (or more rapidly perfused) vascular pathways, possibly as a result of hepatic arteriolar vasodilatation or the development of arteriovenous "shunts." The pattern of distribution in the remaining patients with cirrhosis was characterized by a marked broadening and flattening of the peak (range: 48.1 ± 34.8 to 74.3 ± 46.4 seconds) with increased skewing to the right. These changes may be attributed to a combination of (1) increased heterogeneity of hepatic perfusion which results in a wider range of splanchnic transit times and (2) increased portal transit times due to stasis.

The departure from the normal pattern observed in the five patients with cirrhosis can be attributed to local vascular derangements, not otherwise detectable, that affect the distribution of flow through different parts of the splanchnic bed. Further analysis of these differences may permit a more precise appraisal of structural and functional changes in cirrhotic disease.

Pressor Potentiation of Nor-epinephrine by Tetraethylammonium Chloride and Atropine. JOSEPH A. WILBER and ALBERT A. BRUST,* Atlanta, Ga.

Since provocative test drugs are widely employed in suspected pheochromocytoma, this study was designed to clarify the actions of these agents in the presence of known amounts of circulating catecholamines.

Twenty-one fasting normotensive subjects, free of cardiovascular disease, were studied. Auscultatory blood pressures and pulse responses to histamine base (.025 mg. I.V.), mecholyl (10 mg. S.C.), tetraethylammonium chloride (TEAC 400 mg. I.V.) and atropine sulfate (1.2 mg. I. V.) were recorded during control periods, during constant infusion of l-epinephrine (.085 mcgm. per Kg. per min.) and of l-nor-epinephrine (.085 mcgm. per Kg. per min.). Blood sugar and lactic acid were sampled at appropriate intervals.

Histamine was consistently depressor during control periods (− 20/20 mm. Hg), during epinephrine (− 30/20 mm. Hg) and nor-epinephrine infusion (− 25/20 mm. Hg). With mecholyl, control pressures fell (− 20/25 mm. Hg) and these depressor effects became more prominent during epinephrine (−30/34 mm. Hg) and nor-epinephrine (−48/42 mm. Hg).

Control TEAC responses were slightly depressor (− 12/8 mm. Hg) and were similarly depressor (− 15/5 mm. Hg) during epinephrine. During nor-epinephrine, however, TEAC became dramatically pressor (+ 33/20 mm. Hg).

Blood pressures were unchanged by atropine in control periods and during epinephrine. When atropine was given during nor-epinephrine infusion, blood pressures rose sharply (+ 45/35 mm. Hg) and as with TEAC these potentiated pressor effects were sustained until the infusion was discontinued.

Histamine caused little pulse change, but mecholyl, TEAC, and atropine regularly increased epinephrine tachycardia and abolished nor-epinephrine bradycardia.

Although the test drugs alone did not affect blood glucose and lactic acid, these were elevated by epinephrine. With nor-epinephrine glucose rose slightly but lactic acid remained unchanged.

The results suggest:

1. In pheochromocytoma a positive provocative test with TEAC represents a potentiation phenomenon specific for circulating nor-epinephrine.
2. Since histamine and mecholyl remain depressor during catecholamine infusion, a different mechanism accounts for the pressor paroxysms these agents may induce.
3. Atropine, like TEAC, potentiates nor-epinephrine and should be evaluated as a screening agent for pheochromocytoma.
4. Pressor potentiation of nor-epinephrine by TEAC may involve blockade of sino-aortic regulatory mechanisms or autonomic vasodilator pathways or both. Cardiac output changes have not yet been excluded.
5. Specificity of blood lactic acid for circulating epinephrine is confirmed.

An Inhibitor of Desoxyribonuclease Obtained from Human White Blood Cells. JOHN J. WILL and RICHARD W. VILTER,* Cincinnati, O.

An inhibitor of desoxyribonuclease can be extracted from white blood cells and quantitated in terms of residual desoxyribonuclease activity by means of the alcohol precipitation test of McCarty or the methyl green test of Kurnick. We have investigated the activity and chemical properties of this substance in leukemic and normal white blood cells in order to determine whether, under these conditions, there are differences in enzyme systems affecting desoxyribonucleic acid.

White blood cells of acute and chronic leukemia contain less inhibitor than do cells of normal subjects or of patients with leukocytosis or leukemoid reactions. The lowest inhibitor content is found in chronic lymphatic leukemia and in acute blastic leukemia. In acute leukemia and in chronic myelogenous leukemia the inhibitor content of white blood cells increases as the total white blood cell count and per cent of immature cells decrease, either as a result of treatment or during spontaneous remission. In chronic lymphatic leukemia, remissions do not alter the low inhibitor content of these lymphocytes.

The inhibitor is heat labile, and its activity disappears after 24 to 48 hours' storage at 4° C. It is water soluble, non-dialyzable, and active from pH 3 to 8. The inhibition of desoxyribonuclease is instantaneous and reversible. The inhibitor is adsorbed on barium sulfate and eluted with sodium citrate. The precipitated ribose nucleoprotein fraction of the cells contains the inhibitor activity. The inhibitor activity is lost after tryptic digestion.

Leukemic cells have the potentiality for uninhibited mitosis and disorganized patterns of growth. The deficiency of this inhibitor substance, and the continued activity of desoxyribonuclease that results, may be responsible for instability of nuclear desoxyribonucleic acid, ultimately resulting in altered growth and maturation of leukemic cells.

Mechanisms of Antimicrobial Action by Chloramphenicol. CHARLES L. WISSEMAN, JR., Baltimore, Md. (Introduced by Joseph E. Smadel).

The basic means by which antibiotics interfere with multiplication of microorganisms have been investigated extensively. Our group has been particularly interested in the actual mechanism of such inhibition by Chloramphenicol and the structural requirements of this antibiotic for its action. Previous studies showed that minimal bacteriostatic drug concentrations specifically inhibit microbial protein synthesis, an effect of sufficient moment to account for its antimicrobial action. Other important cellular processes are not affected, *viz.*, synthesis of nucleic acids and at least one polysaccharide, operation of many enzymes and energy production and utilization. Specific antagonism of Chloramphenicol by amino acids which the drug might resemble structurally has eluded us despite statements to the contrary by others. Indeed, reconsideration of the drug structure suggests that it may not resemble any known nutrient closely enough to act as its antimetabolite. Furthermore, systematic correlation of changes in antibiotic activity produced by alterations in the drug molecule (gleaned from numerous published reports) has emphasized the dependence for maximal antimicrobial action on: (a) a specific steric configuration; (b) the amide bond as well as the electronegativity and the molar volume of the terminal substituent in the side chain; and (c) the size of the planor area of the cyclic moiety and the charge of its para or equivalent substituent. From this information, a generalized formula emerges which describes a series of antimicrobial compounds, of which Chloramphenicol remains the most active known. Collectively considered, the information now available points to Chloramphenicol acting either as a specific antagonist to an undescribed cofactor operating in protein synthesis or, less specifically, by occluding the hypothetical matrix upon which amino acids are assembled for polymerization.

Detection and Estimation of Mitral Regurgitation by Indicator-Dilution Technics. EARL H. WOOD,* EDWARD WOODWARD, JR., H. J. C. SWAN, and F. HENRY ELLIS, JR., Rochester, Minn.

Fisher's percutaneous puncture technic was used to introduce an 18T-gauge needle into the left atrium (LA) in patients with valvular disease. An 0.8-mm. O.D. catheter manipulated into the left ventricle (LV) through a special adapter made possible recording of LV pressure and simultaneously LA pressure *via* the lumen remaining between needle shaft and catheter. LA blood was withdrawn continuously through a cuvette oximeter *via* this needle-catheter lumen for dilution curves simultaneously with radial and ear oximeter recordings, while dye in-

jections were made into the LV and, after partial withdrawal of the catheter, into the LA. Rapidly appearing dye was detected consistently in the LA during injections into the LV in patients with mitral insufficiency and also in dogs with mitral insufficiency in amounts apparently related to the severity of the surgically produced regurgitation. In patients with mitral stenosis and in whom insufficiency could not be detected, by gloved finger at surgery, no, or only a small amount of, rapidly appearing dye was detected in the LA; and similarly none was detected in the LA after LV injections in normal dogs.

If cardiac output is constant during successive injections of equal doses of dye into LV and LA, and if uniform mixing: (a) of dye regurgitated through the mitral valve and LA blood occurred following LV injection, and (b) of dye and LA blood after LA injection, then the proportion of dye injected into the LV which regurgitated into the LA is related to the ratio of the areas of the curves: $(\bar{c}\cdot T_p)_{LV}/(\bar{c}\cdot T_p)_{LA}$, in which $\bar{c}$ is mean concentration and T_p is passage time of curves following LV and LA injections, respectively. Results obtained promise a valuable approach to study of valvular insufficiency in man and animals.

Peripheral Venous and Arteriolar Responses to Moderate Reductions in Effectively Circulating Blood Volume in Man. J. Edwin Wood and John W. Eckstein, Boston, Mass. (Introduced by James M. Faulkner).

This study was undertaken to assess forearm venous and arteriolar responses to venous pooling in the legs.

Normal subjects were studied in a tilted (30° head-up) position. Venous pooling was prevented with pneumatic counterpressure leggings during control periods. Leg congestion was produced by deflation of the leggings and inflation of a cuff high on each thigh. Forearm venous distensibility was measured plethysmographically from the increase in volume due to a rise in local effective venous pressure of 1 to 31 mm. Hg produced with a proximal congesting cuff. An increase in venous distensibility indicated venodilatation; a decrease, venoconstriction. Blood flow was calculated from the initial rate of forearm volume increase. A low flow indicated arteriolar constriction; a high flow, arteriolar dilatation. Venous pressure was measured, with a sensitive transducer, in the opposite forearm proximal to an arterial occluding wrist cuff.

Venoconstriction occurred in response to leg congestion in 20 of 21 tests on 10 subjects. This venous distensibility decrease averaged 21 and ranged from 8 to 40 per cent. Blood flow was measured in these 20 tests. After leg congestion arteriolar constriction occurred prior to venoconstriction in 13; venoconstriction occurred first in 1. Arteriolar constriction was present throughout each of the 6 remaining experiments. Leg counterpressure reapplication in 17 experiments resulted in venodilatation in every case, followed by arteriolar dilatation in 12; arteriolar constriction persisted in 5.

Venous pressure was measured in 9 tests and always decreased prior to venoconstriction with leg congestion. In 7 tests, after leg counter-pressure reapplication, brief persistence of venoconstriction with an associated venous pressure overshoot (7 to 24 mm. water) was observed.

Leg congestion in the head-up position usually results in immediate arteriolar constriction and venous pressure decline followed by venoconstriction. Blood volume restoration usually causes in sequence venous pressure overshoot, venodilatation and arteriolar dilatation.

The Mechanism and Significance of Alterations in Serum Glutamic Oxaloacetic and Serum Glutamic Pyruvic Transaminase in Liver and Heart Disease. F. Wróblewski, C. Friend, I. Nydick, P. Ruegsegger, and J. S. LaDue, New York, N. Y. (Introduced by O. H. Pearson).

Glutamic oxaloacetic (GOT) and glutamic pyruvic (GPT) transaminase have been found to be widely distributed in animal and human tissues. GOT is particularly concentrated in cardiac muscle while GPT has its greatest concentration in liver tissue. These enzymes can be readily differentiated.

The serum activity of the enzymes was studied following the production of acute myocardial infarction in dogs and after the infection of mice with hepatitis virus. Similarly, the serum enzyme studies were made during the course of human transmural myocardial infarction and acute hepatitis.

Clinical myocardial infarctions are associated with a 2 to 20 times increase in serum GOT during the first 48 hours following cardiac necrosis while no appreciable increase in GPT is simultaneously noted. Acute hepatocellular injury results in a rise of from 2 to 1,000 times in serum GOT and GPT, the latter being greater than the former.

The mechanism for the increase in serum GOT associated with acute cardiac muscle injury appears to be primarily one of escape of GOT from necrotic cells, while the mechanism for increase of the serum enzymes during acute hepatocellular injury involves, in addition, a metabolic and/or excretory aberration.

The Effect of Irradiation Damage of Albumin-I^{131} on the Rate of Its In Vivo Metabolism with Special Reference to the Validity of Biologic Studies with I^{131} Labeled Proteins. Rosalyn S. Yalow and Solomon A. Berson,* New York, N. Y.

Previous studies from this laboratory have demonstrated that certain preparations of albumin-I^{131} and insulin-I^{131} may be inhomogenous with respect to the rate of metabolic degradation *in vivo* and that the degree of heterogeneity may vary considerably among different preparations. It has also been recently established that the appearance of heterogenous fractions of insulin-I^{131} is a consequence of alteration of the native protein by self-irradiation from the I^{131} present.

In the present study, trace labeled albumin-I^{131} solutions containing 20 mg. per cent albumin were subjected to radiation doses of 50,000 r delivered over a half-hour

period by a 1 Mev peak energy x-ray machine. Aliquots of the same solutions not subjected to x-irradiation were used for control purposes. Following intravenous administration to man the irradiated preparations underwent much more rapid *in vivo* degradation than the control solutions as indicated by a steeper fall in plasma protein bound radioactivity and an almost three-fold increase, during the first 3 days, in urinary excretion of non-protein bound I^{131} released by metabolic degradation of the labeled protein. Increase in the albumin concentration to 500 mg. per cent prior to irradiation protected partly, but not completely, against the damaging effects of irradiation.

The x-ray doses used in these experiments are equivalent to the self-irradiation received in one week by solutions of albumin-I^{131} containing 700 μc I^{131} per ml. Since the preparations used in investigations of serum albumin metabolism commonly receive this order of irradiation, the validity of results obtained with such material merits careful scrutiny. Although irradiation damage can be minimized by giving consideration to the protective effect of high protein concentration as well as to reduction in the cumulative self-irradiation dose, attempts should be made to establish biologic identity between labeled and native proteins in each specific instance if a valid tracer effect is to be accepted.

The Effect of the Intragastric Administration of Whole Blood on the Blood Ammonia, Blood Urea Nitrogen and Non-Protein Nitrogen in Patients with Liver Disease. PHILIP C. YOUNG, CHARLES R. BURNSIDE, and HARVEY C. KNOWLES, JR., Cincinnati, O. (Introduced by Leon Schiff).

Hepatic coma has been observed to frequently follow upper gastrointestinal bleeding in the decompensated cirrhotic. Since the blood ammonia concentration is frequently elevated in patients with hepatic coma, observations were made of the effects of blood ingestion on blood nitrogenous components in normal individuals and in those with liver disease.

Eight normal healthy male volunteers were each phlebotomized of 500 cc. of blood, and the blood was introduced into the stomach through a Levine tube. Eight patients convalescing from liver disease (six with decompensated cirrhosis, one with hepatitis and one with fatty liver) were each given 500 cc. of banked human blood, in a similar manner. Following the ingestion of the blood, determinations of serum urea nitrogen, non-protein nitrogen, and blood ammonia were made at intervals during a 9-hour period. Ammonia was determined by a modification of the microdiffusion method of Conway.

In the normal individuals, blood ammonia levels rose slowly from a mean control value of 25 gamma per cent to a maximum of 48 gamma per cent within 3 hours, and returned to normal by the end of 9 hours. Serum urea nitrogen and non-protein nitrogen concentrations increased slightly during the 9-hour period.

The eight patients with liver disease all exhibited a rapid rise in blood ammonia from a mean control value of 48 gamma per cent to 155 gamma per cent within 2 hours. The ammonia remained at peak levels for an additional hour then gradually returned toward normal, remaining slightly elevated even at the end of 9 hours. The concentrations of serum urea and non-protein nitrogen rose slightly in degree similar to that of the controls. Three of the patients with decompensated cirrhosis became drowsy when ammonia levels were maximally elevated.

It is concluded that the absorption of blood from the gastroenteric tract of patients with liver disease results in abnormally high concentrations of blood ammonia.

Movement of Potassium Into Skeletal Muscle During Spontaneous Attack in Family Periodic Paralysis. KENNETH L. ZIERLER * and REUBIN ANDRES, Baltimore, Md.

In normal man there is a diurnal variation in net potassium exchange between skeletal muscle and extracellular fluid. During the middle of the night potassium tends to move into muscle and at mid-morning to move out of muscle. Since spontaneous attacks in classical family periodic paralysis are apt to occur in the middle of the night it seemed possible that in patients with this disease there were exaggerated diurnal variations in potassium exchange which led to hypokalemia and paralysis. This appears to be the case. Metabolism of forearm muscles was studied in two patients with periodic paralysis and in three normal subjects throughout the night. During development of a spontaneous attack of paralysis potassium moved into muscle at a rate of approximately 1 mEq. per hr. per Kg. muscle. This rate was adequate to explain the observed decrease in serum potassium concentration. During spontaneous recovery from a spontaneous attack, potassium moved from muscle at a rate of about 4 mEq. per hr. per Kg. of muscle, thereby raising the concentration of potassium in serum and terminating the attack. Both these rates are at least five to ten times greater than those seen in normal subjects. Collateral data reveal associated disturbances in glucose, lactate, and CO_2 metabolism, but these do not appear to be etiologically related to the exaggerated potassium movement. It is suggested that unusual permeability of muscle membrane to potassium is a fundamental defect in family periodic paralysis.

Effect of Extracts of Leucocytes and of Synovial Membrane on a Mucoprotein from Cartilage. MORRIS ZIFF,* H. JOEL GRIBETZ, and JOSEPH LOSPALLUTO, New York, N. Y.

The effects of inflammation on the ground substance of cartilage have been studied by examining the action of extracts of leucocytes and inflamed synovial membrane on a mucoprotein isolated from this source. This mucoprotein, which is highly viscous and contains 75 per cent chondroitin sulfate, is the major constituent of the ground substance of cartilage. Changes in viscosity and composition of the mucoprotein were studied.

Extracts of leucocytes from the peripheral blood and of synovial membranes from patients with either rheumatoid arthritis or degenerative joint disease were prepared by standard procedures. When active extracts were incubated at neutral pH with dilute solutions of the mucoprotein, the viscosity of the mucoprotein fell rapidly. Similar effects were produced by treatment with trypsin.

The results of variation of concentration, pH and temperature suggested that the effect of the extracts was an enzymatic one. Extracts of mouse kidney, liver, lung and spleen showed no activity.

Formal titration showed that less than 1 per cent of the total nitrogen was liberated when the viscosity of the mucoprotein was reduced 50 per cent. When the products of enzymatic digestion were filtered through sintered glass funnels, filtrable and non-filtrable fractions were obtained. Although the untreated mucoprotein had a N to Hexosamine ratio of 3.9, the N to Hexosamine ratio in the filtrable fraction was 20, and in the non-filtrable 3.0, indicating that splitting into chondroitin sulfate-rich and chondroitin sulfate-poor fractions had occurred.

Specimens of synovial membranes from patients with rheumatoid arthritis showing active inflammation yielded active extracts, whereas those from patients with degenerative joint disease showed no activity, suggesting that erosion of cartilage in the rheumatoid process may be related to the presence of mucoprotein-splitting enzymes in the inflammatory cells.

Lactic Dehydrogenase in Human Serum. H. J. ZIMMERMAN and H. G. WEINSTEIN, Chicago, Ill. (Introduced by David I. Abramson).

An enzyme which can catalyze the conversion of lactate to pyruvate has been demonstrated in the serum of animals and humans. The serum level of this enzyme has been studied in 50 normals and 300 patients. The procedure used involved the determination of reduced DPN in the presence of serum and lactate with results expressed as micromals DPN $\times 10^3$. The levels of the enzyme did not seem to be affected by the overnight fast. The mean activity of 0.1 ml. of normal serum (181 ± 7) was found to be approximately equivalent to that produced by 15 micrograms of crystallized rabbit muscle lactic dehydrogenase.

Marked elevations were found in 50 per cent of patients with neoplastic diseases, in patients with acute myocardial infarction, in the two patients with infectious mononucleosis, the three with sickle cell anemia and in one with megaloblastic anemia. Moderate elevations were found in some patients with jaundiced cirrhosis and early hepatitis. It is tentatively suggested that increased serum levels of this enzyme represent necrosis of tissue with a normal enzyme content; or release from tissue with an unusually high enzyme content related to the increased rate of glycolysis.

Termination of Ventricular Fibrillation in Man by Externally-Applied Electric Countershock. PAUL M. ZOLL, ARTHUR J. LINENTHAL, WILLIAM GIBSON, MILTON H. PAUL, and LEONA R. NORMAN, Boston, Mass. (Introduced by Samuel L. Gargill).

We have previously reported to this Society the use of external electric cardiac stimulation to resuscitate the heart from ventricular standstill. Now we are reporting the termination of ventricular fibrillation 11 times in four patients, and ventricular tachycardia once, by a new procedure of external application of electric countershock across the intact chest. We used 60-cycle alternating current for 0.15 second at 240 to 720 volts.

In 3 patients with fibrillation after acute myocardial infarction, procainamide for a rapid arrhythmia, and ventricular tachycardia from digitoxin, defibrillation was carried out only after long delays. Countershocks were followed by ventricular standstill, by spontaneous ventricular beats without demonstrable output, or by recurrent fibrillation; respiration ceased, effective circulation was not restored, and the patients died.

The fourth patient, with Stokes-Adams disease, was resuscitated three times from ventricular fibrillation and recovered completely. Ventricular tachycardia followed defibrillation twice; once it stopped spontaneously and once it was terminated by a second countershock. On each occasion, ventricular standstill followed the termination of the ventricular fibrillation or tachycardia. During this standstill external electric stimulation with a cardiac pacemaker produced effective ventricular beats and maintained an adequate circulation until a spontaneous ventricular rhythm returned.

External countershock offered the only means of defibrillation in these cardiac patients, in whom thoracotomy with cardiac massage and direct countershock could not be undertaken. These experiences demonstrate that externally-applied electric countershock is an immediately effective, safe, and clinically feasible procedure for termination of ventricular tachycardia and fibrillation. In the experimental animal we have also stopped atrial fibrillation and atrioventricular nodal tachycardia.

Unexpected cardiac arrest in the operating room is due to ventricular standstill or fibrillation. In either event external electric stimulation and defibrillation comprise a combined technique for resuscitation before recourse to the more formidable procedure of cardiac massage.