The Biosynthesis of the Purine Ring. D. A. GOLDTHWAIT, G. R. GREENBERG, and R. A. PEABODY, Cleveland, O. (Introduced by H. S. Ginsberg).

The purine nucleotides occupy a key role in the biosynthesis of many cofactors, of nucleic acids, and of adenosine triphosphate (ATP). Since Aminopterin and azaserine, both effective tumor chemotherapeutic agents, block the synthesis of purine nucleotides, an understanding of the mechanism of purine biosynthesis should lead to the development of more oncolytic agents. The synthesis of the nucleotide inosinic acid has been studied in an acetone powder extract of pigeon liver. With the proper additions and the omission of $KHCO_3$, early precursors of inosinic acid accumulate. These have been isolated and purified by paper and ion-exchange chromatography. One has been assigned the structure glycine amide ribotide (I), while the other (II) is similar but contains a formyl group. These compounds have since been shown in another laboratory to accumulate in the presence of azaserine. In the sequence of reactions leading to the synthesis of these compounds, ribose-5-phosphate reacts with ATP to form ribose-1-pyrophosphate-5-phosphate (PRPP). This compound has been shown to react with glutamine, but the product is still unknown. PRPP, glutamine, glycine, and ATP plus the Dowex treated dialyzed enzyme react to form I. If formate and tetrahydrofolic acid or citrovorum factor are included II is synthesized. Aminopterin blocks the reduction of folic acid to tetrahydrofolic acid and thereby inhibits the transfer of formate *via* tetrahydrofolic acid to I and also to imidazolecarboxamide ribotide (III) to form inosinic acid. N^{10}-formyltetrahydrofolic acid and not citrovorum factor is the active transformylating cofactor in this system. The reactions between II and III which must include ring closure and addition of two nitrogens and the carbon of CO_2 are unknown. Studies on the mechanism of fixation of CO_2 into the purine ring suggest that Krebs cycle intermediates are not involved.

The Detection of Pulmonary Congestion Utilizing the Valsalva Maneuver. RICHARD GORLIN, JOHN H. KNOWLES, and CLIFFORD F. STOREY, Portsmouth, Va. (Introduced by Eugene C. Eppinger).

The immediate effect of the Valsalva maneuver is to impede thoracic venous inflow with resultant fall in systemic arterial pressure during the strain. With release of the strain, systolic and pulse pressures both increase; *i.e.*, the "overshoot." Both auscultatory and directly recorded systemic arterial pressures have been measured during the Valsalva maneuver in 55 patients, with particular attention directed toward changes during straining. The time of and rate of fall of arterial pressure during strain was altered by body position and certain disease states. Arterial pressures decreased immediately during the strain in standing normals, and in pregnancy, pulmonary disease and mild mitral stenosis. Recumbency resulted in larger pulse pressures in all groups. Recumbency with feet elevated resulted in maintenance of arterial pressure from 2 to 3 seconds in some normals to longer periods in patients with minimal pulmonary congestion. In severe mitral stenosis, constrictive pericarditis, and left ventricular failure, arterial pressure was sustained seven seconds or more in all positions with intensification in recumbency. During the maneuver peripheral venous pressure rose in linear fashion but never exceeded the positive pressure of straining until after the first eight seconds. The poststraining overshoot was absent in general when arterial pressure was sustained during straining. Sustained arterial pressures during the maneuver correlated with prolonged circulation time, decreased vital capacity and total lung volume. Ausculted blood pressure was sufficiently accurate to differentiate between the response with congestion and in the normal and to detect the presence or absence of an overshoot. As such the arterial pressure response to the Valsalva maneuver was utilized as a simple clinical test for the detection of pulmonary congestion. Cardiac dyspnea has been separated from that due to pulmonary disease; pulmonary congestion has been detected where unsuspected; and the effects of cardiac surgery on pulmonary congestion easily evaluated.

Continuous Recording of Thoracic-Aorta Flow in Man. JOSEPH B. GRACE, WILLIAM P. CROWLEY, IRWIN J. FOX, and EARL H. WOOD,* Rochester, Minn.

The dilution technic using constant-rate injection of the indicator is the closest approach to an acceptable method for continuous recording of cardiac output in man. The period during which instantaneous flow can be recorded is, however, limited by recirculation of indicator. If, however, the injection is made into the thoracic aorta, only recirculated indicator reaches the aortic-arch arteries and in identical concentrations to the recirculated indicator which is "contaminating" thoracic-aorta blood as the injection continues. Under these conditions the concentration of recirculated indicator can be recorded from a radial artery and used as a baseline for measurement of injected indicator on its first passage by the sampling site (femoral artery) throughout any duration of constant-rate injection that may be desired. Then if (1) uniform mixing of an indicator injected in the thoracic aorta occurs, (2) the rate of injection is known, and (3) the indicator concentration is recorded continuously at a femoral and radial artery, the instantaneous blood flow down the thoracic aorta can be obtained for the duration of the injection. Constant-rate 60-second injections of T-1824 were made *via* an 80-cm. (0.5 I.D., 1.0 mm. O.D.) catheter into the thoracic aorta while its concentration was recorded from the radial and femoral arteries of 21 subjects. Erratic results for thoracic aortic flow (average: 64, range: 46 to 90 per cent of cardiac output [direct Fick]) indicated that uniform mixing was not obtained. With an end-sealed catheter having six (0.1 to 0.2 mm. diameter) radially spaced holes near the tip and an injection rate of 30 ml. per minute, thoracic aorta flow ranged from 69 to 77 per cent of cardiac output. Flows recorded

during Valsalva and tilting maneuvers showed similar directional changes to mean arterial (subclavian) pressure.

Relationship Between the Stomach and the Adrenal Cortex. SEYMOUR J. GRAY,* LEWIS J. KRAKAUER, RAMON VILLAREAL, COLIN G. RAMSEY, and ROBERT REIFENSTEIN, Boston, Mass.

The present report encompasses evidence of a relationship between gastric activity and adrenal cortical function.

Adrenocorticotrophic hormone or the adrenal glucocorticoids (Compounds E, F, B) produce a considerable increase in gastric juice acid and pepsin both in human subjects and in Heidenhain pouch dogs. The chloride concentration of the gastric juice increases significantly and is accompanied by a fall in sodium and potassium concentration. A single injection of 40 units of intramuscular ACTH to Heidenhain pouch dogs elicits a significant gastric response within four to six hours. A more prolonged stimulation is necessary in humans. Removal of the antrum and vagectomy do not alter the gastric response to the adrenal steroids in humans or experimental animals, indicating a direct action of the glucocorticoids upon the stomach. Mineralocorticoids, testosterone, and estrogens are ineffective.

The urinary uropepsin excretion, as a measure of gastric peptic activity, has been correlated with the urinary excretion of 17-hydroxycorticoids and 17-ketosteroids to investigate the relationship between gastric and adrenal activity.

A diminished uropepsin excretion paralleled by a decrease in urinary steroid excretion was observed in twelve patients with untreated Addison's disease (P less than 0.01). With replacement glucocorticoid therapy the uropepsin returned to normal. Conversely in twelve patients with adrenal hyperfunction (Cushing's disease) a significant increase in uropepsin excretion was accompanied by an elevation in the urinary corticoids (P less than 0.01).

The relationship between the stomach and the adrenal gland is further borne out by a significant correlation of uropepsin and adrenal corticoid excretion in normal subjects. This correlation is significantly implemented during the administration of ACTH, and is of a greater order of magnitude than that observed in the unstimulated normals.

The Biophysics of the Variants of Sickle Cell Disease. ROBERT C. GRIGGS and JOHN W. HARRIS, Cleveland, O. (Introduced by Frederick C. Robbins).

The mechanisms have been investigated concerning the intracellular content of S-hemoglobin and the oxygen tensions at which the erythrocytes sickle, as causally related to the severity of the clinical and hematological manifestations of the various syndromes associated with sickle hemoglobin.

Six patients with sickle trait, six with sickle-C disease, three with sickle-Thalassemia disease and eight with sickle anemia were studied. At various oxygen tensions observations were made on tactoid formation in hemoglobin solutions, and on the number of sickled cells, erythrocyte mechanical fragility and viscosity of whole blood samples. For a sickle hemoglobin concentration of 90 to 100 per cent, as observed in the erythrocytes of the clinically severe sickle anemia, an oxygen tension as high as 60 mm. of mercury produces the biophysical changes *in vitro* of increased numbers of sickled cells, increased mechanical fragility and increased whole blood viscosity. For sickle hemoglobin concentrations of 50 to 80 per cent, as observed in the clinically intermediate sickle-C and sickle-Thalassemia diseases, a lower oxygen tension, 40 mm. of mercury, is required to produce comparable biophysical changes. Both of these oxygen tensions can occur physiologically. However, for sickle hemoglobin concentrations of 24 to 45 per cent, as observed in the clinically benign, non-anemic sickle cell trait, the oxygen tension must be extremely reduced, to a non-physiologic level of 10 mm. of mercury, to produce the above biophysical changes.

The formation of small tactoids in concentrated hemoglobin solutions and the increased mechanical fragility occurred at higher oxygen tensions than are required to effect sickling. These findings suggest that small intracellular tactoids, not large enough to distort the intact cell produce alterations leading to increased mechanical fragility.

These observations indicate that the degrees of anemia and circulatory alterations found in the variants of sickle cell disease are related to the intracellular content of S-hemoglobin and the biophysical changes produced by low oxygen tensions.

A Method for Estimating the Inferior Vena Caval Blood Flow in Man. JACOB GROSSMAN, SHIRLEY RUBLER, HELEN B. HOROWITZ, and RAYMOND E. WESTON,* New York, N. Y.

The modified Fick principle previously employed in the laboratory for the measurement of cardiac output without gas analysis, using PAH as the test substance, has been adapted for the estimation of lower inferior vena caval blood flow. The method involves placing the tip of a double lumen catheter into the right renal vein with the opening of the other lumen in the inferior vena cava above both renal veins. From the plasma concentration of PAH in arterial ($[PAH]_A$) and caval ($[PAH]_{VC}$) blood samples, simultaneously withdrawn at intervals during determinations of renal plasma flow (RPF), the lower inferior vena caval blood flow (LVCF) may be calculated from the following formula:

$$LVCF = RPF \frac{[PAH]_{VC} - [PAH]_{RV}}{[PAH]_A - [PAH]_{VC}} \times \frac{1}{1 - Hct}$$

where $RPF = C_{PAH}/E_{PAH}$, $[PAH]_{RV}$ = renal venous plasma PAH concentration, and Hct = hematocrit.

The estimated LVCF includes largely the venous drain-

age from both lower extremities including the gluteal region, the left gonad and adrenal gland, and, in females, the pelvic reproductive organs.

In eight normal and eight cardiac subjects, the estimated LVCF ranged from 25 to 33 per cent of the cardiac output which was measured during the procedure, although not simultaneously. In patients with decreased cardiac outputs, LVCF was proportionately reduced. When the cardiac output was increased by exercise or pyrogen reaction, a proportionate increase in LVCF occurred. The assumptions and errors inherent in the methods will be discussed.

Nucleic Acid Metabolism in Chronic Leukemia. L. D. HAMILTON, New York, N. Y. (Introduced by Joseph H. Burchenal).

Adenine-8-C^{14} was given intravenously to patients with chronic lymphocytic and chronic myelocytic leukemia. Ribose nucleic acid (RNA) and deoxyribose nucleic acid (DNA) were separated from cells collected over periods of up to 670 days, and the radioactivities of the separated individual purines determined.

In the granulocytic cells the incorporation of adenine as such into the DNA was as extensive as into the RNA, and there was extensive transformation of adenine into RNA and DNA guanine. The retentions of isotope in the adenine and guanine of RNA and DNA were similar—consistent with data from other rapidly dividing tissues. In contrast, in lymphocytes, the incorporation of adenine into DNA (and its transformation into guanine) was less extensive than into RNA.

The decline in activities of both RNA and DNA of lymphocytes could be divided into two phases—an initial more rapid component (DNA adenine of roughly 18 days half-time), and a residual slow component showing a half-time of the order of 300 days. In granulocytes there was no such prolonged retention of the isotope in the polynucleotide purines—activity could not be demonstrated beyond 140 days. The decline in activity of DNA adenine in the first component had a half-time of approximately 9 days, and the second component with a somewhat longer half-time, was a relatively insignificant proportion of the total.

These results suggest that leukemic granulocytes survive for shorter times than leukemic lymphocytes; we cannot yet tell whether the results in lymphocytes represent the survival of leukemic lymphocytes for very long periods, or a specific re-utilization by lymphocytes of large fragments of nucleic acid of their predecessors. The latter interpretation is favored by the relative insignificance of the second component in granulocytes, and the morphological evidence compatible with re-utilization —phagocytosis of lymphocytes by reticulum cells in lymph nodes. Such re-utilization could be a method for blood cell differentiation following transformation of the primitive multipotential cell by phagocytosed DNA.

The Relationship of Platelets to the Serum Potassium Concentration. ROBERT C. HARTMANN and SHERMAN M. MELLINKOFF, Nashville, Tenn. (Introduced by Elliot V. Newman).

A patient with unexplained thrombocytosis (platelet count 1 to 2 million per cmm. of blood) was noted to have a high serum potassium concentration (7.0 to 8.0 mEq. per liter) on routine determination. There was no clinical evidence of or explanation for the apparent hyperkalemia. The white cell count was normal. The serum potassium concentration could be correlated with the platelet concentration of the plasma or blood from which the serum was obtained. Serum from "platelet-free" plasma (<1000 platelets per cmm.) had a potassium concentration of 5.0 to 5.4 mEq. per liter, whereas "platelet-rich" plasma (2 to 3 million platelets per cmm.) yielded serum containing 8.0 to 9.5 mEq. per liter potassium. There was no evidence that the red cells were concerned in the results obtained. An appreciable amount of potassium was released from the platelets *immediately* after coagulation; it was not necessary for the clot to remain in contact with the serum for a prolonged period.

In three of six other patients with thrombocytosis this phenomenon was sufficiently marked so that serum from "platelet-rich" plasma contained from 1.3 to 2.1 mEq. per liter more of potassium than serum yielded by "platelet-free" plasma. There was no evidence that these platelets had a mass appreciably greater than that of normal platelets. In normal human subjects there was no consistent or significant influence of the platelets on the serum potassium concentration even when platelets were artificially concentrated *in vitro* to values approximating those of the patients with thrombocytosis. It is concluded that in some patients with thrombocytosis a false hyperkalemia may be attributed to the abnormal platelets.

The Production of Antibody by Partially Hepatectomized Rats. W. PAUL HAVENS, JR.,* MARY ELLEN SCHLOSSER, and JEAN KLATCHKO, Philadelphia, Pa.

The demonstration by Paschkis *et al.* that certain transmissible tumors had an increased rate of growth following implantation in partially hepatectomized rats suggested that an attempt be made to determine whether a specific stimulus associated with regeneration of liver might account for the enhanced production of antibody by many patients with hepatic cirrhosis. Four experiments, employing 192 adult male rats, were performed. Sheep erythrocytes were injected intravenously immediately after sub-total hepatectomy in 64 animals. Equally numbered groups of normal controls and laparotomized rats were also injected with the same antigen, and the titers of sheep-cell hemolysin were determined six days later. The geometric mean of the hemolysin titers (reciprocals) in the partially hepatectomized rats was 1310, compared with 570 and 520, respectively, in the normal controls and the laparotomized group. This difference is statistically significant, although not great enough (within the limits of accuracy of the test) to claim an increased potential in the partially hepatectomized rats.

There was no apparent relationship between changes in weight and the antibody produced. Approximately 40 per cent of the control and laparotomized groups gained during the experiment, while almost all the partially hepatectomized rats lost weight in significantly greater amounts than those which lost in the other two groups. Hypoalbuminemia and hyperglobulinemia are present for many days after partial hepatectomy or laparotomy. In partially hepatectomized rats, proliferation of the liver occurs, and after six days, when antibody titers were measured in these experiments, the mass of hepatic tissue was approximately its original magnitude. Under these conditions, with such great demands on available proteins, it is of interest that the partially hepatectomized rats made more antibody than their controls although the amounts made were insufficient to warrant the interpretation that any specific stimulating effect of regenerating hepatic cells was operative.

Vascular Adjustments after Phlebotomy in Polycythemic Subjects. HANS H. HECHT,* WALTER GAYLOR, and DAVID STEIN, Salt Lake City, Utah.

Hemodynamic adjustments of fifteen polycythemic patients were studied both before and after bleeding to normal packed red cell volumes. Four subjects had true erythremia, and eleven patients suffered from erythrocytosis with cor pulmonale secondary to emphysema. Microcytosis and reduced mean corpuscular hemoglobin concentration were common in the erythrocytosis group. None of the patients were in heart failure at the time of the study. Bleeding was carried out slowly over two to three weeks with a fall of packed red cell volume of 27 per cent (17–45). The usual methods for determination of respiratory and blood gas analysis, renal clearance, and plasma volume were employed. Pulmonary vascular pressures, right ventricular output and total pulmonary resistance were calculated, using the cardiac catheter.

All hemodynamic and renal data were normal in the erythremic subjects and remained so after bleeding. The absolute changes in the red cell mass were reflected in the plasma volume which rose from 31 ml. per kg. before, to 44 ml. per kg. after repeated bleeding.

In the patients with cor pulmonale no uniform response was obtained. Arterial oxygen content fell in all but one patient. Arterial oxygen saturation remained unchanged for the group with a mean of 66 per cent (53 per cent to 79 per cent) before, and 68 per cent (42 per cent to 84 per cent) after bleeding. Oxygen saturation rose moderately in four subjects, fell in two, and remained unchanged in five. Pulmonary artery pressures, elevated in all subjects with cor pulmonale, generally varied inversely with arterial oxygen saturation or remained unchanged. With the exception of two subjects in whom the onset of heart failure appeared to be precipitated by bleeding, no significant trends were observed in the other respiratory, circulatory, and renal functions studied.

Repeated bleeding may improve subjective symptoms of polycythemia and may lower blood viscosity. It has no predictable effect on the vascular alterations of cor pulmonale.

Lactate Metabolism in Cushing's Syndrome. DOROTHY H. HENNEMAN and JOHN P. BUNKER, Boston, Mass. (Introduced by Henry K. Beecher).

In an effort to clarify the disturbance in carbohydrate metabolism in Cushing's Syndrome, certain aspects of intermediary carbohydrate metabolism have been investigated. Plasma lactate was measured in twelve patients with Cushing's Syndrome and in one female patient with a masculinizing adrenal tumor with coincident features of Cushing's Syndrome. Fasting plasma lactate concentrations were significantly above normal. In three patients so studied the plasma lactate concentrations returned to normal two to three weeks post-operatively. Nine of these patients were studied during 70 to 180 minutes of ether anesthesia for adrenalectomy. All showed marked plasma lactate elevations during anesthesia which were significantly greater than the slight elevation in lactate of other patients under ether anesthesia, including two patients undergoing adrenalectomy for metastatic carcinoma. Pure d-lactate, according to Soffer's technique, was infused intravenously pre-operatively in seven patients with Cushing's Syndrome and all manifested a decreased lactate tolerance. Three dermatological patients treated with large doses of ACTH or cortisone for one to three months failed to show elevations in fasting plasma lactate concentrations or intolerance of d-lactate infusions. These findings suggest a decreased utilization of lactate in Cushing's Syndrome, perhaps due to disturbance in liver function. The failure to demonstrate similar changes after short term induced hyperadrenocorticism suggests that the duration rather than the severity of the disease is responsible for the lactate intolerance.

The Suppression of Urinary Calcium and Magnesium Excretion by Oral Sodium Phytate. PHILIP H. HENNEMAN and EVELYN L. CARROLL, Boston, Mass. (Introduced by Fuller Albright).

Our previous finding of increased calcium absorption as an explanation for the hypercalcuria of sarcoid patients emphasized the need for an agent which would antagonize vitamin D and decrease calcium absorption. The effect of sodium phytate on calcium, magnesium, sodium, potassium, phosphorus, and nitrogen metabolism has now been tested in four balance studies. Sixteen additional patients have been treated with this drug during the past 24 months.

Sodium phytate (inositol hexaphosphate) forms insoluble and apparently unabsorbable complexes with calcium and magnesium. In balance studies the daily oral administration of 9.0 grams caused increases in the daily fecal calcium ranging from 88 to 244 mg. and decreases in the urinary calcium ranging from 55 to 402 mg. (28 to 97 per cent). The same dose concomitantly caused increases in the fecal magnesium ranging from 17 to 88

mg. and decreases in the urinary magnesium ranging from 35 to 62 mg. per day. That the urinary calcium may be decreased to a greater extent than the fecal calcium is increased may be due to the absorption of inorganic phosphate released from sodium phytate by intestinal phytases.

The only adverse effect of the oral administration of 9 grams sodium phytate has been diarrhea which is usually mild and diminishes with time, and, more rarely, nausea and vomiting. It is of physiological interest that oral sodium phytate in the dose administered has only a small effect on calcium and magnesium balances, its main effect being to block absorption of dietary calcium and magnesium.

Sodium phytate may prove of specific therapeutic value in hypervitaminosis D, sarcoid and berylliosis with hypercalcuria, in certain types of renal lithiasis, and, possibly, in hemochromatosis. The latter prediction derives from the previously demonstrated *in vitro* removal of iron from solutions by sodium phytate.

The Effects of Parathyroid Extract on Renal Function in Man. HOWARD H. HIATT and DAVID D. THOMPSON, Bethesda, Md. (Introduced by Ephraim Shorr).

The mechanism of the increase in urinary phosphate which follows the administration of parathyroid extract has been a subject of controversy. We have studied the acute effects on renal function of parathyroid extract and its action after prolonged administration.

In 28 studies on six normal and two hypoparathyroid subjects renal reabsorption of phosphate was calculated at endogenous and elevated serum phosphate levels. Inulin clearances were determined in all studies. The infusion of buffered sodium phosphate permitted calculation of maximal tubular reabsorption of phosphate (Tm P).

Intravenous administration of 200 to 1000 USP units of parathyroid extract (Lilly) produced no immediate measurable change in TM P in five normal subjects in 11 studies, and a 40 per cent depression in Tm in two hypoparathyroid individuals. A second preparation of extract given to one of these hypoparathyroid subjects did not alter phosphate Tm. In all studies, a prompt rise in renal blood flow and urinary phosphate was observed. In the studies in which phosphate Tm was unchanged, the increased phosphate excretion was attributable to the rise in filtered phosphate secondary to an increased glomerular filtration rate.

Administration of 1600 to 4000 units of extract over 48 to 96 hours produced a 20 to 60 per cent fall in phosphate Tm in all normal and hypoparathyroid subjects. The depression in Tm occurred after prolonged administration of all preparations of extract employed. All preparations produced hypercalcemia.

Our studies support the following conclusions:

1. Renal tubular reabsorption of phosphate is depressed by parathyroid extract, although an effect is demonstrable in normal subjects only after prolonged administration.

2. The increased urinary phosphate observed in normal individuals following acute administration of extract is generally attributable to a rise in filtered phosphate.

3. Preparations of extract vary in their capacity to depress phosphate Tm acutely in hypoparathyroid subjects.

Observations of Magnesium Metabolism in Man. A. G. HILLS, D. W. PARSONS, O. ROSENTHAL, and G. D. WEBSTER, JR., Philadelphia, Pa. (Introduced by William A. Jeffers).

Evidence that renal magnesium excretion is influenced by renal sodium chloride excretion was obtained in humans. Seven subjects, each kept on a constant diet throughout, were observed during a control period followed by a 3- or 4-day experimental period in which the only change of regimen was withdrawal of a sizable supplement of enteric-coated sodium chloride. Twenty-four hour urinary excretion of sodium chloride and magnesium decreased during the experimental period in all subjects, the mean change of urinary magnesium being -21.8 ± 3.7 per cent ($P < .01$). Quantitatively similar results (urinary magnesium decrease 28 ± 6.6 per cent, $P < .05$) were obtained in four adrenal-deficient patients in whom maintenance steroids were withdrawn together with the salt supplement. The changes reflected renal tubular activity since the ratio magnesium clearance: glomerular filtration rate fell.

In four of five subjects studied, fecal as well as urinary magnesium decreased. Definite rise of serum magnesium concentration was observed only in adrenal-deficient subjects developing evidence of adrenal insufficiency. Net magnesium retention of the indicated magnitude without change of extracellular magnesium concentration would suggest homeostatic transfers of magnesium out of extracellular fluid in subjects with adequate adrenal function.

Six-day adrenal stimulation with ACTH significantly decreased urinary excretion of magnesium, sodium, and chloride in another subject, and cortisone had similar effects in a totally adrenalectomized hypertensive, sodium chloride intake being constant in both patients. Hormone withdrawal exerted opposite effects.

The data indicate that adrenal cortical hormones influence urinary magnesium excretion only insofar as they affect urinary sodium and chloride excretion. Urinary potassium excretion appears unrelated to urinary magnesium. In experiments effecting change of 24-hour urinary excretion of sodium *or* chloride ion independently, urinary magnesium excretion paralleled urinary chloride, but urinary sodium and magnesium excretion varied in opposite directions.

Abnormalities of Energy Metabolism in Congestive Heart Failure and Their Quantitative Relationship to Circulatory Changes. WILLIAM E. HUCKABEE, Boston, Mass. (Introduced by Chester S. Keefer).

Measurements of tissue anaerobic metabolism were derived from changes in relationships between lactate and pyruvate net increment rates. Balances between aerobic and anaerobic energy turnover constitute measures of

"adequacy" of oxygen supply for the support of intracellular oxidative processes, independent of blood flow or tissue demand taken separately. In 11 normal subjects and 18 patients with heart failure this method was applied to the whole body through analysis of arterial blood, and integrated with cardiac catheterization data.

Cardiacs relied on chemically anaerobic mechanisms to meet cellular energy requirements much more than normal subjects, with no overlapping of data from the two groups. In normals 92 to 98 per cent of tissue energy demands in mild exercise were met by circulatory response. Calculations showed that patients with diseased hearts increased cardiac output by only 30 to 60 per cent as much as called for by metabolic needs; and only 50 to 80 per cent of oxygen required by the tissues was delivered, despite increased blood oxygen extraction; the remainder of total energy expenditure was supplied by peripheral tissue anaerobic mechanisms (61 to 185 ml. O_2-equivalents per min.). Thus O_2 consumption gave a grossly erroneous estimate of exercise severity in patients.

Results of this estimate of "circulatory adequacy" were not predictable from observed changes in O_2 consumption, $(A\text{-}V)O_2$ or cardiac pressures, whether these were greatly altered or entirely normal, as in six cases of Class I heart disease and nine of post-valvulotomy mitral stenosis, which showed metabolic abnormalities of characteristic types.

Conclusions: 1) Insufficiency of blood supply to the body is a possible basic mechanism in the syndrome of heart failure, regardless of cardiac output or even of venous blood oxygen.

2) Major compensatory responses to circulatory insufficiency include not just increase in vascular pressures and blood oxygen extraction, but also significant utilization of energy sources other than oxygen (anaerobic glycolysis) to maintain life processes, an abnormality extremely sensitive to cardiac impairment.

Method for the Prevention of Ventricular Fibrillation during Hypothermia. CHARLES A. HUFNAGEL, PIERRE RABIL, and JOHN C. ROSE, Washington, D. C. (Introduced by Laurence H. Kyle).

The use of hypothermia for open heart procedures offers direct correction of complicated defects, but the high rate of ventricular fibrillation during ventriculotomy below 26° C. has been a deterrent to its application. This study attempts to compare the value of intravenous Prostigmin,® S-A nodal block with Xylocaine, and Prostigmin® plus S-A nodal block in preventing ventricular fibrillation in dogs with temperatures below 26° C.

The animals were divided into four groups. In group I a standard procedure was established. The rectal temperature was lowered to between 24 and 26° C., both venae cavae were occluded for 15 minutes, and a right ventriculotomy was performed. Under these conditions ventricular fibrillation occurred in at least 90 per cent of the animals. In group II an identical procedure was carried out except that at 30° C., .025 mg. Prostigmin® per pound of body weight was given intravenously. This was repeated with each degree of temperature fall thereafter until 26° C. In this group ventricular fibrillation was infrequent, and when it occurred, defibrillation was easily accomplished. In group III, immediately following opening of the chest, the S-A node was injected with 5 cc. of 1 per cent Xylocaine. Fibrillation occurred with approximately the same frequency as in group II. In group IV Prostigmin® was given as in group II, and following thoracotomy the S-A node was blocked with Xylocaine as in group III. Caval occlusion and ventriculotomy were carried out as in all groups. Ventricular fibrillation occurred in only one animal in this group.

Group IV animals showed a high rate of permanent recovery as well as protection against ventricular fibrillation. The combination of intravenous Prostigmin® in large doses and infiltration of the S-A node with Xylocaine appeared to provide better protection against ventricular fibrillation than either method alone.

The Origin of the Early Systolic Click of the Pulmonary Artery. HERBERT N. HULTGREN and JOHN J. KELLY, JR., San Francisco, Calif. (Introduced by Arthur L. Bloomfield).

An early systolic sound, frequently clicking in character and closely following the apical first sound, is occasionally heard at the pulmonic area in patients with pulmonary hypertension. During the past four years we have studied 45 cases exhibiting the early systolic click of the pulmonary artery with particular reference to its mode of production. The following observations have been made:

1. The first sound-click time is related, as pointed out by Leatham, to the duration of isometric contraction of the right ventricle. Isometric contraction times, measured by means of intracardiac pressure tracings and electrokymography in 100 patients with varying degrees of pulmonary hypertension, were found to be prolonged in proportion to the elevation of pulmonary artery diastolic pressure.

2. Three patients have been studied in whom a shortening of the P-R interval was associated with a delay and an increased intensity of the first sound but no change in position or intensity of the pulmonary sound.

3. Two patients without systolic murmurs exhibited loud Graham Steell murmurs which continued through the first sound and ended an instant before the pulmonary click.

4. Phonocardiograms recorded from the exposed heart during surgery revealed the click to be most intense over the main pulmonary artery and faint over the right ventricle or distal pulmonary artery. An abrupt checking of systolic expansion was palpable at the pulmonary valve ring but not over the artery.

5. Unsuccessful attempts have been made to reproduce the sound by suddenly tensing in a perfusion apparatus pulmonary arteries obtained at autopsy.

These observations indicate that the early systolic click of the pulmonary artery is not due to A-V valve closure

but that it is probably produced by sudden early systolic tensing of the fibrous pulmonary valve ring. Transmission to the chest wall is facilitated by pulmonary artery dilatation.

Sodium, Potassium, and Magnesium Balance during Recovery from Congestive Heart Failure Due to Cor Pulmonale. LLOYD T. ISERI and IVAN J. MADER, Detroit, Mich. (Introduced by William O. Maddock).

Metabolic studies conducted during recovery from heart failure due to low and high (beri-beri) output heart disease have previously shown uptake of cellular K and Na, and frequent loss of water. Calculations have also indicated inactivation of cellular base during this period.

A complete metabolic balance study of Na, K, Cl, Mg, N, and H_2O, using a constant low sodium diet of known analyzed composition, was undertaken during recovery in one case of cor pulmonale and in two cases of low-output failure. Measurement of cardiac, pulmonary, and renal hemodynamics, obtained by cardiac catheterization technique before and after recovery, established the diagnosis in the case of cor pulmonale.

Metabolic studies in all three patients, including the one patient with cor pulmonale, showed a marked loss of extracellular water, Na, and Cl, a slight gain of intracellular water, a marked gain of intracellular sodium, and a moderate gain of intracellular potassium. Two patients showed a gain of intracellular magnesium, but one patient showed a loss. All three patients showed a marked inactivation of cellular base.

These studies indicate that intracellular changes during recovery from congestive heart failure due to cor pulmonale are similar to those found in beri-beri and in low-output heart disease. The hypothesis that increased osmolarity of intracellular base following cardiac and circulatory insufficiency may be responsible for the train of events leading to congestive cardiac edema appears to apply in cor pulmonale as well as in other heart diseases.

Uridine Compounds: Their Role in Hormonal Glucuronide Formation. KURT J. ISSELBACHER and JULIUS AXELROD, Bethesda, Md. (Introduced by Seymour S. Kety).

The significance of uridine compounds in biologic reactions has become increasingly apparent in the past few years on the basis of the work of Leloir, Dutton, Storey, and Kalckar. These studies have included the isolation from mammalian tissues of uridine diphosphate (UDP) derivatives—including UDP-glucose, UDP-glucuronic acid, and UDP-N-acetyl glucosamine. The exact functions of these compounds in biologic systems remains to be elucidated.

It is known that the metabolites of adrenal, thyroid, and sex hormones are excreted in significant amounts as glucuronide conjugates, but the mechanism of their formation has remained obscure. Since free glucuronic acid or its lactone do not function as direct precursors of these glucuronides, we have investigated the possible role of UDP-glucuronic acid in hormonal glucuronide synthesis.

When tetrahydrocortisone is incubated with a cell-free preparation of mammalian liver, the addition of UDP-glucuronic acid results in the formation of tetrahydrocortisone glucuronide and the liberation of UDP. The enzyme catalyzing this transfer reaction is localized in the microsomal fraction of the liver cell and has been partially purified. In a similar manner we have obtained glucuronide formation of other hormones such as tetrahydro-hydrocortisone and L-thyroxine, and of non-hormonal substances such as phenolphthalein and morphine.

It would appear that UDP-glucuronic acid is an "active" form of glucuronic acid and an essential cofactor for the synthesis of glucuronides by the mammalian organism.

A Hemorrhagic State Following Multiple Whole Blood Transfusions. DUDLEY P. JACKSON and JULIUS R. KREVANS, Baltimore, Md. (Introduced by C. Lockard Conley).

A serious hemorrhagic disorder has occasionally followed the administration of multiple blood transfusions. To determine the cause, 29 adult patients and 5 newborn infants who received one or more compatible whole blood transfusions were studied. Sixteen adults who received more than 5,000 ml. of whole blood within 48 hours developed thrombocytopenia following the transfusions, and 12 exhibited clinical evidences of abnormal bleeding. In 14 cases, platelets were reduced to 60,000 per cmm. or less. Other studies of hemostatic function were performed in 7 patients who developed a hemorrhagic state. Fibrinogenopenia was encountered in only one instance, and evidence of increased fibrinolytic activity was not found. Some of the 13 adult patients who received less than 5,000 ml. of whole blood within 48 hours developed mild thrombocytopenia, but none had abnormal bleeding. In five consecutive cases of exchange transfusion for erythroblastosis fetalis, all of the infants developed severe thrombocytopenia, and 2 displayed clinical evidences of abnormal bleeding. The thrombocytopenia that was observed in these patients was related to the amount of whole blood transfused and to the rate of infusion. Its development was not prevented by the use of non-wettable blood bank equipment nor by the use of freshly drawn blood. Direct transfusion, using multiple silicone-coated syringes, failed to prevent the occurrence of thrombocytopenia in one case. Thrombocytopenia developed even though splenectomy was performed in one instance. The platelet depression persisted for three to five days following transfusion in most cases.

Thrombocytopenia has been produced in dogs following the administration of large amounts of compatible blood from donor dogs. Auto-transfusion of comparable quantities of whole blood administered by the indirect technique did not produce thrombocytopenia.

Alterations of the Plasma Protein Mosaic in Pneumococcal Infections in Rabbits. RALPH F. JACOX* and ALEXANDRA FELDMAHN, Rochester, N. Y.

A type 1 pneumococcal "dermal pneumonia" was produced in adult male rabbits and the sequence of plasma protein alterations was determined at daily intervals utilizing a cationic detergent fractionation method (Jacox, R. F., J. Clin. Invest., 32: 661, 1953; Jacox, R. F., J. Lab. & Clin. Med., 44: 885, 1954). Two days after the injection of pneumococci the rabbits developed a striking rise of plasma fibrinogen and alpha globulins. These fractions remained at high concentrations for eight days and by the tenth day slowly returned toward control levels. Between the second and tenth days of infection, plasma albumin was depressed and then rose slowly toward normal levels during the second and third week. The plasma beta-gamma fraction was decreased at the height of infection and then returned to normal or slightly elevated values during convalescence.

Five of thirteen rabbits with untreated pneumococcal infections developed a severe illness as judged by the intensity of pneumococcal bacteremia. Five others had a milder illness with a transitory and mild bacteremia. While each of these groups showed similar alterations in the plasma protein mosaic, those severely ill had significantly less elevation of the plasma alpha globulins within the first 24 hours after onset of infection. The severely bacteremic animals thereafter, developed a significantly greater rise of plasma alpha globulins and fibrinogen and a greater fall of plasma albumin than occurred in the mildly bacteremic group. Administration of penicillin 48 hours after the injection of type 1 pneumococci in another group of rabbits, partially inhibited the development of the plasma protein alterations observed in the untreated series.

These experiments indicate that the stimulus for synthesis of fibrinogen and alpha globulins and for depression of the albumin level is directly related to the intensity of stimulus produced by the infecting agent. The data also suggest that an *early* failure of the rabbit to synthesize plasma alpha globulins may predispose the animal to the development of a more severe bacteremia.

Erythrocyte Destruction and Hemoglobin Turnover in Severe Acquired Hemolytic Anemia Studied with N^{15}-Glycine. G. WATSON JAMES, III* and LYNN D. ABBOTT, JR., Richmond, Va.

The response of the bone marrow to a continuing demand for erythropoiesis and hemoglobin synthesis has been studied in two elderly patients with severe acute acquired hemolytic anemia.

A 75-year-old man with 6.3 gm. per cent hemoglobin, 1.44 million red cells and 52 per cent reticulocytes; and a 70-year-old woman with 5.6 gm. per cent hemoglobin, 1.39 million red cells, and 60 per cent reticulocytes were in a stabilized hematologic state as evidenced by serial hemograms during the period of study. The nitrogen of the heme of their erythrocytes was labeled by the oral feeding of *one* gram of N^{15}-glycine (31 atom per cent excess), thus affording a means of following the disappearance of red cells from the individual's own circulation. The marked reticulocytosis was evidence of the rapid erythropoiesis. In the absence of a hemolytic process erythrocytes formed during an accelerated erythropoiesis with reticulocytosis live a normal life span (Clin. Res. Proc., 1953, 1, 64).

Approximately five times as much heavy nitrogen (N^{15}) was incorporated into the heme of these subjects when compared with a normal male given the same amount of glycine. The rapid rate and pattern of disappearance of the isotope from the circulating heme could be interpreted as due to random destruction of erythrocytes with an extremely short half life of about five days, indicating a mean life-time for the major portion of the red cells of about 7 days, and a turnover rate for hemoglobin of about 15 per cent per day. From total circulating hemoglobin by T-1824 technique it was calculated this represented hemoglobin synthesis and destruction of about 36 gm. per day. This may represent a close approximation to the limiting value for hemoglobin synthesis, and is further evidence of the remarkable functional capacity of the bone marrow.

Mechanisms of the Defect in Neuromuscular Function in Familial Periodic Paralysis. RICHARD J. JOHNS, ÅKE LILJESTRAND, DAVID GROB,* and A. MCGEHEE HARVEY,* Baltimore, Md.

A patient with familial periodic paralysis was studied during and after attacks of paralysis. Both spontaneous attacks and attacks precipitated by glucose administration were studied. Muscle action potentials in response to supramaximal ulnar nerve stimuli were recorded from the abductor of the fifth finger or adductor of the thumb. The isometric twitch tension of the adductor of the thumb was also recorded. The electrocardiogram and serum potassium levels were followed concurrently.

The normal steps in neuromuscular transmission are: 1) Liberation of acetylcholine by the nerve ending in response to a nerve impulse; 2) Depolarization of the motor end-plate by the acetylcholine, producing the end-plate potential; 3) initiation of the muscle action potential by the end-plate potential; 4) Propagation of the muscle action potential along the muscle fiber giving rise to muscular contraction. The sensitivity of the motor end-plates to depolarization by acetylcholine was tested by the intra-arterial injection of acetylcholine. This test demonstrated that during paralysis the end-plates were highly resistant to depolarization by acetylcholine. Other evidence showed that this resistance to depolarization was not due to the presence of a competitive (curare-like) block at the neuromuscular junction.

During paralysis the electromyogram showed abnormalities of depolarization and repolarization of the muscle fibers. Records of action potentials obtained from successive points along the muscle indicated interference with the normal propagation of depolarization along the muscle fibers. The electrocardiogram showed only

marked abnormalities of repolarization. With recovery from the paralysis, either spontaneously or due to administration of potassium chloride, all the abnormalities disappeared.

Thus, there appear to be at least two causes contributing to the paralysis seen in this disease. First, there is partial neuromuscular block due to resistance of the motor end-plate to depolarization by acetylcholine. Second, there are abnormalities of propagation of the muscle action potential.

Correlations of Serial Renal Biopsies and Other Data in Patients with the Nephrotic Syndrome. ROBERT M. KARK,* ROBERT C. MUEHRCKE, CONRAD L. PIRANI, and VICTOR E. POLLAK, Chicago, Ill.

The progression of pathologic changes in kidneys of patients ill with the nephrotic syndrome was studied by serial renal biopsies, and compared with biopsies from non-nephrotic controls. Histologic data were correlated with changing clinical status, clinical laboratory data, and gross and discrete renal function tests in a continuing study of the pathophysiology and natural history of different renal diseases.

Forty adults who developed the nephrotic syndrome (criteria of Leiter) were biopsied. Serial biopsies (up to four in 6 patients) were done in 14. Diagnoses included: glomerulonephritis (14); diabetes mellitus (11); lupus erythematosus disseminatus (7); renal vein thrombosis (2); possible lipoid nephrosis (2); primary renal amyloidosis (1); and undiagnosed (3). In glomerulonephritis, the earliest detectable lesions were interstitial edema and tubular cellular degeneration. Subsequent biopsies showed development of glomerular changes. Azotemia was associated with interstitial edema and tubular changes, disappeared as these improved, and reappeared when glomeruli became damaged. Changes seen during massive diuresis following corticotropin therapy will be demonstrated.

There was no correlation between the degree of proteinuria and extent of glomerular involvement. In fact, proteinuria was inversely related to the glomerular damage. The concept that protein is filtered by glomeruli and reabsorbed by tubules was supported by finding proteinaceous material in Bowman's space around normal glomeruli in biopsies from non-nephrotic patients without proteinuria. Usually there was good correlation between renal function tests and structural changes. These findings, together with previous data, relate the pathophysiology of the nephrotic syndrome to diffuse renal interstitial edema.

The Occurrence of Acid Mucopolysaccharides in Human Leukocytes. GRACE P. KERBY,* Durham, N. C.

The ground substance of connective tissue has as its major component high polymeric compounds called acid mucopolysaccharides, composed of repeating units of disaccharides of hexuronic acid and hexosamine. In most instances, the hexosamine is acetylated, the disaccharide esterified with sulfuric acid, and the compounds loosely bound to protein. The compounds exhibit metachromatic staining. The reaction of ground substance to any form of injury plays an important role both in the type of response to and the recovery from injury. Regulatory mechanisms are unknown. Because of the important role played by the leukocyte in inflammatory reactions of all kinds, and because of the failure of certain types of tissue reaction to injury (*i.e.*, the Shwartzman phenomenon) to occur in the absence of leukocytes, the acid mucopolysaccharides in human leukocytes are worthy of study.

Human leukocytes yield a non-dialyzable, metachromatic-staining material which contains hexuronic acid and hexosamine. Expressed in terms of hexuronic acid content, leukocytes from males yielded 1180 ± 52 (S.E.) gamma, females 900 ± 48 gamma of the material per 10^{10} leukocytes (said by Rossiter to equal 4 gm. wet weight of leukocytes). The difference between the yield from the leukocytes of males and of females was slight but highly significant ($p = < 0.01$). Chromatographically the major component of the material from human leukocytes had the same R_f value as did the major component of both a similar material isolated from human urine and a commercial brand of chondroitin sulfate. The material from leukocytes was homogeneous in this R_f range and in this respect was unlike both the human urine material and the commercial chondroitin sulfate.

The Relation of Steroid Structure to the In Vivo Reactivation of Influenza B Virus. EDWIN D. KILBOURNE, New Orleans, La. (Introduced by C. Thorpe Ray).

Evidence has been presented that injection of chick embryos with cortisone results in reactivation to a state of infectivity of thermally inactivated influenza B virus. This effect is mediated by the action of cortisone on the host cell of the cell-virus complex, and not by a direct effect upon the virus. Investigation of the specificity of this effect has revealed that it is not confined to cortisone but may be achieved also by the administration of other 11-oxygenated corticosteroids (Compounds A, B, F) and by such biologically diverse C-21 steroids as progesterone, desoxycorticosterone (DOC), and Reichstein's Compound S. However, specificity of the reaction is implicit in the failure of 11αhydrocortisone (11 epi-F) and 11αhydroxy progesterone to induce reactivation or increase of virus. Thus, abnormal orientation of the C-11 hydroxyl group is equated with inactivity of compounds whose stereoisomers (11-β-hydroxy-) are highly active in effecting viral reactivation.

Despite this evidence of the importance of oxygenation at C-11, the activity of compounds desoxy- at that position (DOC, progesterone) has been repeatedly demonstrated. These apparently divergent findings may be reconciled by an hypothesis that β-hydroxylation at the 11th carbon atom may be effected by the chick embryo. An endogenous 11-β-hydroxylase might thus convert DOC and progesterone to 11-oxy-steroids of the gluco-

corticoid type, but would be ineffectual in the conversion of inactive compounds already hydroxylated in the α orientation at C-11.

Production of Renal Ischemia and Proteinuria in Man With Intravenous L-norepinephrine and epinephrine. S. EDWARD KING and DAVID S. BALDWIN, New York, N. Y. (Introduced by Joseph Post).

Clinical manifestations of vasomotor instability and pronounced systemic reactions to postural change and other environmental influences with demonstrable renal ischemia are frequently observed in subjects with intermittent proteinuria. In this study renal ischemia and proteinuria have been induced in man through the intravenous administration of l-norepinephrine and epinephrine (USP).

Observations of glomerular filtration rate (GFR), renal plasma flow (RPF), urine volume and protein excretion were made under basal hydropenic conditions in 10 subjects with l-norepinephrine and 4 with epinephrine (USP), administered intravenously at constant infusion rates varying from 15 to 45 μg. per min.

Proteinuria was induced by l-norepinephrine in 8 out of 10 subjects and by epinephrine in all 4. Small doses of l-norepinephrine were employed in the 2 subjects failing to exhibit proteinuria. Proteinuria persisted for periods up to 50 minutes following discontinuation of these amines while renal functions were returning to control levels. No difference of response was observed between normal subjects and those with known intermittent proteinuria.

Marked reduction in RPF occurred particularly with larger doses of l-norepinephrine (mean 57 per cent) and with epinephrine (mean 51 per cent). The GFR was reduced following larger doses of l-norepinephrine (mean 38 per cent) and after epinephrine (mean 32 per cent).

Increased renal vascular resistance was preponderantly afferent with l-norepinephrine, and afferent and venular with epinephrine.

In 5 normal subjects, the maximal tubular reabsorptive rate for glucose (TmG) was unaffected by l-norepinephrine infused at a rate of 30 μg. per min. This suggests that the renal ischemia induced was diffuse.

Proteinuria accompanied by renal ischemia was induced in man by intravenous l-norepinephrine and epinephrine. These data suggest that the adrenal medullary hormones play a role in production of transient proteinuria in various stressful conditions.

Steroid Hormonal Metabolites of Meconium. RALPH A. KINSELLA, JR. and FAITH ELLEN FRANCIS, St. Louis, Mo. (Introduced by Ralph A. Kinsella).

Recently, we reported the isolation and crystallization of estriol from human meconium, thus establishing excretion by human intestinal route of this metabolite. Further studies have indicated the excretion in meconium of 17-ketosteroids. However, no evidence for the excretion of significant amounts of water soluble ketonic corticoids has been found by Zaffaroni's paper chromatographic analysis for substances reducing ammoniacal silver or by analysis with Porter and Silber's reagent for 17-hydroxy alpha-ketols. The extraction procedure consisted of preparation of a butanol extract, evaporation of the solvent, suspension of the residue in water, extraction of the aqueous suspension with ligroin and with ethyl ether. After incubation with bacterial betaglucuronidase, extraction with ethyl ether was carried out. Sulfate conjugates of the estrogens were hydrolyzed with phenol sulfatase and the estrogens were removed by extraction with ether. Hydrolysis of the sulfate conjugates of the 17-ketosteroids was carried out by continuously extracting with ether for 24 hours at pH 0.7. The extract of each hydrolysate was subjected to Girard's separation procedure. A benzene solution of the ketonic fraction was repeatedly extracted with water to remove corticoids and then with alkali to remove phenols. Ten mg. of 17-ketosteroids per kilogram of meconium were obtained after hydrolysis with beta-glucuronidase and 2 mg. per kilogram after continuous extraction at pH 0.7. Preliminary paper chromatographic analysis of the neutral fractions indicated that the bulk of the 17-ketosteroids was relatively more polar than testosterone, with the exception of the principal component of the sulfate fraction which moved about twice as fast.

Effect of Mild Steady State Exercise on Cerebral and General Hemodynamics of Normal Untrained Subjects. JEROME KLEINERMAN and SALVATORE M. SANCETTA, Cleveland, O. (Introduced by Roy W. Scott).

The effect of mild exercise (120 to 135 foot pounds per minute) on the general and cerebral hemodynamics has been studied in 10 normal untrained subjects in the prone position, employing a variable resistance bicycle ergometer. The amount of work which would insure a steady state within 10 minutes and maintain it for an additional 20 minutes as gauged by oxygen consumption was predetermined the day before the study. Cerebral blood flow (CBF) was determined by the nitrous oxide method of Kety and Schmidt, the cardiac output by the direct Fick principle and pressures transduced *via* Statham strain gauges.

There were significant increases in mean minute ventilation, oxygen consumption per M^2 (123 cc. to 221 cc., $p = < .001$) and total AV difference, with significant decreases in pulmonary vascular (242 to 190 c.g.s., $p = < .05 - > .01$) and total peripheral resistance (1346 c.g.s. to 1087 c.g.s., $p = < 0.5 - > .01$). Mean minute respiratory rate increased from 15.6 to 20.3 per min. and heart rate from 81 to 87 beats per min. Mean pulmonary and brachial artery pressures showed no significant changes.

The CBF fell from a mean of 56 cc. per 100 gm. per min. at rest to 49 cc. in exercise ($p = < .05 - > .01$). The cerebrovascular resistance concomitantly increased from 1.8 to 2.0 units ($p = < .05 - > .01$). The cerebral oxygen consumption did not change significantly during exercise (3.2 cc. per 100 gm. per min. at rest, 3.1 cc. per 100 gm. per min. in exercise). The respiratory quotient

of the brain similarly was unchanged in exercise (.91 at rest, and .92 in exercise). The decreased CBF is probably dependent on the fall in arterial pCO_2 during exercise. At rest the $ApCO_2$ was 43 mm. Hg as compared to 41 mm. Hg during exercise ($p = < .05 - > .01$). From these observations it appears that the brain does not participate in the general body decrease in peripheral resistance during mild exercise.

The Action of Iodoacetate on the Electrical and Mechanical Activities of the Isolated Perfused Frog Heart. MORRIS KLEINFELD, EDWARD STEIN, and JOHN MAGIN, New York, N. Y. (Introduced by Charles E. Kossmann).

Microelectrodes were inserted into the interior of single ventricular fibers of the isolated perfused frog heart and the effects of 5×10^{-4} M iodoacetate on the intracellular potential, the indirect electrocardiogram and the direct cardiac output were recorded. Prominent changes in both the electrical and mechanical properties were observed. The most consistent, early change was a shortening of the action potential duration (APd) which was progressive. Coincident with this a shortened S-T interval and abnormal T wave were observed in the indirect electrocardiogram. Of lesser frequency was a prolongation of the P-R interval. A decreased height in the membrane action potential (MAP) with impaired depolarization occurred relatively late. The change in mechanical activity was represented by a progressive decrease in cardiac output, stroke volume and heart rate. Auricular activity persisted for sometime after ventricular arrest. The alterations in mechanical activity were usually paralleled by changes in the repolarization phase of the action potential (shortened APd) of the ventricular fiber. The addition of substrates such as pyruvate (0.0055 M) and acetate (0.0055 M) to the IAA treated heart produced only a partial recovery and delay of ultimate deterioration of cardiac activity. The result was similar when adenosine triphosphate (1.0×10^{-4} M) was added.

The results obtained confirm the previous observations of the effect of IAA on the mechanical activity of the heart but are at variance with the findings and conclusions with regard to the electrical activity. The recording of several parameters of the electrical potentials in contrast to only the height of the surface action potential as measured by Gardner *et al.*, revealed an early effect on the electrical properties which mirrored the changes in cardiac output.

The inability of added pyruvate, acetate, and ATP to restore the cardiac activity suggests that under the conditions of these experiments IAA has other actions in addition to that of a specific inhibitor of triose—phosphate dehydrogenase. In line with Hodgkin's theory of membrane permeability it is postulated that the enhanced repolarization phase is associated with an increased permeability of the cellular membrane to K. Observations of Love *et al.* of an increased outflow of K and net gain of Na in human erythrocytes following exposure to IAA lends support to this postulate.

The Protective Effect of Hypercalcemia in the Hyperkalemic State. MILTON J. KRAININ, CHARLES L. WHISNANT, and JOSEPH S. WILSON, Atlanta, Ga. (Introduced by Arthur J. Merrill).

Renewed clinical interest has been shown in the possible protective action of calcium against the toxic effects of hyperkalemia. Previous experimental data appear to show that when serum calcium levels are high there may be marked increases in potassium tolerance. Improvement in the electrocardiographic abnormalities of hyperkalemia shortly after the intravenous injection of calcium gluconate has been demonstrated clinically.

The procedure in this study was to infuse intravenously a solution of potassium chloride into two groups of normal anesthetized dogs. One of the groups received calcium gluconate simultaneously. Plasma potassium and calcium as well as other electrolytes and electrocardiograms were followed at frequent intervals in both groups until death. Results representing 20 dogs in the control group and 20 dogs receiving calcium gluconate showed that the average venous plasma potassium level shortly prior to death in the control group was 11.2 ± 1 mEq. per L. and that in the group receiving calcium gluconate was 12.2 ± 1.5 mEq. per L. ($P < .02$). At the time these samples were drawn, calcium levels in the group receiving calcium gluconate averaged 19.3 mg. per 100 ml. Those not receiving calcium required an average of 143 mg. KCl per lb. to produce death while those receiving calcium required 173 mg. KCl per lb. This difference was not statistically significant ($P > .6$). Similar results were recorded in a group of ten dogs in whom both ureters had been ligated three days before the above procedure was carried out. Five dogs acted as controls and five were given calcium.

It is concluded that increased serum calcium levels appear to offer slight protection against hyperkalemia under the conditions of the experiments.

The Effect of Thyroid Disease on the Metabolism of Calcium[45] in Man. STEPHEN M. KRANE, GORDON L. BROWNELL, and JOHN B. STANBURY,* Boston, Mass.

Increased calcium excretion resulting in negative calcium balance has been observed in patients with hyperthyroidism; decreased calcium excretion has been observed in patients with myxedema. This study was designed in an attempt to define these changes using radioactive calcium (Ca^{45}).

Ca^{45} (5 microcuries) was administered intravenously to four patients with hyperthyroidism, two with myxedema and one normal, all of whom were on constant low calcium diets. Specific activities (per cent administered dose Ca^{45} per gram stable calcium) were determined on serum, urine, and feces. Semilogarithmic plot of urine and serum specific activities versus time suggests curves composed of three decreasing exponential functions. Dif-

ferences among patients were most marked in the third slope which appeared by the second day. This slope ranged from 24.3 to 28.8 per cent per day in the hyperthyroid patients and was 8.1 per cent per day in the normal patient. The values were 6.3 and 7.2 per cent per day in the myxedema patients and rose to 15.0 and 17.4 per cent per day 6 months after treatment with thyroid.

At an arbitrary time of nine days following the administration of Ca^{45}, a calcium space was calculated:

$$\frac{\text{(\% administered dose remaining after 9 days)}}{\text{urine specific activity day 9}}$$

This value ranged from 47.3 to 247.7 grams in the hyperthyroid patients and 10.8 grams in the normal patient. In the myxedema patients it was 5.0 and 5.4 grams, before treatment, and 13.0 and 18.2 grams after treatment.

Since the marked fall in serum and urine specific activities in patients with hyperthyroidism is not accounted for by increased excretion rate of Ca^{45}, it appears that more Ca^{45} enters the body calcium spaces, more rapidly than normal. The reverse is found in patients with myxedema.

The Effect of Intravenous Heparin on Lipemia-Induced Angina Pectoris in Patients with Coronary Artery Disease. PETER T. KUO and CLAUDE R. JOYNER, JR., Philadelphia, Pa. (Introduced by Charles C. Wolferth).

Lipemia was induced in 20 patients with coronary artery disease and angina pectoris by a standard fat meal. Electrocardiogram; ballistocardiogram; pneumogram; plasma lactescence; serum cholesterol, phospholipid, and fatty acid; and serum lipoprotein (paper electrophoresis) were studied before, at intervals after the test meal, and 15 minutes after intravenous administration of heparin.

One to four attacks of angina pectoris were regularly precipitated on different occasions, in each of eight patients in this series by the ingestion of the fatty meal. These patients developed a total of 19 attacks of anginal pain three to five hours after meal, when their postprandial lipemia was near its peak level as indicated by plasma lactescence and serum lipid determinations. Electrocardiographic changes of acute myocardial ischemia, deterioration of the ballistic complexes, and an increase in the rate and depth of the respiration were observed during each attack.

Thirteen anginal attacks ceased five to seven minutes after the intravenous administration of 5 to 25 mg. of heparin irrespective of the previous duration of the anginal pain. This was accompanied by reversion of the electrocardiogram, ballistocardiogram, and pneumogram to their respective controlled levels. More rapid relief was observed on five occasions with nitroglycerine. One patient developed prolonged substernal distress four and a half hours after the fatty meal, with persistent electrocardiographic and ballistocardiographic changes. He did not respond to heparin or nitroglycerine therapy. Two anginal attacks were not influenced obiectively or subjectively by saline injections, but were subsequently relieved by heparin.

In one patient, the anginal pain was initially relieved by nitroglycerine, but he experienced a recurrence of symptoms 35 minutes later. This was controlled by 5 mg. of heparin intravenously.

Heparin alters plasma lactescence and serum lipoprotein migration on filter paper. The relation of these to the relief of angina remains to be determined.

The Metabolism of Intravenously Infused C-14 Labelled Cholesterol in Man Before and After the Induction of Myxedema. GEORGE S. KURLAND and JUDITH L. LUCAS, Boston, Mass. (Introduced by Benjamin Alexander).

The metabolism of cholesterol has been studied in two patients with angina pectoris before and after the induction of myxedema by I-131. C-14 labelled cholesterol was administered intravenously, and the decrease in specific activity of plasma cholesterol was followed for 149 to 300 days. The specific activity of the plasma free and total cholesterol, the red blood cell cholesterol and stool radioactivity in myxedema have been compared with the values in the same patient when euthyroid.

The induction of myxedema resulted in a rise in serum cholesterol from approximately 235 and 400 mgm. per cent to 400 and 650 mgm. per cent, respectively. In both patients, there was an initial period of about 13 days of relatively rapid decrease in plasma cholesterol specific activity. During this period, the disappearance curves of C-14 activity in the same patient when euthyroid and when myxedematous were quite similar. For the duration of the experiments, the rate of diminution of cholesterol specific activity in myxedema was much slower than in the euthyroid state. The half-time of disappearance from 30 to 104 days after infusion increased, in Case I, from approximately 65 days when euthyroid to 160 days following the induction of myxedema, and in Case II, it increased from 68 to 100 days; estimates of the size of the "exchangeable cholesterol pool" showed no change. The effect of the administration of desiccated thyroid and of changing cholesterol levels on the subsequent course of cholesterol radioactivity will be discussed.

The time for equilibration of labelled plasma free cholesterol with red blood cell cholesterol did not change in myxedema. On the other hand, the rate of appearance of C-14 labelled plasma cholesterol esters was much slower in myxedema than during the euthyroid state.

The data are regarded as consistent with decreased synthesis of cholesterol and cholesterol esters in myxedema.

ADH and Impaired Water Tolerance: Implications of Alcohol Diuresis in Certain Disease States. EZRA LAMDIN, CHARLES R. KLEEMAN, MILTON E. RUBINI, and FRANKLIN H. EPSTEIN, New Haven, Conn. (Introduced by John P. Peters).

Increased production, or diminished destruction, of posterior pituitary hormone (ADH) have been invoked to explain the impaired water metabolism characterizing

certain diseases. Experimental support for this hypothesis is, however, scanty. To assess the role of the posterior pituitary in such states, ethyl alcohol, a known transient inhibitor of ADH release, was imbibed by recumbent patients who failed to excrete normally a standard water load. Diagnoses included: cirrhosis, cor pulmonale, and congestive failure (with hyponatremia, edema, and/or ascites); obstruction of the superior vena cava; Addison's disease; and hypopituitarism. Alcohol was also given to water-loaded normal subjects in the head-up tilted posture—a state in which antidiuresis supervenes despite hypotonic expansion of body fluids.

Single doses of ethanol (33 to 55 grams), which provoke water diuresis in semirecumbent normals, did not affect the antidiuresis of the upright-tilted posture, and produced no increased urine flow in 10 of 11 recumbent patients.

Multiple doses (33 grams at hourly intervals) were used to prolong the inhibitory effect of ethanol on the neurohypophysis. After the second or third dose, the antidiuresis of upright-tilting was overcome, urine flow approaching diuretic values observed in the supine position before tilting. Of two cirrhotics with ascites given multiple doses, one responded equivocally, while the other, responding to the first dose with slightly increased urine flow, diuresed briskly after the second.

Conclusions: 1) Stimuli unrelated to tonicity of the extracellular fluids provoke the release of ADH from the pituitary; 2) Although other factors are undoubtedly operative, a diuretic response to alcohol implicates ADH as at least partly responsible for the impaired water excretion characterizing certain diseases; 3) Varying responses to the inhibiting action of alcohol suggest that the magnitude and intensity of this action of ADH probably differs from person to person and from disease to disease.

The Relation of Age to Certain Measures of the Performance of the Heart and the Circulation. MILTON LANDOWNE,* MARTIN BRANDFONBRENER, and NATHAN W. SHOCK, Baltimore, Md.

Estimates of resting circulatory performance have been made in a series of 67 men clinically free of cardiovascular disease, aged 19 to 86. Peripheral resistance (R) increased with age, as evidenced by a decline in cardiac output measured by dye dilution, unassociated with a decrease in mean brachial intra-arterial pressure. With respect to the heart, there was an agewise decline in estimated external left ventricular work (W) at rest, since the increase in mean systolic pressure was not comparable to the decrease in stroke volume, and the heart rate decreased. The increase in estimated systolic duration implies a further decrease in power (P), *i.e.*, rate of work performance. While the reduction in work may reflect lessened circulatory requirement, heart size did not diminish, and diastolic duration was increased. These indications of a decreased stroke work relative to diastolic volume specify a less favorable cardiac performance and therefore are evidences of a reduction in the reserve of the left ventricle as a muscular pump.

Estimated effective central elasticity (E) showed a striking age correlation ($r = .76$), as the relative rate of pressure fall in diastole increased with age, despite the increase in peripheral resistance. Residual individual variability in elasticity of ± 30 per cent limits the accuracy of predicting stroke volume from blood pressure curves by means of an age dependent factor for elasticity. Convenient approximations describing over-all results are R (mm. Hg min./Liter) $= 18e^{(.011 \pm .0014)a}$; W (gm. m./beat) $= 108e^{-(.005 \pm .0018)a}$; P (watts) $= 3.9e^{-(.009 \pm .0017)a}$; E (dynes/cm.^{5}) $= 900e^{(.016 \pm .0017)a}$ where a = age minus 50 yrs.

Effect of Folic Acid on Serum Vitamin B_{12} Concentrations in Pernicious Anemia. ARNOLD A. LEAR, Boston, Mass. (Introduced by Shu Chu Shen).

Therapy with folic acid alone may have a neuropathic effect in pernicious anemia, in which during relapse the serum vitamin B_{12} level is characteristically low. A study was therefore made of the effect of folic acid on serum vitamin B_{12} concentrations.

Twenty-seven patients with pernicious anemia who had been receiving 15 μg. of vitamin B_{12} intramuscularly every four weeks for two or more years were selected. During the first six months, 20 were given 30 μg. and 7 were given 20 μg. of vitamin B_{12} intramuscularly every two weeks. During the next six months, all received the same vitamin B_{12} dosage and in addition took 5 mg. of folic acid by mouth daily. Serum vitamin B_{12} levels were estimated microbiologically, using *Euglena gracilis,* initially and after 6 and 12 months.

Initially 14 of the 27 patients had serum vitamin B_{12} levels less than 100; average 202; range, 10 to 1050 $\mu\mu$g. per ml. After the first six months, only one patient had a level of less than 100; average, 451; range, 97 to 1360. After the second six months (supplemental folic acid), the average level was 279; range, 116 to 745. At this time, individual serum vitamin B_{12} levels in 18 of the 27 patients were 15 per cent or more lower than after the first six months. Results indicate that injection of 15 μg. of vitamin B_{12} every four weeks may be insufficient to saturate body vitamin B_{12} stores. Administration of folic acid may lower the serum vitamin B_{12} level possibly by accelerating the utilization of vitamin B_{12}. No neuropathic effects were observed.

The Volume of Distribution and Turnover of Endogenously Labelled Human Thyroid Hormone from Euthyroid and Hyperthyroid Donors. RICHARD P. LEVY, LUTHER W. KELLY, JR., GRAHAM W. COOPER, and WILLIAM MCK. JEFFERIES, Cleveland, O. (Introduced by Walter H. Pritchard).

The fate of endogenously labelled thyroid hormone obtained from two euthyroid patients has been compared with that obtained from two hyperthyroid patients, when administered to recipients in a euthyroid state. Recipients included subjects with normal thyroids which were

blocked by methimazole, preventing reutilization of I^{131}, and an athyroid patient maintained in a euthyroid state with desiccated thyroid. Serum PBI, total I^{131}, PBI^{131}, and urinary and fecal radioactivity were followed. From this data the volume of distribution and turnover rate of labelled hormone, the total exchangeable hormonal iodine, and the total daily hormonal iodine turnover were calculated.

It was found that approximately 24 to 30 hours were required for equilibration of the tracer hormone with the exchangeable hormonal pool, regardless of the donor. After this the rate of urinary plus fecal excretions equalled the rate of plasma fall in radioactivity, indicating equilibrium with an ultimate volume of distribution. When labelled hormone from a hyperthyroid donor and from a euthyroid donor was administered to the same recipient on different occasions, the daily hormonal iodine turnover rates were practically identical, although the volumes of distribution were different. When labelled hormone from the same hyperthyroid donor was administered to two recipients the volumes of distribution were similar, but the turnover rates were different. In one instance the volume of distribution attained by tracer hormone from a euthyroid donor was greater than that reached by tracer hormone from a hyperthyroid donor, in another it was smaller.

Hence the source of labelled hormone did not consistently influence its behavior in the recipients. It also appears that daily thyroid hormonal iodine turnover (utilization) may be the same in a single subject with repeat studies under similar conditions, whereas different euthyroid persons may exhibit rates of utilization which vary over a considerable range.

The Effects of Thyroid Hormone on the Distribution Kinetics and Degradation of Iodo-albumin. C. G. LEWALLEN, J. E. RALL*, M. BERMAN, and H. HAMEL, Brookhaven National Laboratory and New York, N. Y.

To assess the role of thyroid hormone in albumin metabolism, seven patients with thyroid carcinoma and myxedema were studied with I^{131} iodo-albumin. Plasma specific activity and urinary excretion were followed up to 60 days. Iodide or antithyroid agents were administered to exclude label from thyroid tissue.

The plasma and urine curves were resolved by graphic analysis into three exponentials. With more frequent early collections, five exponentials were obtained from the urine curves. Significant disparity between retention and plasma slopes revealed an interval essentially non-feedback pool. External counts over liver and thigh in three patients were confirmatory.

The model found to be compatible with the data and used for analysis was a three-compartment doubly-open system consisting of plasma in parallel with one fast and one slow extravascular compartment providing for internal non-feedback loss and for external loss through a delay pool. A complete solution of this model was possible in one study.

Four patients were studied while myxedematous and after administration of thyroid hormone. There was in myxedema an average increase in total exchangeable albumin of 31 per cent, in extravascular albumin of 65 per cent, in fractional rate of transcapillary transport of 90 per cent, and in fractional rate of feedback to plasma of 24 per cent. In myxedema, the fractional rate of degradation was reduced by 47 per cent.

The metabolic status of two patients was changed during tracer studies by withdrawing or administering triiodothyronine. A rapid and marked effect in the rate of iodo-albumin degradation was clearly shown.

In a donor-recipient experiment performed to insure biologically homogeneous iodo-albumin, kinetic analysis favored the rapidly exchanging extravascular compartment as the major site of degradation.

The studies indicate that thyroid hormone exerts a profound effect on compartment size, degradation rate, and transcapillary transport of iodo-albumin in man.

Mechanisms Regulating Aldosterone Production in Man. GRANT W. LIDDLE, FREDERIC C. BARTTER,* LEROY E. DUNCAN, JR., JOAN K. BARBER, and CATHERINE DELEA, Bethesda, Md.

Although aldosterone has recently been shown to play a part in renal sodium conservation in edematous patients, the factors governing its production have not been defined. Studies designed to elucidate these factors have been carried out in human subjects, maintained under controlled metabolic conditions. Aldosterone production was estimated from the urinary excretion of methylenechloride soluble material having aldosterone-like activity as assayed in adrenalectomized dogs. Other indices of adrenal cortical function were followed, as were body weight, serum, urine, and, in some, fecal electrolytes, and endogenous creatinine clearance. Results were as follows: Sodium deprivation, with weight loss, consistently increased aldosterone excretion. Administration of potassium chloride resulted in increases of aldosterone output in those subjects in whom it induced sodium loss.

In sodium-depleted subjects, expansion of body fluids with water given with Pitressin® Tannate produced decreases in aldosterone and potassium excretion and increases in sodium excretion despite hyponatremia, suggesting that volume changes and not changes in concentration or in total body sodium mediate the effect of sodium on aldosterone secretion. Expansion of the vascular compartment with albumin without concomitant increase in total body water did not consistently decrease aldosterone excretion.

Reports that aldosterone output is increased in edematous patients have been confirmed. Infusions of physiologic saline in such subjects produced marked decreases in aldosterone output. ACTH caused increases in aldosterone output, which were significant, albeit small in proportion to concomitant increases in 17-hydroxycorticoid output. Abrupt withdrawal of ACTH resulted in transient falls in aldosterone excretion to values below pre-ACTH levels. Large doses of corti-

sone did not decrease aldosterone excretion significantly in sodium-depleted subjects.

The Effects of Triiodothyronine on the Biosynthesis of Plasma Lipids from Acetate-1-C^{14} in Myxedematous Subjects. S. R. LIPSKY, P. K. BONDY,* E. B. MAN, and J. S. MCGUIRE, JR., New Haven, Conn.

The incorporation of acetate-1-C^{14} into the free and ester cholesterol and the triglyceride and phospholipid fatty acids of the plasma was studied in three women with severe myxedema before and after a single dose of triiodothyronine. A significant fivefold decrease in the utilization of acetate for cholesterol biosynthesis occurred in these patients in the untreated state when compared with a series of six "normal" subjects. Although the total plasma cholesterol was elevated in all cases the diminution in labelling was found to be a reflection of reduced hepatic synthesis rather than the effect of simple dilution by an increased pool of circulating cholesterol. In one patient followed for 17 days, a decrease in peripheral utilization of the cholesterol was noted as evidenced by some delay in the rate of disappearance of the labeled sterol from the plasma.

The formation of the fatty acid fractions from acetate was only slightly retarded in two of the three subjects. In the third patient whose plasma was grossly lactescent and contained abnormally large quantities of neutral fat, synthesis was significantly reduced.

After an interval, 0.5 mg. of triiodothyronine was administered to these patients. Two days later, when the BMR and plasma lipids were rapidly returning toward normal, the isotope studies were repeated.

The rate of cholesterol synthesis returned to normal in one individual, and was still delayed in the two other patients. Normal rates of fatty acid synthesis were now noted in all patients.

It is concluded that in myxedematous subjects there is a decrease in hepatic synthesis of cholesterol as well as a defect in the peripheral utilization of this substance. With treatment, normal disposal rates prevail, cholesterol levels in plasma decrease, and synthesis returns toward normal.

Studies on Protein Synthesis in the Liver. JOHN W. LITTLEFIELD, ELIZABETH B. KELLER, JEROME GROSS, and PAUL C. ZAMECNIK,* Boston, Mass.

It is known that the initial *in vivo* incorporation of intravenously injected radioactive amino acids into rat liver proteins takes place largely in the microsome fraction of the hepatic cell (the submicroscopic particles), and that ribonucleic acid is concentrated in this cell fraction as well. We have separated the microsome fraction into two components: sodium desoxycholate-soluble material containing 85 per cent of the protein of the original microsome fraction, and sodium desoxycholate-insoluble material containing 15 per cent of the original protein and essentially all the original ribonucleic acid. Purest samples of the latter contain equal amounts by weight of protein and ribonucleic acid, and may be termed "ribonucleoprotein particles." Under the electron microscope, unfixed preparations of such samples appear as essentially homogeneous dense granules averaging 240 A in diameter; in the ultracentrifuge, suspensions of such samples migrate as a sharp peak with sedimentation constant of 47 S.

We have studied also the *in vivo* incorporation of intravenously injected DL-leucine-C^{14} and L-valine-C^{14} into the two components of the microsome fraction and the soluble protein of the rat liver cell. Maximal incorporation into the ribonucleoprotein occurs within a few minutes after injection, while the bulk of microsomal protein and the soluble protein of the cell are labeled more slowly and progressively. One can estimate from such data that only a very small fraction of the total amino acids in the ribonucleoprotein are turning over rapidly. With the cell-free incorporation system (Zamecnik and Keller) we obtained evidence that the labeling of ribonucleoprotein is not merely a rapidly established equilibrium between free amino acids and those incorporated but is an irreversible step in protein synthesis.

Antidiuretic Content of Rat Hypothalami Under Different Experimental Conditions. C. W. LLOYD,* and P. O. OLDFORD, Syracuse, N. Y.

Antidiuretic activity of hypothalami and pituitaries of rats has been measured under different experimental conditions. Moderate dehydration (48 hours) without starvation produced an increase in the hypothalamic antidiuretic material. Intracarotid hypertonic saline solution was necessary to cause a decrease in hypothalamic antidiuretic substance. Maintenance on a liquid diet produced a decrease of antidiuretic activity. The hypothalami of hypophysectomized animals contained very little antidiuretic material shortly after hypophysectomy but there was essentially the same amount as in intact animals after a longer postoperative interval. Dehydrating for 48 hours produced a decrease and hydration caused an increase in antidiuretic activity of hypothalami of hypophysectomized animals.

The hypothalami of animals with diabetes insipidus, resulting from removal of the posterior lobe with maintenance of anterior lobe function, contained less antidiuretic substance than was found in totally hypophysectomized animals after the same postoperative interval. The administration of hydrocortisone caused a very large increase in the antidiuretic substance of the hypothalami of intact, hypophysectomized and adrenalectomized animals although more steroid was required in adrenalectomized rats. These data are compatible with the hypothesis that antidiuretic substance of the hypothalamus plays a part in the release of ACTH by the anterior lobe.

Atypical Pressor Responses to Standard Exercise. GORDON A. LOGAN and ROBERT A. BRUCE,* Seattle, Wash.

Variations in blood pressure response in cardiac patients during exercise testing have been analyzed for different cardiovascular diseases. Blood pressure data

were derived from sphygmomanometric recordings of brachial artery pressure at minute intervals during the resting, exercise, and recovery phases of a standard test requiring patients to walk for 10 minutes at 1.7 mph and 10 per cent grade on a treadmill ergometer. Incomplete periods of exercise, presence of auricular fibrillation, variations in complications, treatment and age of patients are recognized as factors limiting interpretation and comparison of results.

In 16 normal subjects, systolic pressure increased from 20 to 45 mm. Hg (mean 25) by the third minute of exercise, remained unchanged during further exercise and gradually returned to normal during the recovery period. Diastolic pressure remained unchanged throughout.

Forty-five patients with predominant mitral stenosis showed only 5 mm. Hg mean systolic rise on exercise. During early recovery there was significant rebound. whereas diastolic pressure remained constant throughout. Following surgery, exercise systolic pressure approached normal and recovery rebound was nearly eliminated. These post-surgical changes were not related to surgical benefit. Analysis related to degree of exercise intolerance, cardiac deceleration during recovery, and presence of auricular fibrillation was done.

Eleven patients with aortic stenosis showed similar systolic hypotension during exercise, and slight rebound during recovery.

Sixteen hypertensive patients showed normal rise in systolic pressure, despite widened pulse pressure, during exercise. Evaluation immediately after drug-induced acute relative hypotension showed reduction in systolic, diastolic, and pulse pressures and increase in exercise tolerance.

Slight differences were found preoperatively in 13 patients with coarctation of the aorta.

Significant systolic and diastolic hypotension was apparent in 18 patients with patent ductus arteriosus with incomplete alleviation following surgery in 4 patients.

Mean diastolic pressure was constantly slightly reduced in 16 patients with interauricular septal defect.

Relationship of Aldosterone in Urine to Sodium Balance and to Some Other Endocrine Functions. JOHN A. LUETSCHER, JR.* and ROBERT H. CURTIS, San Francisco, Calif.

Increased sodium-retaining activity has been found in urine extracts from patients with nephrosis, heart failure, hepatic cirrhosis, and toxemia of pregnancy, when urine sodium excretion is low. Sodium-retaining activity is diminished during diuresis when urine sodium increases. Aldosterone has been isolated from one active urine extract from a child with nephrosis: in the other extracts, a material of similar chromatographic behavior is associated with the sodium-retaining activity.

Standardized methods of extraction of urine yield between 1.5 and 3.0 μg. of aldosterone per day from urine of normal subjects on unrestricted diets. Aldosterone was not found in Addison's disease or after bilateral adrenalectomy.

The output of aldosterone is not significantly affected by situations associated with changes in output of 17-ketosteroids or 17-hydroxy-corticoids, for example, in hypopituitarism, myxedema, adrenogenital syndrome, certain patients with Cushing's syndrome and normals given ACTH. Elimination of dietary sodium is followed by increased aldosterone output without appreciable change in 17-ketosteroids or 17-hydroxycorticoids.

An unexpectedly high output of aldosterone in relation to urine sodium has been observed in sodium-losing nephritis and in congenital adrenal hyperplasia. In all other cases studied, an inverse correlation exists between daily output of aldosterone and of sodium in urine, both in health and in disease. Thus the output of aldosterone appears to reflect a normal mechanism for sodium conservation, which under an abnormal stimulus causes accumulation of sodium in certain phases of disease. The stimulus to increased secretion of aldosterone is not clearly defined. It is possible that increased blood and urine levels might result from decreased destruction of aldosterone, which is rapidly inactivated by human and rat liver slices.

The Physiological Role of Adrenal Salt Hormone (Aldosterone) in Man. JOHN P. MACLEAN, MIN CHIU LI, MORTIMER B. LIPSETT, BRONSON RAY, and OLOF H. PEARSON,* New York, N. Y.

Adrenalectomized humans remain eucorticoid and in salt balance when receiving 50 to 75 mg. of cortisone acetate daily, together with a normal diet. Data will be presented to demonstrate that these patients cannot achieve sodium balance with a sodium intake of 20 mEq. a day, and that a salt depletion syndrome occurs. In contrast, 4 hypophysectomized patients with intact adrenals, maintained on 50 mg. of cortisone actetate daily, achieved sodium balance by the fifth day, with an intake of less than 10 mEq. daily. A salt depletion syndrome did not occur after two weeks on this regimen. The hypophysectomies were judged functionally complete by separate studies: 1) Development of acute adrenal insufficiency within 7 days after cortisone was withdrawn; 2) Clinical and laboratory evidence of myxedema; 3) Absence of pituitary gonadotrophin (FSH) in the urine.

The effect of aldosterone has been studied in an adrenalectomized human. Cortisone was withdrawn and acute adrenal insufficiency developed in 36 hours. In a subsequent cortisone withdrawal study, aldosterone, 900 μg., was given intramuscularly in divided doses over a 36-hour period. At this time an identical picture of acute adrenal insufficiency was manifest.

These observations indicate that the secretion of adrenal salt hormone responds to changes in the electrolyte milieu without the necessary intervention of the pituitary gland. Thus, the adrenal salt hormone in man appears to be necessary for the prevention of sodium depletion during severe dietary restriction or excessive extrarenal losses. Its role, therefore, is minor in comparison with hydrocortisone which is necessary for life maintenance.

The Response to Water Loading in Renal Failure. E. GORDON MARGOLIN and JOHN P. MERRILL,* Boston, Mass.

To determine the responsiveness of patients with chronic renal impairment to water loading, a series of cases on near-constant solute intakes was studied. In most instances the fluids were administered by mouth over periods of 2 to 5 days, but in several cases the additional fluids were given intravenously. The adequacy of physiological adjustment to water-loading was roughly correlated with the degree of disease as estimated by the levels of blood urea nitrogen.

In general, it can be stated that the response to the water loads was blunted; that is, urinary concentration dropped slowly if at all, while urinary output rose gradually during the challenge and returned to control levels only slowly after withdrawing the extra fluid load. The gain in solute excretion by the water-loading was minimal and seemingly decreasing as the challenge continued. The most effective time of day for unloading extra water by these patients was in the early morning hours. The patients tended to gain weight due to positive water balance and to develop signs of water intoxication consisting of bloating, generalized discomfort, nausea and vomiting and loss of desire for water. The handling of individual solutes, particularly sodium, chloride, and urea, closely paralleled the over-all dilution of the urine; potassium and phosphate, however, failed to follow this pattern.

Study of the dynamics involved would implicate hypotonic expansion of plasma volume with resultant increase in GFR in the increased total solute output. The ability to dilute the urine under this stress suggests tubular function of some degree.

This study points out the limitations of excess water administration in the management of patients with chronic uremia.

Effects of Fasting and Feeding on Pathways of Hepatic Glycogen Synthesis. PAUL A. MARKS and BERNARD L. HORECKER, New York City and Bethesda, Md. (Introduced by Alfred Gellhorn).

Studies with fasted animals have shown that $C^{14}O_2$ incorporated into liver glycogen is distributed predominantly and equally into carbon atoms 3 and 4. This finding was interpreted as indicating that glycogen synthesis occurred by a reversal of anaerobic glycolysis. Recent evidence has suggested that alternate pathways of glucose metabolism exist and that these may play a role in glycogen formation.

It has been known that fasting markedly affects overall hepatic carbohydrate metabolism. The present studies provide evidence that 1) the pattern of CO_2 incorporation into glycogen differs in fasted and fed rats; and 2) pathways other than the formation of glycogen *via* phosphopyruvate contribute to glycogen synthesis. Liver slices from rats fasted or fed a 58 per cent glucose diet were incubated with lactate and $C^{14}O_2$. Glycogen was isolated and degraded to determine the distribution of isotopic carbon. While carbon atoms 3 and 4 become equally labelled in liver slices from fasted animals, in those from fed animals position 3 had a lower specific activity than position 4. When glycerol was included in the incubation medium with fasted rat liver slices, the distribution of tracer was similar to that found with fed rats. The lower specific activity of position 3 suggests that glycogen formation in *fed* rats involves a condensation of dihydroxyacetone phosphate derived from endogenous precursors (unlabelled) and newly formed 3-phosphoglyceraldehyde prior to the complete equilibration of these trioses. Fed rats, in contrast to fasted rats, have a large source of dihydroxyacetone phosphate precursors, *i.e.*, glycogen and triglycerides of fatty acids.

In most experiments, small amounts of isotope were also incorporated into carbon atoms 1 and 2, and, to a lesser extent, 5 and 6. This asymmetric incorporation of isotope cannot be explained by a simple reversal of anaerobic glycolysis and suggests that under certain conditions, other pathways play a role in hepatic glycogen formation.

Effect of Growth Hormone and ACTH on Plasma Phospholipid Levels and Phospholipid Turnover in Man. E. A. MCCULLOCH, C. J. BARDAWILL, A. BRITTON, and K. J. R. WIGHTMAN, Toronto, Ont. (Introduced by J. A. Dauphinee).

A study of phospholipid turnover in the plasma of a series of patients has been carried out, using P 32 as a tracer. The curve of specific activity of phosphorus in the phospholipid fraction at various intervals after the administration of P 32 has been found to be remarkably constant in a given patient when studied repeatedly, although individual differences among patients may be quite marked. This individual constancy allows each patient to act as his own control, and gives significance to changes in response to treatment which may not be very large.

We have found that opposite alterations in the specific activity curve and the total plasma phospholipid are produced by ACTH and growth hormone. Preliminary observations in acromegaly appear to confirm the growth hormone effect. This consists of a fall in total plasma phospholipids, and a lowering of the specific activity curve. To date this is the only consistent metabolic effect of administered growth hormone that we have been able to detect in the human. A study carried out in an adrenalectomized patient during treatment with cortisone and on another occasion with desoxycorticosterone acetate gave results comparable to those reported when adrenalectomized dogs were similarly treated. Treatment of the intact patient with ACTH produced a rise in the total plasma phospholipid, but did not alter the shape of the specific activity curve. This is taken to indicate a net increase in phospholipid turnover.

These changes are probably mediated in the liver, and their exact significance is not known. The suggestion might be put forward that the change occurring after growth hormone represents an anabolic effect

(after Zilversmit). So far we have not detected any such change in other endocrinopathies.

Skin as an Electrolyte Reservoir in the Experimental Nephrotic Syndrome. JACK METCOFF,* IRENA ANTONOWICZ, SILVESTRE FRENK, JOHN CRAIG, JORGE MARTNER, and JOHN JAMES, Boston, Mass.

A nephrotic syndrome was produced in 24 immature male rats by injection of aminonucleoside (9-6 dimethylamino purine, 3-d amino ribose) for 12 days. Characteristic features and renal lesions were similar to those of the nephrotic syndrome in children. Body composition measured directly by tissue analysis and indirectly by balance study, was compared with that in 24 pair-fed controls. Both groups received NaCl loads orally. Isotope (Na_{24}, and Br_{82} or Cl_{36}) was administered to animals in each group 24 hours before sacrifice.

NaCl loading caused marked salt and water retention with negative potassium balance in nephrotic rats, but virtually unchanged balances in the controls. After sacrifice, nitrogen, electrolyte, and isotope analysis was performed on whole depilated skin, whole carcass, viscera and muscle. Analytical data were referred to non-collagenous dry fat-free solids. Carcass showed relatively small increases in total water, sodium and chloride content. The potassium content of edematous muscle was unchanged, but the intracellular potassium concentration (based on chloride distribution) was significantly reduced due to shift of water intracellularly.

Skin, containing 16 to 18 per cent of total body solids, showed the most striking changes, accounting for more than ½ of non-ascitic edema and ⅛ of tissue retained isotope. The very large amounts of potassium, sodium, and chloride found in these skins could not be satisfactorily assigned to either the extra or intracellular phases by conventional calculations. The data suggest that either sodium or potassium, in addition to chloride, is "bound" in skin. The magnitude of these quantities may define a specific obstacle in the interpretation of balance measurements.

Studies on Destruction of Red Cells by Canine Autoantibodies in Normal Dogs and in a Dog with Naturally Occurring Auto-immune Hemolytic Disease. GERALD MILLER, FRANK W. FURTH, SCOTT N. SWISHER,* and LAWRENCE E. YOUNG,* Rochester, N. Y.

Extensive studies have documented a naturally occurring form of auto-immune hemolytic disease in a dog. The canine disorder is an exact counterpart of the disease occurring in human beings, manifested by profound anemia, spherocytosis, autohemagglutination and agglutinability of the red cells by antiglobulin (Coombs) serum. Temporary remission following administration of ACTH was accompanied by qualitative change of the panagglutinin in the dog's plasma and an *increase* in titer of the panagglutinin.

Exchange transfusion permitted collection of plasma with high content of panagglutinin. Effects of transfusion of this plasma were studied in four experiments, involving three normal recipient dogs. *In vitro,* the panagglutinin strongly sensitized recipient animals' erythrocytes for the antiglobulin test. Following transfusion, the recipients' cells were *not* sensitized for the antiglobulin reaction, but coating of these erythrocytes could be demonstrated by loss of ability to react *in vitro* with samples of the plasma infused. These "blocked" cells showed sphering and increased osmotic fragility, and their life span was shortened as determined by Cr^{51} survival in each of two normal dogs.

Studies on the diseased animal and normal recipient dogs suggest that spherocytosis and hemolysis may be conditioned by factors other than the amount of antibody demonstrable on the cells or in the plasma, and that the "autoantibodies" of the canine disease can initiate hemolysis in normal animals. Although the actual mechanisms of hemolysis initiated by these antibodies still await elucidation, it seems clear that these mechanisms are operative in normal dogs transfused with plasma from the diseased dog.

It is probable that the beneficial action of ACTH in the dog (and perhaps in humans) was not mediated through suppression of antibody formation, but in part, at least, through an effect on the erythrocyte-antibody reaction.

An Analysis of the Rate and Volume of Forced Expiration as a Measure of the Mechanical Nature and Extent of Physiological Impairment Caused by Disorders of Ventilation. WILLIAM F. MILLER, ROBERT L. JOHNSON, JR., and NANCY WU, Dallas, Texas. (Introduced by Edward L. Pratt).

The 0.5 second expiratory capacity and total vital capacity determined during the course of a single maximal expiratory effort are accurate expressions of both the rate and volume of displaceable air and thereby constitute measures of obstructive and restrictive ventilatory disturbances. In order to determine the extent to which these measures estimate not merely displaceable lung volumes but alveolar ventilation as well, the $\frac{0.5 \text{ sec. EC} \times 100}{\text{TVC}}$ (EC ratio) and TVC were compared with arterial pCO_2, indexes of intrapulmonary gas distribution and lung volumes in 150 normal subjects and patients with respiratory disorders.

Ventilatory disturbances were designated as *obstructive* when the EC ratio was decreased below 60 per cent while the TVC exceeded 80 per cent of predicted; *restrictive* when the EC ratio exceeded 60 per cent and the TVC was reduced below 80 per cent; and *combined* when both were reduced. The extent of the impairment of these functions reflected the magnitude of the ventilatory disturbance.

The results of classification and quantitative evaluation of ventilatory disturbances in this manner correlated well with measured lung volumes. In the obstructive group both the total capacity TC, (135 ± 35 per cent) and the residual volume, RV (149 ± 32 per cent) were increased. In the restrictive group the reduced TC (74 ± 11 per

cent) paralleled the reduction in the TVC while the RV was normal (94 ± 29 per cent). Combined disorders revealed reduced TC (87 ± 14 per cent) but the RV (136 ± 50 per cent) varied in a manner predictable by the EC ratio, depending on the extent of airway obstruction present.

Similarly alterations of the EC ratio correlated excellently with measures of alveolar ventilation: arterial pCO_2 ($r - 0.83$), index of intrapulmonary mixing ($r - 0.86$) and the ratio $\frac{RV}{TC}$ ($r - 0.91$).

Thus, analysis of the 0.5 sec. component of the rapid expiratory volume provides a precise evaluation of both the nature and extent of mechanical ventilatory defects as well as the degree to which alveolar ventilation is impaired.

On the Importance of Available Base on Acetazoleamide Induced Renal Excretion of Carbon Dioxide. HERSCHEL V. MURDAUGH, Durham, N. C. (Introduced by J. D. Myers).

In respiratory acidosis due to CO_2 retention it would appear desirable to seek an extrapulmonary route for CO_2 excretion. Past attempts to produce adequate urinary loss of CO_2 using the carbonic anhydrase inhibitor, acetazoleamide, proved disappointing perhaps because of lack of a sustained effect (acetazoleamide fastness) and production of a metabolic acidosis. In considering this failure of acetazoleamide the following questions materialized: (1) What effect does available base have on acetazoleamide fastness? (2) As the renal excretion of CO_2 is increased by acetazoleamide, is the pulmonary output of CO_2 actually decreased? and (3) Is therapy of CO_2 retention by acetazoleamide and sodium loading practicable?

In evaluating the effect of available base on acetazoleamide fastness the following studies were performed. The effect of acetazoleamide on total urinary CO_2 excretion was determined in normal human subjects in the control state, and the subjects then were made acetazoleamide fast either by receiving the rice-fruit diet plus acetazoleamide for five days or by ingestion of ammonium chloride. In such acetazoleamide fast states, total urinary CO_2 excretion was diminished following acetazoleamide, but the effect of the drug was restored by intravenous sodium lactate (14 grams in physiological saline). The data, in mM total CO_2 per min., are as follows: For the rice-fruit diet group, control—0.439 ± 0.059, after diet—0.181 ± 0.025, after sodium lactate—0.265 ± 0.046, after sodium lactate and acetazoleamide—0.522 ± 0.114; for the ammonium chloride group, control—0.523 ± 0.088, after ammonium chloride—0.180 ± 0.039, after sodium lactate and acetazoleamide—0.501 ± 0.152.

The second question was approached by determining pulmonary and urinary outputs of CO_2 before and after acetazoleamide plus sodium lactate, with caloric intake constant in control and test periods. Urinary total CO_2 excretion increased by 1.16 ± 0.05 (S.D.) mM per min., and pulmonary output of CO_2 decreased by 1.34 ± 0.21 (S.D.) mM per min.

With such encouraging findings the effect of acetazoleamide with sodium load in patients with CO_2 retention is now being evaluated.

A New Type of Antibody and a "New Hypothesis" for Antibody-Antigen Interaction. VICTOR A. NAJJAR* and JEAN FISHER, Baltimore, Md.

Antisera to yeast alcohol dehydrogenase were produced in rabbits by multiple injections given subcutaneously. The antibody reacted readily with the enzyme to form a precipitate (antibody-enzyme complex) which still possessed considerable enzymatic activity. The antibody did not react with liver alcohol dehydrogenase. A study of the kinetics of the precipitin reaction at 0° C. showed a fast rate followed by a much slower one proceeding in a linear manner with time. This indicated the presence of two distinct types of antibodies.

When antibody was added to a constant amount of enzyme a point was reached where all the enzyme was precipitated by a definite amount of antiserum. Further additions of antiserum, however, resulted in further precipitation of antibody and further inhibition of the enzymatic activity of the complex. This occurred even when antiserum additions were made after the supernatant had already contained many times the amount of antibody necessary to precipitate the enzyme. These observations led to the hypothesis that the two types of antibodies present were antibodies to the enzyme and antibodies to the complex. Injection of the complex resulted in antiserum which reacted with the complex faster than it did with the enzyme as measured by rate of turbidity formation and rate of inhibition of enzymatic activity.

It was possible to absorb the antibodies to the complex by exhaustive addition of the antigen-antibody until no further reaction occurred. Following this the addition of the (enzyme) antigen resulted in immediate precipitation of antibody.

Requirement for Antibody and Complement in Phagocytosis of Starch In Vitro. ROBERT A. NELSON, JR.* and JACQUELIN LEBRUN, Institut Pasteur, Annexe de Garches, France.

Starch granules have been employed widely as an inert substrate for *in vitro* studies on phagocytosis. In contrast to the concept that starch is inert the present data demonstrate that two components of "normal" serum are essential for phagocytosis of the starch granule. The heat-stable component of serum conforms to the definition of antibody since 1) it is a serum globulin; 2) it is specifically and rapidly adsorbed to starch at 0° and is not eluted by repeated washings with saline; 3) it appears in increased quantity in animals injected with starch; and 4) once combined with starch, it induces specific immunological phenomena, *i.e.*, opsonization, agglutination and immune-adherence. The heat-labile component of serum appears to be complement. By papain

treatment of the starch-antibody-complement complex, it is proved that both serum components act on the starch granule and not on the phagocytes.

The constituent of the starch granule which is antigenic has not yet been defined.

A brief survey has indicated that antibody to starch is found in "normal" sera from several different species, including man.

Studies on a Second Outbreak of a Newly Recognized Infectious Exanthem. FRANKLIN A. NEVA, Pittsburgh, Pa. (Introduced by I. Arthur Mirsky).

An epidemic of an unusual exanthem which occurred in Massachusetts, in 1951, has been reported recently. Evidence was presented indicating the etiological relationship between this disease and a group of viruses isolated from these patients.

The initial observations have been corroborated and extended by studies of a second outbreak of a similar clinical entity, in Pittsburgh, during 1954. At least eighteen cases occurred, during two weeks, in June, among thirty families in a residential suburb. Six additional cases were detected, from July to September, in households in four different areas of the city. Multiple infections in six households were observed with adults as well as children affected.

Clinically, children showed fever for one or two days which was either followed by, or associated with, a pink maculo-papular skin eruption lasting two to three days and evident over the face, trunk, and extremities. Systemic signs and symptoms were usually minimal. By contrast, systemic manifestations were prominent in adults with chills and fever, severe headache, and muscle aches and pains. A skin rash which appeared after defervescence was recognized only in four of the eight adults studied.

Viruses have been isolated, with tissue culture techniques, from eleven of thirteen patients, representing both the suburban epidemic and sporadic cases. Most isolations have been from feces, but in one patient the agent was recovered from the blood. Based upon tissue culture host range, the agents resemble the group of viruses isolated from the Boston outbreak. Preliminary serologic tests indicate that Pittsburgh patients developed neutralizing antibodies to both local and Boston strains of virus.

These findings confirm the existence of an infectious exanthem differing from the common exanthemata. They support, furthermore, indications of an etiologic relationship between this disease and the agents isolated from specimens obtained during the acute phase of illness.

The Relation of Valve Function to the Genesis of the Sharp First Sound in Mitral Stenosis. HENRY T. NICHOLS, WILLIAM LIKOFF, HARRY GOLDBERG, and PHILIP LISAN, Philadelphia, Pa. (Introduced by Joseph DiPalma).

This study defines the genesis of the sharp first sound of mitral stenosis by correlating the mechanical function of the deformed valve with the laws that determine the duration and pitch of sound.

Events in the closure of normal and stenotic mitral valves were recorded in a group of patients undergoing cardiac surgery. Duration and pitch of the first sound were tabulated independently in these same patients prior to operation. The time relation of the first sound to the ventricular isometric period was determined by: (a) recording sounds simultaneously with pressure determinations during right heart catheterization, or (b) recording sounds from the surface of the heart when left ventricular pressures were taken at surgery.

Two events in the closure of the normal valve, the contacting force of the cusps, and intra-atrial displacement of the leaflets, were modified in mitral stenosis. The force was decreased consistently, and displacement was more abrupt or was eliminated entirely.

The first sound was sharp and delayed in relation to the isometric period when ballooning was abrupt. This type of valve acoustically functions as a two-dimensional stretched membrane, and it was concluded that the sharp, short sound ensued because of the decreased length and mass, and increased inertia and tension of the leaflets.

The first heart sound was normal in duration, pitch, and timing when extensive leaflet calcification prevented intra-atrial displacement. This type of valve functions acoustically as a diaphragm in which elasticity alone determines pitch.

Following mitral commissurotomy, the alteration in the sharp first sound is related to a buffered ballooning effect not to the size of the orifice. A sharp sound may appear when it was not present, if the valve is converted from a diaphragm to a two-dimensional stretched membrane with abrupt leaflet displacement.

The investigation suggested that the manner and degree of intra-atrial displacement is the mechanical event in the genesis of the sharp sound, and is an expression of changes which determine the vibratory qualities of the valve.

The Mechanism of Ammonia Excretion. JACK ORLOFF and ROBERT W. BERLINER,* Bethesda, Md.

Ammonia excretion is conditioned by factors involving intracellular production and net transport into urine. The urine pH is the major determinant of transport in the case of ammonia and certain exogenous weak bases. Thus it is possible to distinguish factors influencing ammonia production from those influencing transport by comparing the simultaneous excretion of ammonia and specific organic bases. Infusion of amino acid precursors of ammonia markedly enhanced ammonia excretion and increased urine pH; the decrease in titratable acidity was less than the rise in ammonia excretion. These data together with demonstration of a reproducible relationship between urine pH and rate of ammonia excretion when no substrate was administered, indicate that NH_3 diffuses into the urine and accumulates as non-diffusible NH_4^+. The weak bases can be considered to enter the urine by a similar process. The rate of excretion of ammonia

was found independent of urine flow when urine was acid but not when urine was neutral or alkaline. This is interpreted as indicating that ammonia enters the urine at a rate limited by ammonia production throughout the acid range. At higher pH's rate of production is non-limiting and rate of excretion is dependent on equilibration of the ammonia in tubule urine with that in tubule cells. Since the relationship between pH and rate of excretion extended into the most acid range, it is assumed that the more acid the urine the greater the length of tubule over which the urine is acid, and therefore the greater the segment of tubule which can contribute maximally to ammonia output. Since rate of excretion rather than concentration was a function of pH even in the alkaline range, these substances must enter urine at a site proximal to final water transport.

Studies on Headache: The Appearance of the Bulbar Conjunctiva Vessels in Patients with Vascular Headache of the Migraine Type. ADRIAN M. OSTFELD, New York, N. Y. (Introduced by Harold G. Wolff).

Observations of the bulbar conjunctiva in patients during headache and headache-free periods were made using a Poser slit lamp at a magnification of 47.5 × and camera attachment. Twenty seven patients were observed on one hundred and sixty occasions, and over two hundred photographs were made.

During the hours preceding headache an euvascular state was usual. Moderate arteriolar and venular constriction was also noted in some patients. During a migraine headache, dilatation of arterioles and venules, and increase in the number of patent capillaries were predictably noted on the headache side. Conjunctival edema was manifested by blurred appearance of tissue locally and indistinctness of the vascular structures. Slowed flow, sludging of blood, and venular hemorrhagic accumulation were frequently recorded. Vascular dilatation and conjunctival edema persisted as long as local scalp tenderness was present. Changes similar to those of migraine were seen unilaterally in two patients with atypical facial neuralgia. During subsidence of migraine headache as the result of intravenous infusion of nor-epinephrine, dilated arterioles constricted and showed increased segmental spasm. Also, edema disappeared. The caliber of venules was not immediately significantly altered.

Observations on patients who had had frequent and severe unilateral headache for many years exhibited tortuosity and moderate dilatation of venules which persisted even during headache-free periods. In muscle contraction headache, constriction of relevant arterioles and venules with blanching of capillary beds was observed. Arterioles showed striking segmental spasm and flow was slowed in vessels of all calibers.

Cranial artery pressure pulse waves were obtained using a glycerine pelotte, piezoelectric pulse wave attachment and direct writing electrocardiograph type recorder. High amplitude smooth contoured pulse waves from temporal and supraorbital arteries indicative of arteriolar dilatation accompanied conjunctival minute vessel dilatation during migraine headache. Low amplitude pulsations with increased number and size of reflected waves indicating vasoconstriction accompanied constriction of conjunctival minute vessels during muscle contraction headache.

The Effects of Isotonic and Hypertonic Salt Solutions on the Renal Excretion of Sodium. SOLOMON PAPPER, LAWRENCE SAXON, and HENRY W. COHEN, Boston, Mass. (Introduced by Maurice B. Strauss).

The natriuretic response to acute sodium loading administered as isotonic saline and hypertonic saline was studied in eight normal subjects. It was hoped that in this manner some information concerning the relative importance of acute changes in filtered sodium and acute changes in extracellular body fluid volume, as stimuli for sodium excretion might be obtained.

While taking 137 mEq. of sodium chloride daily, each subject was studied as follows. After 2 hours of control observation, 338 mEq. of sodium chloride was given intravenously over a 90-minute period. The salt was given as 0.9 per cent saline on one occasion and 5.0 per cent on another. In five of the subjects an additional experiment was performed infusing the same quantity of sodium as an isotonic "balanced" chloride-bicarbonate solution. Observations were continued for 2½ hours after the infusion was stopped.

There was no significant change in creatinine clearance associated with salt loading. Serum sodium concentration was increased more when 5 per cent salt was used than when either the 0.9 per cent saline or the isotonic-balanced solution was administered. The 0.9 per cent saline evoked a natriuretic response equal to or greater than that caused by the infusion of 5 per cent saline in six of the eight subjects. In the other two subjects the strong salt solution produced the greater increase in sodium excretion. The isotonic-balanced solution was as potent a natriuretic stimulus in two of the five subjects, a definitely weaker stimulus in two and a questionably weaker stimulus in one.

These data are consistent with the view that both increased filtered load of sodium and increased extracellular fluid volume are stimuli for sodium excretion. Furthermore, isotonic saline was at least as effective as hypertonic saline, in inducing renal sodium excretion.

Dynamics of Acute Failure of the Left Ventricle During Neurogenic Hypertension. J. L. PATTERSON, JR.* and H. G. LANGFORD, Richmond, Va.

The dynamics of the acutely failing left ventricle were investigated by the induction of acute myocardial necrosis and sudden hypertension. Injections of zinc hydroxide (F. H. Meyers) were made into the left ventricular myocardium, in nine dogs, and hypertension induced by electrical stimulation of the cerebral motor cortex for 60 to 180 seconds. Pressures were measured with strain gauges before and after dextran infusion, and cardiac output by dye dilution. Five normal animals given dextran and 40 animals previously studied served as controls.

Despite necrosis of 15 to 50 per cent of the left ventricle, mean aortic pressure rose with cortical stimulation by 64 mm. Hg, compared with 80 mm. Hg in the controls, primarily due to systemic vasoconstriction since cardiac output was unchanged. Calculated cardiac work increased 21 per cent in the experimental animals, 42 per cent in the controls. Pulmonary arterial and capillary (wedge) pressures showed small rises in the experimental and control animals during stimulation.

After infusion of 20 to 30 ml. dextran per kilo., stimulation produced increases in aortic pressures up to 240 and 210 mm. Hg in control and experimental dogs. Cardiac output increased 45 per cent in both groups over predextran values. Dextran infusion resulted in slight wedge pressure rise in controls, with little further change during stimulation. In the experimental dogs, infusion alone produced 11 mm. Hg rise in capillary pressure. An additional 13 mm. increase on stimulation resulted in several pressures over 40 mm. Hg. One experimental animal had no increase in pulmonary capillary pressure but a 14 mm. Hg rise in left atrial pressure.

These observations show that a severely injured left ventricle can support acute hypertension and large increases in cardiac work for brief periods but at the price of high filling pressure. They further indicate that increase in blood volume plays a critical role in the phenomena of acute left ventricular failure.

Some Problems in Interpreting the Significance of Total Peripheral Resistance. LYSLE H. PETERSON, Philadelphia, Pa. (Introduced by R. D. Dripps).

The dynamics of hemodynamics is incompletely understood. One important aspect of the complex subject involves the interpretation of so-called total peripheral resistance. Its magnitude is commonly assumed to be given by the ratio: mean intra-aortic pressure minus mean "central" venous pressure/mean cardiac output. Even if that simple assumption were true, *i.e.*, no energy added by the skeletal and respiratory pumps, the *distribution* of the resistance is not indicated. The localization of the resistance is, however, important to an understanding of the physiology, pharmacology, and diseases of the cardiovascular system. One approach involves the measurement of pressure drop. For this to have meaning it would be necessary to measure simultaneously the blood flow as well as the pressure drop along all vessels in the cardiovascular system. Even if such a task were possible the summation of values would be further complicated by the fact that the resistances are in series, parallel, and series-parallel. We have used another approach, favored by physicists and engineers, to study complex circuit parameters including resistance. This method involves the analysis of the pressure pulse which results when an artificial stroke volume, of known parameters, is introduced into the aorta. By so doing we have been able to differentiate the resistance which is distributed along the arterial system from the total peripheral resistance. This is the pre-arterial or, more strictly the pre-elastic resistance. This resistance offered by the arterial system itself is least when blood pressure is "normal" but increases rapidly with hyper- or hypo-tension. We have concluded that the magnitude of these changes are such that prearteriolar resistance may have an important role in normal and abnormal cardiovascular behavior.

The Miscible Pool and Turnover Rate of Hydrocortisone in Man. RALPH E. PETERSON and JAMES B. WYNGAARDEN, Bethesda, Md. (Introduced by Harry Eagle).

Data on total daily adrenal hydrocortisone production have not been available. Attempts to estimate its production by measurement of urinary steroid metabolites of hydrocortisone have been unsuccessful because of inadequacies of hydrolytic and colorimetric methods. Applying conventional tracer techniques, we have administered hydrocortisone-4-C^{14} intravenously in tracer amounts in 10 per cent ethanol (200 to 500 micrograms; 1 to 2.5 microcuries). Plasma samples were collected over 4-hour period and extracted with dichloromethane. The dichloromethane was evaporated dry, the residue applied to paper and chromatographed in a Bush-type system. Hydrocortisone was eluted, and its specific activity determined by assaying aliquots for radioactivity and hydrocortisone by sulfuric acid-ethanol fluorescence. From linear semilogarithmic plots of specific activity *vs.* time, the metabolic pool of hydrocortisone and rate of turnover was calculated. In eight studies in five normal subjects the pool of hydrocortisone ranged from 1.3 to 2.4 mg. with turnover rates of 0.30 to 0.67 pools per hour (0.66 to 1.10 mg. per hour). Projected to daily endogenous hydrocortisone production, figures of 16 to 26 mg. per day were obtained, with mean of 21.3 mg. Hydrocortisone spaces, calculated from pool size and plasma concentration, were 15 to 20 liters. Two patients with cirrhosis of the liver had turnover rates of 4.7 and 7.2 mg. per day, with increased pool in one with ascites. Two patients with hypothyroidism had turnover rates of 3.5 and 13.8 mg. per day. A normal subject under influence of continuous maximal ACTH stimulation had pool of 9.07 mg., with turnover of 181 mg. per day. In normal subject given 60 mg. metacortandracin over 36-hour period prior to study, hydrocortisone production 8 to 12 hours after the last dose was 1.2 mg. per day. This method offers promise for more precise measurement of adrenal function.

The Effect of Carbon Dioxide on the Concentration of Calcium in Ultrafiltrate of Serum Obtained by Centrifugation. ANANDA PRASAD and EDMUND B. FLINK, Minneapolis, Minn. (Introduced by F. W. Hoffbauer).

An ultrafiltrate of serum was obtained by centrifugation of serum in Visking® casing suspended in gauze bags in large centrifuge tubes. Centrifuging 10 ml. serum at 2500 r.p.m. for 75 minutes produced 2.5 to 3.0 ml. ultrafiltrate. The blood was collected and serum separated anaerobically using mineral oil seal. The Visking® casing and centrifuge tube for its suspension were flushed

with 5 per cent carbon dioxide and 95 per cent oxygen (I). In other experiments blood was drawn without anaerobic precautions (II) and in a third set of experiments the serum was saturated with carbon dioxide by bubbling the same mixture through the serum or by using higher concentration of carbon dioxide for flushing the centrifuge tube (III). Calcium was determined by the Kramer and Tisdall method both on serum and on ultrafiltrate. pH was determined on the serum residue in the casing at the end of centrifugation in (I) and (III).

When the diffusible calcium in normals is expressed as per cent of total calcium the mean and range of values in the three procedures are as follows: 53.3 per cent (47 to 56) in (I); 52 per cent (42.7 to 58.4) in (II); 64.2 per cent (57 to 72) in (III).

There is a significant increase in diffusible calcium when the serum is exposed to an excess of carbon dioxide (III) although the pH is kept in the same range as I. This suggests that the excessive carbonic acid produces an artefact and that the normal diffusible calcium expressed as per cent of total calcium actually is 53 per cent ± 5.

Relationship of Corticotropin, Intermedin, and Growth Hormone to Metabolic Activities of Pituitary Extracts. M. S. RABEN * and E. B. ASTWOOD,* Boston, Mass.

Purification of pituitary extract by adsorption on oxycellulose yielded a product potent in corticotropin, intermedin, and extra-adrenal metabolic effects concerned with fat and carbohydrate metabolism. The product did not contain growth hormone, which was concentrated from the fraction not adsorbed by oxycellulose. Fractionation of the oxycel-adsorbed product on columns of carboxylic resin or oxycellulose, or by countercurrent distribution or, less satisfactorily, by precipitation methods, separated a fraction, constituting about 10 per cent of total, of greater intermedin potency and greatly diminished corticotropic and metabolic activity, and a corticotropic-metabolic fraction with much diminished intermedin activity. The latter fraction did not yield to attempts to separate the corticotropic from the metabolic activity and from the residual intermedin activity and no differences in susceptibility of the corticotropic and metabolic activities to inactivation were found. Treatment with 0.1N NaOH at 100°C. increased intermedin potency 10 to 30 times (tested in *Rana pipiens*) and greatly increased duration of action. Treatment for 1 minute potentiated intermedin without appreciable loss of metabolic activity; after 15 minutes, corticotropic and metabolic activities were largely lost while intermedin activity was still enhanced. Metabolic and intermedin activities were also found present in alpha-corticotropin, corticotropin-beta, and corticotropin-B, fractions isolated from the oxycel product in three different laboratories. Both oxycel material and purified growth hormone preparations caused in mice increased liver fat, ketosis, lowering of blood sugar, and protection against insulin hypoglycemia, but the oxycel fraction was many times the more potent. The oxycel fraction was not found to be diabetogenic in the dog, while growth hormone preparations were diabetogenic but the ratio of growth to diabetogenic activity varied widely. The overlapping activities which were found may result from inadequate separation of contaminants but the possibility is also considered that the several metabolic activities of corticotropin and growth hormone are side effects of obscure physiological significance.

The Effects of Hydrocortisone on Water Diuresis and Renal Function in Man. LAWRENCE G. RAISZ, WILLIAM F. MCNEELY, LAWRENCE SAXON, and JACK D. ROSENBAUM,* Boston, Mass.

An enhanced diuretic response to water in subjects receiving cortisone has been previously described. In order to investigate the mechanism of this change the acute effects of a 5 to 6-hour intravenous infusion of 200 mg. of hydrocortisone were observed in three subjects during maximal water diuresis. In two of these, serial water diureses were studied before, during, and after the oral administration of 160 to 280 mg. of hydrocortisone daily for 10 to 14 days. The influence of oral cortisone, 300 mg. daily, was similarly studied in another subject. Each study included determination of electrolyte excretion, clearances of inulin (GFR), paraaminohippurate (ERPF), total osmols (C_{osm}) and "free" water (C_{H_2O}). All subjects were healthy men, maintained on low sodium diets.

During oral hydrocortisone administration maximal water diuresis increased progressively. By the second week C_{H_2O} had risen from 9 and 16 ml. per min. to 17 and 23 ml. per min. in the two subjects, and the per cent of filtered water excreted from 10 and 14 to 16 and 19 per cent, respectively. GFR and ERPF increased irregularly to maximal values 10 to 20 per cent above control levels. Sodium excretion, C_{osm}, and body weight all increased slightly. After hydrocortisone was discontinued C_{H_2O} returned to pretreatment values in 3 to 5 days. Similar changes were observed with oral cortisone.

Intravenous hydrocortisone produced smaller increases (2 to 4 ml. per min.) in C_{H_2O} with a larger rise in GFR and decreased sodium excretion.

The observed increase in C_{H_2O} without fall in C_{osm} implies increased delivery of water and solute to the distal tubule where the extra solute, but not the water was largely reabsorbed. Such an increased delivery could be a consequence of increased GFR alone; however, the lack of correlation between changes in GFR and C_{H_2O} indicates an additional direct effect of hydrocortisone to diminish proximal reabsorption.

The Effect of Clotting on the Spontaneous Activation of Plasmin. OSCAR D. RATNOFF,* Cleveland, O.

The biological significance of the proteolytic activity which is potentially available in human plasma is undetermined. Plasmin, the proteolytic enzyme or enzymes effective at neutrality, is capable of digesting fibrinogen, fibrin and other plasma components. However, in freshly shed blood, plasmin appears to be in an inactive form,

plasminogen. Under certain conditions *in vitro,* plasmin may become active "spontaneously," or upon addition of such agents as chloroform, tissue particles or streptokinase, a principle elaborated by hemolytic streptococci.

Earlier experiments demonstrated that the activation of plasminogen by streptokinase was greatly potentiated by blood coagulation. Evidence has now been obtained that the *spontaneous* activation of plasmin is also potentiated by the clotting process.

A globulin fraction of native plasma, prepared in silicone tubes without the addition of anticoagulant, remained fluid in silicone-coated tubes but clotted readily when mixed with crushed glass. This fraction contained fibrinogen, prothrombin, proaccelerin, proconvertin, plasminogen and many other substances. After 24 hours, the unclotted or clotted globulins were mixed with casein, and the amount of casein digested was determined in silicone tubes. Much more casein was digested by the clotted globulin than by the unclotted globulin. In control experiments, globulin prepared from serum instead of plasma developed no more proteolytic activity in glass than in silicone tubes, and no more than the same subject's unclotted native globulin in silicone tubes.

Thus it appeared that the clotting process activated plasmin from its precursor. This correlated with the rapid lysis of fibrin which occurred in the globulin which had been clotted in the presence of glass, compared with the stability of fibrinogen in the unclotted globulin kept in silicone. These experiments suggest that the proteolytic activity of plasma may be activated when clotting occurs, permitting the destruction of fibrin. The possible importance of this phenomenon *in vivo* is apparent.

Modification of the Gastric Secretory Response to Histamine During Varying Behavioral States: Observations on an Infant with a Gastric Fistula. FRANZ REICHSMAN, GEORGE L. ENGEL,* and HARRY L. SEGAL, Rochester, N. Y.

It is generally believed that histamine acts directly on the peripheral secretory mechanism of the stomach. There is some evidence that the magnitude of response to a standard dose of histamine is related to the peripheral reactivity of this secretory mechanism, as influenced by stimuli arriving *via* normal physiological channels.

Observation of a 1½ year infant with a congenital atresia of the esophagus and a gastric fistula provided an opportunity to examine the relation between behavioral states and gastric secretion, including the responses to histamine. Over a period of five months, behavior and gastric secretion were studied during 59 observation periods, a total of 161 hours, yielding more than 600 specimens of gastric juice. Histamine phosphate, 0.1 mg. per 10 kg. of body weight was administered subcutaneously nine times, yielding 55 specimens of gastric juice.

In general it was found that the mean rate of secretion of total HCl (mEq. per min.) when the child related actively to the experimenter in a friendly or aggressive way was more than twice as much as when she was withdrawn, depressed, or asleep ($P < 0.01$). Histamine actively stimulated HCl secretion during the outgoing states, but was relatively ineffective during the depressed, withdrawn state or sleep. Thus, in three experiments when relating actively to the experimenter the total secretions of HCl were 2.21, 2.35, and 2.39 mEq. in an hour; in three experiments when withdrawn, 0.16, 0.49, and 0.54 mEq. in an hour. When the 55 specimens of gastric juice were classified according to the behavior manifest during the period of collection of each specimen, the same relationship was established. Thus, specimens obtained during periods of withdrawal, depression, and sleep revealed significantly lower rates of total HCl secretion (mEq. per min.) than those during an active relationship with the experimenter ($P < 0.01$).

The Nephropathy of Potassium-Depletion: A Clinico-Pathologic Entity. ARNOLD S. RELMAN* and WILLIAM B. SCHWARTZ,* Boston, Mass.

A study of five adults with severe chronic potassium-depletion has defined the clinical and pathological features of a previously unrecognized renal disorder apparently caused by potassium deficiency.

Potassium-depletion in each instance had resulted from chronic diarrhea of various etiologies. Urinary excretion of potassium, measured in four patients, was below 10 mEq. per day, indicating efficient renal conservation. Severe hypokalemia was the rule, but no patient was dehydrated or sodium-depleted, and none had significant acid-base disturbances. One patient noted marked polyuria and polydipsia, and two had intermittent ankle edema. Routine urinalyses revealed minimal inconstant albuminuria and cylindruria in three.

BUN and serum creatinine were normal or only slightly elevated, but PSP excretion was low, and all patients exhibited Pitressin®-resistant hyposthenuria. Clearances of inulin, endogenous creatinine and PAH were significantly reduced. In the one patient from whom renal venous blood was obtained, the PAH extraction ratio and Tm PAH were low.

Full replacement of potassium deficits in three patients resulted in gradual restoration of normal renal function in two, and nearly complete recovery in the third. Potassium replacement was incomplete in the remaining two patients; renal function was unimproved after a year in one, while in the other concentrating ability improved but the clearances of inulin, creatinine, and PAH did not.

Renal biopsies were obtained in the latter two patients. In each case there were striking hydropic and degenerative changes, confined chiefly to the convoluted tubules, without significant glomerular or vascular disease. These lesions resemble the vacuolar tubular changes described by earlier workers in patients dying of various diarrheal diseases, and they are not unlike the renal changes produced by experimental potassium-depletion in rats. Despite absence of significant functional improvement following partial repletion, repeat biopsies showed distinct evidence of healing. This observation, taken together with the functional recovery in the other patients, suggests that the disease is probably reversible.

Effects of Hydrocortisone on Carbohydrate Metabolism of a Patient with Renal Glucosuria (DeToni-Fanconi Syndrome). ALBERT E. RENOLD, E. RUDOLF FROESCH, JOHN B. REARDAN, and JOHN T. FINKENSTAEDT, Boston, Mass. (Introduced by George W. Thorn).

The measurement of hormonal effects on carbohydrate metabolism in man is hampered by the efficient mechanisms which maintain constant blood glucose levels and nearly complete glucose reabsorption from the glomerular filtrate. Incomplete tubular reabsorption of glucose leads to a persistent drain on blood glucose which is proportional to the filtered glucose load. With incomplete tubular reabsorption, urinary glucose excretion in the fasting state has been assumed to reflect gluconeogenetic activity. The effects of hydrocortisone on glucose excretion have been studied in a patient with severe congenital renal glucosuria, regularly excreting 30 to 40 per cent of the filtered glucose load at blood glucose levels below 100 mg. per cent. Two requirements in methodology had to be met: (1) Urinary glucose determinations were made with a specific enzymatic method (using glucose oxidase) to eliminate interfering non-glucose reducing substances; (2) Glucose was enzymatically removed to facilitate inulin measurements in urine specimens containing large amounts of glucose. Hydrocortisone was administered intravenously to the fasting patient (25 mg. per hour for 8 hours). Inulin and creatinine clearances were determined throughout. Urinary glucose excretion rose from an average of 11 mg. per minute to a maximum of 37 mg. per minute. The greater portion of this increase could be related to the increased filtered glucose load and is assumed to represent increased hepatic gluconeogenesis in view of the progressive increase in blood glucose levels. In addition, however, *a definite decrease in tubular glucose reabsorption was noted* (from 18 mg. per minute to 7 mg. per minute). These changes will be related to nitrogen, phosphate, and potassium excretion. Similar studies in normal subjects will be reported and animal studies attempting to elucidate the mechanism of decreased tubular glucose reabsorption will be discussed.

Renal Excretion of Carbon Dioxide in Normal Subjects and in Subjects with Carbon Dioxide Retention Following Blockage of Bicarbonate Reabsorption in the Renal Tubule. ALBERT L. RUBIN and WARREN S. BRAVEMAN, New York, N. Y. (Introduced by Thomas P. Almy).

It is known that patients with carbon dioxide retention and respiratory depression may improve clinically after the administration of Diamox®, a carbonic anhydrase inhibitor. In association with this improvement, arterial pCO_2 falls. The pCO_2 of the urine has been observed to rise as renal bicarbonate excretion increases. If the rise in urinary pCO_2 were of sufficient magnitude, it might account for the fall in arterial pCO_2 seen in these patients with chronic carbon dioxide retention after Diamox® is given.

Urinary excretion of sodium, potassium, bicarbonate, and chloride ions, and of carbon dioxide was measured in six normal subjects, and in five subjects with chronic carbon dioxide retention, before and after the administration of Diamox®.

A strikingly similar pattern of response was obtained in the six normal subjects. After Diamox® administration, all exhibited a rise in urinary pCO_2 which paralleled the increased excretion of bicarbonate ion. The six patients with chronic carbon dioxide retention, all of whom improved clinically and showed a significant fall in blood pCO_2 after Diamox® was given, exhibited increases of urinary pCO_2 of the *same* order of magnitude as the normal subjects. This is not of sufficient magnitude to account for the fall in arterial pCO_2 that is observed. Therefore we believe that renal excretion of carbon dioxide is not a significant factor in lowering the arterial pCO_2 in patients with carbon dioxide retention given Diamox®. Some other compensating mechanism must be postulated.

Glutamic-Oxalacetic Transaminase Levels in Experimental Tissue Damage. L. A. RUDOLPH, R. DUTTON, and J. A. SCHAEFER, Syracuse, N. Y. (Introduced by R. H. Lyons).

This study involved investigation of serum glutamic-oxalacetic transaminase levels, using the method described by Karmen, in dogs subjected to infarction and ischemia of various organs. Venous blood was drawn daily before and after each surgical procedure. Eight animals were subjected to myocardial infarction, while branches of the pulmonary artery were ligated in six. Ligation of the renal, splenic, and mesenteric vessels was done in groups of three dogs. Elevations of serum transaminase activity in peripheral venous blood in these studies were characterized by peak levels within twenty-four hours which fell promptly to normal, with the exception of ligation of the lobar vessels where a secondary rise was noted.

Those experiments which produce infarctions involving small areas of myocardium did not always result in increased activity in the peripheral blood above the normal range. There was a similarity between the pattern of response in these experimental infarcts and the clinical cases of myocardial infarction. Generally, there was a rough correlation between the degree of tissue involvement and level of enzyme activity.

Nine animals were subjected to temporary occlusion of splenic, renal, pulmonary, mesenteric, and coronary vessels for short periods of time. Ischemia of this short duration produced no elevation of levels in the peripheral blood.

This study suggests that increases in serum enzyme activity are a manifestation of infarction of the tissues studied, which are not a characteristic of any particular organ.

Evidence for Circus Movement in Atrial Flutter in Man. DAVID A. RYTAND,* DAVID L. BRUNS, and SILVIO J. ONESTI, JR., San Francisco, Calif.

If the mechanism of human atrial flutter involves rapid stimulation from an ectopic focus uninfluenced by return-

ing excitation, the rate probably should be relatively independent of atrial size. But if the mechanism includes a continuing wave of excitation (circus movement), the rate should be reduced by (a) slower conduction velocity or (b) lengthened pathway as might accompany atrial dilatation.

The atrial flutter rate is relatively slow in patients with right (but not left) atrial dilatation. In 7 patients in this clinic and 9 more obtained from the literature and by personal communications, the mean rate with right atrial dilatation was 210 per minute (cycle, 0.28 sec.). Without dilatation, 93 other patients with flutter had a mean atrial rate of 284 (cycle, 0.21 sec.). The difference of 0.07 sec. in cycle duration is statistically significant. Patients receiving quinidine were excluded, as were those without electrocardiographic undulations typical of flutter as distinct from paroxysmal atrial tachycardia.

Evidence was obtained which favored lengthened pathway of a hypothetical circus wave rather than depressed conduction velocity as an explanation for this clinical finding. Observations with esophageal electrodes in 2 patients with flutter and dilated atria yielded velocities of approximately 125 and 167 cm. per sec. as the wave travelled upward, even more rapid than values about 84 cm. per sec. found similarly in others with flutter but without atrial dilatation. Such acceleration of velocity, expected with longer pathways, is contradictory to the depression predictable if atrial anoxia or high intra-atrial pressure were the cause of the observed slow rates.

Finally, exploration of the anterior chest walls of these 2 patients strongly suggests a downward descent of excitation, as if the wave were returning.

These observations represent evidence in support of the circus movement hypothesis as a mechanism in human atrial flutter.

A Physiological Explanation for Results of Water Loading Tests. F. SARGENT, II, R. E. JOHNSON,* A. A. PANDAZI, I. J. LICHTON, and T. W. NIELSEN, Urbana, Ill.

To investigate whether or not water loading tests can be used to estimate dehydration, simultaneous measurements were made of deuterium oxide space by a dilution technique after oral dosage, serum and urine osmotic pressures by freezing point depression, and urinary excretion of a test dose of water (20 ml. per kg. body weight; excretion measured for four hours; correction for basal excretion; subjects reclining, not smoking). For two weeks during a six-week study, 99 healthy young men in groups of four or more subsisted on controlled regimens of unlimited or limited water intake (910 ml. per day); low osmotic load (pure carbohydrate); high osmotic load (high protein—low salt, or moderate protein—high salt); or intermediate osmotic load (medium protein or medium salt). Measurements were made initially and again after 12 days. The water test dose was not retained in normally hydrated individuals, regardless of osmotic load; or in dehydrated, osmotically depleted subjects. It was retained in dehydrated subjects whose osmotic excretion was moderate to high. The hypothesis is defended that, in healthy subjects, the body excretes a test dose of water when the normal total body osmotic concentration is in danger of depression, but retains water when the osmotic concentration will not be depressed below normal by the test dose. The clinical implication of this hypothesis is that the response of a patient to the water loading test of Robinson, Power, and Kepler may be determined primarily by state of hydration and osmotic balance; and not specifically by endocrine or renal function.

The Survival of Normal Ceruloplasmin in Patients with Hepatolenticular Degeneration (Wilson's Disease). I. HERBERT SCHEINBERG,* DONALD T. DUBIN, and RUTH S. HARRIS, New York, N. Y.

Wilson's disease is characterized by a deficiency of a specific plasma protein, ceruloplasmin. This deficiency could be caused by an increased rate of destruction, or loss of the protein, on the one hand, or by a decreased rate of synthesis of the protein, on the other, in comparison with normal subjects. The present work was undertaken in an attempt to distinguish between these mechanisms.

Ceruloplasmin was administered by means of incomplete exchange transfusions to three subjects. Two of these were girls, aged 13 and 21, with Wilson's disease who possessed less than four per cent of the normal level of ceruloplasmin. The third subject, a healthy one-year-old brother of the younger girl, possessed less than two per cent of the normal level of this plasma protein.

The concentrations of ceruloplasmin which were attained in these subjects were determined at intervals following the transfusions. The half life time of the administered ceruloplasmin, calculated from these measurements, was 3.4, 4.4, and 4.7 days, respectively, in the one, 13 and 21 year-old subjects.

If the rate of synthesis (grams per day) of ceruloplasmin in any of these subjects were assumed to be the same as that in normal subjects, the latter would be required to have ceruloplasmin whose half life time was at least 110, 118, or 170 days in order to maintain normal plasma levels of the protein. Half life times of such magnitude are sufficiently greater than those known for other plasma proteins to cast serious doubt on their being correct. Therefore, there is probably a diminished rate of synthesis of ceruloplasmin in the subjects studied in comparison to the normal.

Effect of Salt Restriction on Sodium Excretion in Sweat. IRVING L. SCHWARTZ, NIELS A. THORN, and ALVAN R. FEINSTEIN, New York, N. Y. (Introduced by Vincent P. Dole).

The sodium concentration of human sweat is known to be reduced by administration of desoxycorticosterone and also by physiologic and pathologic hyperactivity of the adrenal cortex.

Previously we have presented evidence that sodium

excretion in sweat involves two processes, one, delivery of sodium into a precursor solution, the other, reabsorption from this precursor. We believe that both of these processes require cellular work because studies with weak electrolytes show that only the un-ionized molecular species can enter the sweat by simple diffusion. The question is, therefore, whether this steroid-induced reduction in sweat sodium concentration is due to diminished sodium input or enhanced reabsorption. The former implies reduced cellular work, the latter, increased.

The rate of sodium input into the precursor, I_{Na}, and the rate of reabsorption, R_{Na}, increase with rising sweat flow. Therefore, both I_{Na} and R_{Na} have been measured at a standard sweating rate of 10 grams M^2 minute. At this flow I_{Na} exceeds R_{Na} in all tubules; thus R_{Na} represents maximal reabsorptive capacity.

Four patients were studied during periods of high (175 mEq. per day) and low (5 mEq. per day) dietary sodium intakes. During the high sodium period, I_{Na} averaged 753 ($\pm$ 96.3) μEq. per M^2 minute and R_{Na} averaged 238 ($\pm$ 54.9) μEq. per M^2 minute. Restriction of sodium decreased I_{Na} to 343 ($\pm$ 48.2) μEq. per M^2 minute and also decreased R_{Na} to 78.5 ($\pm$ 19.0) μEq. per M^2 minute.

These findings, in conjunction with similar observations after administering desoxycorticosterone, suggest that the salt-active steroids decrease sodium reabsorption. The diminished input is of sufficient magnitude to lower the sodium concentration of the final product despite the decreased reabsorption. Thus, the steroids decrease the cellular energy expended for both the delivery and the reabsorptive processes.

The Precordial Electrocardiogram: Is it Predominantly Derived from the Heart Muscle Immediately Beneath the Electrode? GEORGE E. SEIDEN and ROBERT A. KEISMAN, Philadelphia, Pa. (Introduced by Thomas E. Machella).

It has been widely believed that a QRS complex recorded in a chest lead is predominantly derived from the electrical activity of the subadjacent portion of heart muscle. This assumption has been tested experimentally in a study of 10 normal subjects and 20 patients with heart disease and obvious electrocardiographic abnormalities, each with a QRS configuration or deformity peculiar to a localized area of the precordium. In each individual a mirror of the chest lead pattern was found at a site remote from the precordium. The precordial signal and its mirror were electrically "bucked" against each other with an appropriate cancellation technique. The quality of the cancellation is expressed by the residual voltage as a fraction of a weighted average of the two cancelling signal voltages. In no instance was the residual greater than 15 per cent. Stated otherwise the great preponderance of the precordial signal was *not* local, but rather the manifestation of a generally distributed electrical field.

In obtaining these cancellations no effort was made to use a reference terminal approximating the electrical heart center, since such a reference is entirely unnecessary for cancellation. In 9 of the 30 patients in which it was attempted, the location of the site of the mirror pattern was predictable from data obtained in human torso models.

It would seem reasonable to believe that a peculiar precordial pattern would be a local phenomenon, but when the identical peculiarity is consistently found at a distant and predictable point, the concept of a localized current generator producing localized precordial patterns must be re-examined.

Experimental Chronic Pyelonephritis in the Rat. ALVIN P. SHAPIRO, ABRAHAM I. BRAUDE, and JENNIE SIEMIENSKI, Dallas, Texas. (Introduced by Carleton B. Chapman).

It is widely accepted that chronic pyelonephritis leads both to hypertensive vascular disease and to an inflammatory destruction of the kidney that persists after infection disappears. Because this has been difficult to prove in human subjects, an effort has been made to develop an experimental model for studying chronic pyelonephritis.

In a previous report, we described a method for producing acute hematogenous pyelonephritis in rats by intravascular injection of *Escherichia coli* followed by massage of the kidneys. The resulting infection usually subsides within six weeks. To produce chronic pyelonephritis, therefore, rats were given four injections of *E. coli* during seven months. Periodically, urine obtained by cystotomy was examined and the BUN determined. Blood pressures (tail plethysmographic method) were measured every two weeks.

Twelve rats survived and were sacrificed after eleven months. Kidney cultures at autopsy revealed *E. coli* in only one animal, although earlier urine examinations had consistently disclosed bacteruria and pyuria. All, however, demonstrated typical features of chronic bilateral pyelonephritis. Kidneys were distorted, lobulated by scars, and differed in size, with thinning of cortex and thickening of pelvic walls. Interstitial inflammation composed of lymphocytes and plasma cells extended throughout cortex and medulla. Tubules were dilated, and contained colloid and "pus" casts. Connective tissue was increased markedly with periglomerular fibrosis, although glomeruli were uninvolved. Vascular lesions were absent, and blood pressures never became elevated. Despite extensive renal damage, sufficient function remained to prevent azotemia. Control rats, and those subjected to renal massage or bacterial inoculation alone, did not develop significant lesions.

It is concluded that 1) chronic bilateral pyelonephritis has been established experimentally; 2) persisting renal inflammation without infection was demonstrated; 3) the inflammatory reaction did not result directly in vascular disease or hypertension within the period of study. Development of hypertensive vascular disease may require additional disturbances or still greater renal destruction.

Body Composition in Normal and Abnormal States. WILLIAM SIRI and NATHANIEL I. BERLIN, Berkeley, Calif. (Introduced by John H. Lawrence).

The proportions of body fat, water and solids need broader investigation to evaluate the significance of alterations in body composition engendered by certain diseases and particularly the influence of fat in degenerative disease. Fat and lean body mass calculated from either total body water or body density normally are accurate but the method is invalid for abnormal hydration and perhaps obesity. The restriction is removed, however, if both water and density are determined. Using this procedure, gross body composition was investigated in several hundred healthy subjects and fifty patients with blood dyscrasias. Normal subjects included both sexes, ages 21 to 90, and body fat from 4 to 60 per cent. Total body water was measured with tritium. Body density was obtained by a new method developed especially for its toleration by aged and ill subjects; a helium dilution technique for which accurate corrections for thermal and respiratory gas effects were developed. Studies on healthy subjects were examined for variability of body composition in the adult population, for age trends in body constituents, and dependence of extracellular fluid on non-essential fat. Studies on patients were directed toward correlating changes in fat, water and lean tissue as an aid to differential diagnosis and management of disease. Concurrent blood volume studies with Cr^{51} and P^{32} labeled red cells on 30 of the normal subjects suggest a closer correlation with fat-free body weight than with total weight. Preliminary analysis gives a red cell volume of 36 ml. per Kgm. of lean body and 4 ml. per Kgm. of fat. Similar studies on 20 patients with polycythemia and leukemia show abnormal blood volumes to be somewhat better differentiated when expressed in percentage of lean body weight.

Metabolic Factors Influencing the Susceptibility of Mice to Staphylococcal Infection. J. MACLEAN SMITH and RENÉ J. DUBOS, New York, N. Y. (Introduced by Maclyn McCarty).

The course of infection induced in mice by intravenous injection of staphylococci has been studied by the quantitative determination of the fate of the cocci in the different organs and by the fatality rate of the animals. Using both methods of study it has been possible to show that host susceptibility can be increased by various metabolic disturbances.

The feeding of desiccated thyroid, thyroxin or 2:4 dinitrophenol increases the severity of a standard infection. This increase cannot be brought about by feeding di-iodo tyrosine or iodine. The cocci survive in the organs of thyroid treated mice for a longer period than in the control animals. The enhancing effect of thyroid extract on experimental infections obtains also with a number of other bacterial and mycotic pathogens.

Short periods of fasting also increase the susceptibility of mice to staphylococcal infection. A period of 36 hours is sufficient to cause increase in susceptibility. Resistance to infection becomes normal again after 24 hours of normal diet following a period of fasting. The administration *ad lib* of 5 per cent glucose solution as drinking fluid during fasting increases susceptibility. Administration of 1 per cent sodium lactate in a similar fashion corrects partially the infection enhancing effect of fasting.

These experimental findings may be of significance in the analysis of factors which interfere with treatment in certain staphylococcal infections in man.

Further Experiences with Serum Quinidine Concentration as a Guide in the Treatment of Chronic Auricular Fibrillation and Flutter. MAURICE SOKOLOW,* San Francisco, Calif.

Conversion to sinus rhythm was attempted 215 times in 179 patients with chronic auricular fibrillation or flutter. Normal rhythm was achieved in 52 per cent of the 94 cases with rheumatic heart disease, 80 per cent of the 85 non-rheumatic cases, and 71 per cent of the total group; successful conversion occurred at a mean serum concentration of 5.7 micrograms per ml.

Serum quinidine concentrations were determined frequently during the administration of progressively larger doses of the drug. In different individuals, a given daily dosage resulted in a wide range of serum levels, and often did not correlate well with myocardial or gastrointestinal toxicity. On the other hand, a good correlation was found when toxicity was related to serum concentrations, of which 650 were available for study. Toxicity severe enough to require cessation of the drug rose from 1 per cent for levels under 6 micrograms per ml., to 8 per cent, 16 per cent, 25 per cent and 38 per cent for levels from 7 to 10 micrograms per ml., respectively.

The daily dose necessary to effect conversion was 3 Gm. or less in 80 per cent in those cases which converted. On a dose schedule of 3 Gm. per day, peak serum concentrations in 59 instances averaged 7.4 micrograms per ml. but 37 per cent exceeded 8 micrograms per ml., and 20 per cent were less than 5 micrograms per ml. This variation of serum concentration must be appreciated because 77 per cent of the successful conversions occurred at levels below 8 micrograms per ml. while toxicity increased sharply when this level was exceeded. Ninety per cent of the conversions occurred at levels below 10 micrograms per ml.

The best clinical management, therefore, demands caution and a reconsideration of the indication for conversion with quinidine when the serum concentration reaches 8 micrograms per ml. and sinus rhythm has not been restored. Increasing the dose beyond this point leads to a sharp rise in the incidence of serious toxicity, while the chances of conversion are considerably less than they were at the beginning of treatment.

The Effect of Altered Thyroid Function on the Localization of Radioactively Labeled Pituitary Extracts in the Thyroid and Other Tissues. MARTIN SONENBERG and WILLIAM L. MONEY, New York, N. Y. (Introduced by Rulon W. Rawson).

A pituitary preparation rich in thyrotrophic and gonadotrophic activity has been labeled by dissolving the hormone preparation in S^{35}-labeled concentrated sulfuric acid at 0° C. The radioactive preparation was then diluted and the non-protein bound radioactivity was removed by dialysis against isotonic saline.

Such radioactive sulfur labeled pituitary preparations lost completely their capacity to increase the weight of chick thyroids. However, they did retain some of their original gonadotrophic activity. Following injection into rats of such radioactively labeled pituitary preparations, the greatest concentrations of radioactivity were found in the thyroid, testes, retro-orbital tissues, liver, spleen, and kidney. The concentration of radioactivity decreased from the initial maximum at 5 minutes. At 24 hours the concentration of radioactivity in the thyroid, retro-orbital tissues, and testes were 45, 12, and 8 times greater than that found in blood, respectively. Control injections containing S^{35}-labeled inorganic sulfate and an unlabeled pituitary preparation showed insignificant concentrations of radioactivity in the thyroid, retro-orbital tissues, and testes.

Thyroid function was altered to determine the effects on the localization of radioactivity after administration of similarly labeled pituitary hormone preparations. The effects of surgical thyroidectomy, treatment with thiouracil or iodine-poor diet or thyroxine or large doses of sodium iodide were included in this study. The administration of the labeled hormone preparation to animals on an iodine-poor diet resulted in an increased localization of radioactivity in the thyroid. There was increased localization of radioactivity in the retro-orbital tissues of animals maintained on an iodine-deficient diet or on thiouracil. Other methods of altering thyroid function failed to influence the localization of radioactivity after the administration of S^{35}-labeled pituitary preparations.

Thromboplastin Inactivation in Human Blood. THEODORE H. SPAET, San Francisco, Calif. (Introduced by Paul M. Aggeler).

It has been demonstrated that human blood generates a potent thromboplastic activity during clotting. Since this activity is not present in stored serum, a mechanism must be available for its removal. In the present study the basic technique employed was derived from the thromboplastin generation test of Biggs and Douglas. When this test is performed with diluted reagents as originally described, the formation of thromboplastic activity reaches a peak within two minutes, and there is little reduction of activity even after one hour of incubation. If the plasma and serum reagents are used undiluted, thromboplastin generation is normal, but appreciable loss of activity occurs within 14 minutes. These findings suggest an active removal mechanism rather than intrinsically unstable thromboplastic activity.

An assay method has been devised to measure the ability of a specimen to inactivate blood thromboplastic activity. The unknown specimen is mixed with sodium citrate, and added to the thromboplastin generating mixture five minutes after the beginning of the test. The resulting mixture is tested for thromboplastic activity after an additional 30 minutes of incubation. Both plasma and serum inactivate thromboplastin, and their ability to do this is not reduced by heating to 56° C. for 30 minutes, by barium sulfate adsorption, or by ether extraction. Of the Cohn fractions, only fraction V inactivates thromboplastin. However, hypoalbuminemic sera behave similarly to normal serum. Sera of patients with hemophilia and PTC deficiency have no increased ability to reduce thromboplastic activity.

Intestinal Secretion, Absorption and Excretion of Cholesterol in Man. MALCOLM M. STANLEY* and SAMUEL H. CHENG, Boston, Mass.

A special diet, chiefly skim milk solids with added vegetable fat (60 mg. cholesterol, 101 gm. fat daily), allowed cholesterol to be excreted unchanged in the stools. Conversion to other digitonin-precipitable sterols in the intestine was avoided. Stool cholesterol excretion was estimated by a chromic oxide inert indicator method. Cholesterol secretion into the gut was estimated from dilution of ingested radioactive cholesterol. Absorption was calculated either from intake plus secretion minus excretion, or by application of the inert indicator method to the excreted radioactive cholesterol.

Three healthy men, 29 to 38 years old (fasting serum cholesterols *163 to 239* mg. per cent, *72 to 74* per cent esters), secreted 1.9 to 2.2 gm., absorbed 1.5 to 1.8 gm., excreted 0.35 to 0.64 gm., daily. Other subjects had values expressed as above: (*1*) F, 47, *carcinoma head pancreas, complete biliary obstruction* (*375* mg. per cent, *18* per cent esters), 0.17, 0.08, 0.14; (*2*) M, 43, *familial xanthomatosis, diabetes mellitus, coronary disease, recent high-fat diet treatment duodenal ulcer* (*425* mg. per cent, *71* per cent esters), 3.1, 2.7, 0.47; (*3*) M, 40, *non-tropical sprue* (*119* mg. per cent, *70* per cent esters), 0.26, 0.15, 0.17; (*4*) M, 21, *chronic active regional enteritis, shortened small intestine, jejuno-transverse colostomy, severe malnutrition* (*60* mg. per cent, *65* per cent esters), 0.72, 0.34, 0.44; (*5*) M, 67, *idiopathic hyperlipemia* (*387* mg. per cent, *64* per cent esters), 1.5, 1.2, 0.37; (*6*) F, 62, *chronic cholangiolitic hepatitis, slight regurgitation jaundice* (*450* mg. per cent, *68* per cent esters), 2.1, 1.4, 0.74.

With extremely low intake most cholesterol measured was endogenous. Intestinal exchange of endogenous cholesterol is a very active process which involves daily amounts equivalent to a significant portion of total readily exchangeable body cholesterol. Considerable variations occur in some diseases of cholesterol metabolism. Measures which can appropriately influence one or more phases of this process are logical in treatment of some of these disorders.

Physiologic and Therapeutic Effects of the Intravenous Administration of Streptococcal Fibrinolysin-Activating Principle (Streptokinase) in Man. MARIO STEFANINI * and LUCY SALOMON, Boston, Mass.

Following their partial purification, Streptokinase-Streptodornase (Varidase®) preparations have been injected intravenously in humans (Federation Proc., 1954, 13, 125). Doses up to 10,000 units per kilo weight were administered over a 1 to 4-hour period, with absent or mild febrile reaction and moderate hypotensive effect. Injection was followed by increased plasma fibrinolytic activity; lowered plasma fibrinogen, profibrinolysin and antifibrinolysin level for a period of 30 minutes to 12 hours following the infusion. Changes in platelets, prothrombin and labile factor levels were variable, and usually moderate. Bleeding tendency (oozing at the site of venous or finger puncture) was often noted at the height of plasma lytic activity. These results have recently been confirmed and extended by Tillett *et al.* (J.C.I., 1955, 34, 169).

Forty-nine normals and patients have received purified Varidase® intravenously during the past four years. Fourteen constituted the original "physiologic" series. Additional 35 patients were given Varidase® intravenously in the attempt of determining lysis of the intravascular clot. Thirty were suffering from deep thrombophlebitis, 3 with coronary thrombosis, 1 with mesenteric thrombosis and one with thrombosis of the right central retinal vein. They received purified Varidase® for a total dose of 1,000 units per kilo weight per hour: (a) as continuous infusion for four hours, or (b) in two separate infusions of two hours' duration each, with a 6-hour intervening period.

Previously reported changes in the hemostatic mechanism were confirmed. The following clinical response was observed: (a) prompt, considerable decrease of inflammatory reaction, with reduction of pain and edema in patients with deep thrombophlebitis; (b) patient with mesenteric thrombosis recovered; partial success was obtained in the case of thrombosis of the central retinal vein; no appreciable effect was observed in coronary thrombosis, possibly, some reduction in pain; (c) embolic complications (pulmonary infarction) occurred in one patient. Clinical and experimental results will be presented in detail.

Rate of Peripheral Utilization of Thyroid Hormone in Hypermetabolism without Primary Endocrine Disease. KENNETH STERLING,* and ROBERT B. CHODOS, Syracuse, N. Y.

Established clinical precept recognizes that hypermetabolism associated with leukemia, hyperpyrexia and pulmonary disorders may, in some instances, simulate Graves' Disease despite absence of evident primary pathology of the thyroid gland.

The rate of disappearance of intravenously injected I-131 labeled l-thyroxine has been employed to measure the rate of peripheral degradation or utilization of thyroid hormone. Normal volunteers degraded 45 to 60 micrograms of organic iodine daily; myxedematous patients, 10 to 20 micrograms; and typical cases of Graves' Disease, partially controlled on anti-thyroid drugs and/or iodine therapy, degraded 100 to 200 micrograms per day.

Intravenous injections of 1 mg. of l-thyroxine in myxedematous patients failed to alter the slope of the radiothyroxine disappearance curve, prior to the onset of the calorigenic effect.

Patients with leukemia or fever were selected for study as euthyroid subjects with peripheral hypermetabolism, on the basis of clinical appearance and high basal metabolic rate, but normal protein-bound iodine concentration. Such patients degraded approximately 100 micrograms of iodine per day, a figure in excess of the normal range.

These phenomena suggested a secondary or compensatory hyperactivity of the thyroid gland in response to increased metabolic activity in the peripheral tissues. It is inferred that peripheral tissue metabolism has an important role in determining the hormone degradation rate.

The Correlation Between Serum Potassium and the Weight of the Adrenal Glomerulosa in Rats. H. C. STOERK, A. I. KNOWLTON,* and E. N. LOEB, New York, N. Y. and Rahway, N. J.

Circumstantial evidence suggests that the adrenal glomerulosa is concerned with the elaboration of Aldosterone. Earlier studies have indicated that changes in the glomerulosa occur with variations in sodium intake. In this study the relationship of serum Na and K concentrations to glomerulosa weight was studied in rats fed a wide range of Na and K. Nine combinations of three dietary levels of Na (0.001, 0.05, 1.0 per cent) and K (0.001, 0.16, 0.7 per cent) were fed for two weeks to nine groups of six rats. Nine identical groups were injected with 2 mg. of DCA daily. At sacrifice serum Na and K were determined with the flame photometer, the adrenals weighed and serially sectioned. Planimeter measurements of enlarged projections of the glomerular zone served for the calculation of its weight.

None of the nine combinations of dietary Na and K caused appreciable changes in serum Na concentrations. However, there was marked hypokalemia in the groups with low and hyperkalemia in those with high K intake. At all levels of dietary K an increase in dietary Na caused a depression of serum K. Since no differences obtained between the DCA treated and untreated groups they were combined for evaluation. From 36 serum K determinations, each derived from the pooled blood of three rats, and from 36 average weights of the glomerulosa in the same animals, a direct relationship was found between the weight of the glomerulosa zone and the level of serum K. The glomerulosa weights ranged from 3.0 to 15.8 mg., serum K levels from 3.0 to 7.8 mEq. per L. The correlation was highly significant ($r = 0.6$, $p = < 0.01$).

Reconciliation of Theories of Freezing-Thawing Hemolysis of Erythrocytes. TIMOTHY R. TALBOT, JR. and STANLEY C. GLAUSER, Philadelphia, Pa. (Introduced by Thomas Fitz-Hugh, Jr.).

Lovelock's theory states that freezing-thawing hemolysis is the result of high salt concentration caused by

separation of pure ice from solution. Another theory suggests that freezing-thawing hemolysis results principally from the formation of ice crystals outside the erythrocyte, thus causing mechanical disruption.

We have found support for Lovelock's theory from temperature-time curves which were recorded for sucrose solution, saline, plasma, and erythrocytes. These curves showed fractional freezing most marked when erythrocytes were present. This theory predicts that the lower the temperature, the greater the salt concentration and therefore the greater the hemolysis. Hemolysis would be zero at 0° C. and increase with decreasing temperature until −50° C. where there would be 100 per cent hemolysis. Any temperature below −50° should lead to 100 per cent hemolysis according to this theory.

The second theory finds support in our hands from: (a) the fact that materials which prevent hemolysis at −79° C. strongly bond hydrogen and distort the ice lattice; (b) our data showing that high molecular weight polyoxy compounds prevent hemolysis due to freezing at −79° although these compounds do not enter erythrocytes. This theory also predicts that there should be a sharp increase in hemolysis as the temperature goes from −60° C. to −45° C., and that from −45° C. to 0° C. there should be 100 per cent hemolysis.

Curves of hemolysis *versus* temperature show a maximum in the region −45° C. to −55° C. When temperatures from −50° C. to −192° C. are used the range from 0° C. to −40° C. is passed rapidly and the salt effect is small compared to the effect of ice crystals. However, from 0° C. to −40° C. the effect of ice crystals is small compared to the effect of salt.

It is therefore suggested that both mechanisms are operative, with damage due to high salt concentration predominating from 0° C. to −50° C. and disruption due to ice crystals predominating at lower temperatures.

Selective Inhibition of Virus Multiplication by β-D-Ribofuranosides of Benzimidazoles. IGOR TAMM, New York, N. Y. (Introduced by Frank L. Horsfall, Jr.).

Virus multiplication clearly depends on the metabolism of the infected cell. However, it is now evident that the biosynthetic demands of the virus are not identical with those of the cell. One area of probable difference is nucleic acid synthesis. This may be approached through inhibition by chemical compounds of known structure.

The benzimidazole derivatives used were made available by Dr. Karl Folkers of Merck and Company, Inc., and were synthesized with the idea that they might affect nucleic acid metabolism. Certain of these compounds inhibit markedly the multiplication of influenza A and B viruses, and of mumps and vaccinia viruses in various host tissues. They act after the virus has entered the cell but before new virus particles appear.

The very high inhibitory activity of di- and trichlororibofuranosyl compounds on influenza virus multiplication is dependent on: 1) β-D-ribofuranose at N1 in the imidazole ring; 2) multiple chlorine substituents in the benzenoid ring; activity increases as the number of chlorine atoms is increased.

The 5,6-dichloro-1-β-D-ribofuranosyl derivative inhibits influenza virus synthesis more than the metabolic activities of host tissue. Its inhibitory effect on influenza virus is greater than that on vaccinia virus. Selectivity of action of both kinds is dependent on β-D-ribofuranose in the imidazole ring. Any departure from this structure causes lowering of both influenza virus inhibitory activity and selectivity of action. The pentose moiety of the inhibitor molecule is thus identical with that in ribonucleic acid of host cells and with that in the nucleic acid of the influenza virus particle, but different from the β-D-desoxyribose moiety in the nucleic acid of vaccinia virus.

The findings show that with certain compounds virus inhibitory activity and toxicity vary independently. Through appropriate modification of the structure, virus inhibitory activity can be increased without increasing tissue toxicity to the same extent.

A Comparison of Electrocardiographic, Electroencephalographic, and Chemical Effects of Rubidium and Potassium in Dogs. R. TARAIL, T. E. BENNETT, and W. K. NOELL, Buffalo, N. Y. (Introduced by David K. Miller).

Discrepancies exist among chemical, clinical, and electrocardiographic phenomena of potassium intoxication in man. Therefore other metallic ions which simulate potassium intoxication were sought. Rubidium was chosen because of physiologic and chemical similarities.

Isotonic solutions of either rubidium (six dogs) or potassium chloride were injected intravenously into ten pentobarbitalized dogs (9.4 to 16.0 kg.) at rates between 0.49 and 0.70 ml. per kg. per minute. The mean lethal dose of rubidium appeared significantly greater. Both ions produced cardiotoxic death. The electroencephalogram was unchanged until terminally when attenuation and discontinuity suddenly supervened. Respiratory and electroencephalographic arrest were invariably simultaneous, occurring between 29 and 60 minutes, and antedated electrocardiographic cessation. The classical terminal electrocardiographic events of potassium intoxication (intraventricular block and bradycardia) also resulted from rubidium but were overshadowed by prolonged ventricular tachycardia-ventricular flutter and by intraventricular block with less bradycardia.

The earliest electrocardiographic correlate of potassium poisoning was peaking and increased inversion or uprightness of T waves (Lead II) within 2 to 12 minutes at increments of serum potassium of 0.7 to 4.1 milliequivalents per liter above control values. Fading of the P wave, auriculoventricular and intraventricular blocks followed. Rubidium induced analogous changes and long portions of electrocardiograms were indistinguishable from those of potassium intoxication. But within 10 to 23 minutes ventricular premature systoles developed, and later characteristically alternated with non-premature beats, eventuating in long episodes of ventricular tachycardia and ventricular flutter in contrast to the much less significant role of ventricular hyperirritability after potassium administration.

Elevations in serum potassium proceeded in apparently linear fashion during potassium infusions to terminal concentrations of 16.7 to 18.0 milliequivalents per liter.

Both rubidium and potassium invariably provoked impressive reciprocal diminution of serum sodium.

Rubidium poisoning may engagingly mimic potassium but distinctive differences are evident.

The Production of Localised Necrotizing Angiitis by the Combined Effects of a Synthetic Acid Polymer and Endotoxin. LEWIS THOMAS,* New York, N. Y.

In previous investigations it was observed that the endotoxins of gram negative bacteria act synergistically with three heparin-like synthetic acid polymers, sodium polyanethol sulfonate (Liquoid®), sodium polyvinyl alcohol sulfonate, and dextran sulfate. Simultaneous intravenous injections of endotoxin and acid polymer, in amounts which have no demonstrable effect when given singly, cause widespread occlusion of small blood vessels by fibrinoid material, resulting in bilateral cortical necrosis of the kidneys, fibrinoid necrosis of the coronary arteries, and other lesions resembling those of the generalized Shwartzman reaction. During the period when intravascular fibrinoid deposition is occurring, the plasma fibrinogen falls to very low levels. In heparinized animals, fibrinoid does not appear, no diminution of fibrinogen occurs, and no lesions develop in the kidneys or other organs.

In order to explore the possibility that fibrinoid may consist of precipitated fibrinogen in combination with acid polymer, the local effect of Liquoid® in skin tissue was studied in animals simultaneously receiving endotoxin by vein. Within 24 hours after injection, nodules appeared at the site of Liquoid® injection. Throughout the nodules, the small and medium-sized arteries showed severe degrees of polyarteritis, with infiltration of all coats of the wall by polymorphonuclear leucocytes. Dense masses of homogeneous eosinophilic material resembling fibrinoid appeared within the arterial walls, and pools of similar material surrounded the arteries. After 3 or 4 days, the cellular infiltrate in the vessel walls became predominantly mononuclear, with large masses of cells occupying the intima. After 10 days, the lesion became converted to a granulomatous type of arteritis, with numerous multinucleated giant cells.

The observations indicate that polyarteritis nodosa is associated with deposition of precipitated fibrinogen in and around the walls of arteries, due to the presence of an acid polymer in the surrounding tissue. A possible mechanism which may lead to increased precipitability of fibrinogen in animals treated with endotoxin, demonstrable by cold-precipitation of the fibrinogen by heparin, will be discussed.

The Relationship of Serum Levels of Calcium and Phosphate to Parathyroid Function in Man. DAVID D. THOMPSON and HOWARD H. HIATT, Bethesda, Md. (Introduced by Robert F. Pitts).

A precise definition of the role of calcium and phosphate in the regulation of parathyroid function has been hindered by the fact that a change in the concentration of either of these ions in the body fluids usually leads to an alteration in the concentration of the other.

We have demonstrated that renal phosphate reabsorption can be correlated with the amount of parathyroid extract administered. Therefore, if variations in serum calcium or phosphate were to affect parathyroid function, an alteration of phosphate Tm might be expected.

In our studies the inulin clearance was measured and buffered phosphate was infused in sufficient quantity to saturate the renal tubular mechanism for phosphate reabsorption (phosphate Tm).

In normal subjects phosphate Tm was maximal in association with a depressed serum phosphate produced by prolonged administration of aluminum hydroxide gel. This effect was independent of the calcium intake. Reduction of serum phosphate in a hypoparathyroid individual did not alter phosphate Tm.

In a normal subject five successive daily phosphate infusions resulted in a decrease in phosphate Tm from 102 micromols per min. to 0 micromols per min. In contrast, two hypoparathyroid individuals showed minimal changes in phosphate Tm on similar regimens. To ascertain whether the fall in serum calcium which accompanied the elevation in serum phosphate was responsible for the observed change in Tm, disodium calcium ethylenediamine tetraacetic acid (EDTA) was administered intravenously for five days. Reductions in serum calcium comparable to those seen with phosphate infusions resulted. However, no rise in serum phosphate occurred and there was no change in phosphate Tm. Elevations of serum calcium produced by infusions of calcium gluconate also failed to alter phosphate Tm.

The data suggest that variations of serum inorganic phosphate rather than of serum calcium regulate the secretion of a parathyroid hormone affecting renal phosphate reabsorption.

The Effect of Pantothenic Acid Deficiency on Gastric Secretion and Motility. G. H. M. THORNTON, W. B. BEAN,* and R. E. HODGES, Iowa City, Ia.

A study of gastric function was carried out on three human volunteers in whom pantothenic acid deficiency was induced by simultaneous administration of a synthetic deficient diet and a metabolic antagonist to panthothenic acid. The response to histamine, hypoglycemia and a modification of the serial test meal (Hunt, J. N., and Spurrell, W. W.: J. Physiol., 1951, 113, 151) were used to measure the secretory function of the stomach. The test meal data were also used to determine the effect of the experiment on gastric motility. These tests were carried out before and during the development of deficiency and were repeated after recovery.

The secretory response in each test was expressed as volumes of parietal and non-parietal components of gastric juice in milliliters and total quantity of pepsin in Anson units. In two of the three subjects the development of deficiency was associated with a marked fall in all constituents of gastric juice except the pepsin response to the test meal. Restoration of function during the final control period was complete. The gastric emptying pattern of each subject remained the same throughout the experimental and control periods.

Pantothenic acid deficiency is believed to have interfered with the metabolism of the parietal cells. This

may have been an indirect effect of diminished secretion of adrenal cortical hormones, or may indicate that Coenzyme A is essential to the mechanism of hydrochloric acid secretion by the parietal cells.

The Effect of a Low Sodium Diet and the Effect of Nor-epinephrine on the Electrolyte Composition of Arterial Wall. LOUIS TOBIAN, JR.,* Minneapolis, Minn.

Normal rats on a diet containing .3 per cent sodium were compared to similar rats on a diet containing .003 per cent sodium. The "low sodium" rats had 32.6 mEq. of sodium per 100 gm. of aorta solids compared to 37.0 mEq. in the "high sodium" rats, a highly significant difference. While the sodium content of the aorta decreased 12 per cent after a low sodium diet, the chloride content was practically unchanged. Moreover, sodium and chloride concentrations in serum were similar in the two groups. Sodium lost from the aorta during the low sodium diet is mainly intracellular. The low sodium diet also produces a significant rise in the intracellular phosphorus content of aorta, but no change in potassium. Changes in artery composition may account for the protective action of low sodium diets against various forms of hypertension.

In rats with renal hypertension the aorta has a significantly elevated potassium and phosphorus content. When a low sodium diet lowers the elevated blood pressure to normal, the aorta potassium and phosphorus also revert significantly toward normal.

Fourteen dogs infused with nor-epinephrine in saline were compared with five saline controls. One femoral artery was obtained before each infusion; the other during the infusion. Nor-epinephrine produced a highly significant fall in the artery potassium content, the decrease varying from 4 per cent to 59 per cent (average 27 per cent). The controls showed an average increase of 17 per cent in artery potassium content. Nor-epinephrine also produced a significant increase in artery sodium content, an average gain of 1.7 mEq. per 100 gm. solids, while the controls were losing 0.4 mEq. However, five "nor-epinephrine" dogs scarcely gained any artery sodium while losing considerable artery potassium. The mEq. gain in sodium after nor-epinephrine averaged only ¾ the loss of potassium. Nor-epinephrine didn't significantly change the water and chloride in arteries.

Enzymatic Metabolism of Steroids. GORDON M. TOMKINS, Bethesda, Md. (Introduced by Henry Barnett).

The major metabolic fate of the steroid hormones consists of, first, hydrogenation of the double bond of ring A to form the dihydro compound, followed by reduction of the 3-keto group to give the inactive 3-alcohol which is then excreted in the urine. In this manner, cortisone is first reduced to dihydrocortisone which is further reduced to tetrahydro cortisone.

In order to investigate these reactions in greater detail, purified enzyme preparations from mammalian liver have been obtained which are capable of carrying out the reductive metabolism of a wide variety of steroids in a stepwise manner. In the initial attack on the steroid nucleus, hydrogenation of the double bond of ring A, the hydrogen donor is either reduced di- or triphosphopyridine nucleotide (DPNH or TPNH) depending on which steroid is serving as the substrate. The enzymes catalyzing this reaction are highly specific. Thus evidence based on enzyme purification procedures suggests that separate enzymes are involved in the reduction of the androgens, the adrenal steroids, and cholesterone, and that even within one class (*e.g.*, the corticoids), different compounds are metabolized by separate enzymes.

The second reaction under investigation concerns the reduction of the 3-keto group of androgenic and cortical steroids with the formation of the corresponding 3-alcohol. In the second reaction, the reduction of the keto group, in contrast to the preceding one, all the steroid substrates are metabolized by a single enzyme, and either DPNH or TPNH can serve equally well as a hydrogen donor.

The studies reported here provide the basis for a sensitive, specific enzymatic assay for a considerable variety of steroids.

Flexion Deformities in Adrenal Insufficiency. W. P. VANDER LAAN, Boston, Mass. (Introduced by Joseph Hayman, Jr.).

Flexion deformities of the extremities ("tendon contractures") have been observed in Addison's disease treated with desoxycorticosterone. It has been considered a toxic manifestation of treatment. Another interpretation is offered based on observations in three cases in which flexion deformities were noted prior to treatment.

A 41-year-old woman had typical Addison's disease and "tightness" in the thighs with inability to straighten the legs or hips. Leg motion was painless within 20° of flexion but extension beyond this caused pain. Traction resulted in inflammation of the knees and fever. The deformity grew more severe although normal electrolyte levels were maintained. No abnormality of joint or muscle was established by x-rays, electromyogram, muscle biopsy, or creatine excretion levels. Finally a 90° flexion deformity of the legs was noted. Desoxycorticosterone was stopped when cortisone became available; with 10 mg. daily doses improvement occurred. Prior to death from influenza she had recovered to her original state. Autopsy disclosed adrenal atrophy, moderate atrophy of hamstring muscles, no joint disease, and normal tendons and sciatic nerves.

A 75-year-old woman had "arthritis" of legs of 9 years' duration. The legs and hips were held in extreme flexion. This could be overcome with extreme difficulty. Hypoglycemia and hypochloremia were noted. Autopsy disclosed pituitary destruction and no abnormality of the legs.

A 57-year-old woman had an enlarged sella turcica and evidence of hypoadrenalism. The legs were kept at 25° flexion; further extension caused severe pain. With 25 mg. cortisone daily the legs became normal.

These observations suggest flexion deformities occur spontaneously in adrenal atrophy and pituitary insufficiency, probably because of hydrocortisone deficiency.

It is likely that desoxycorticosterone, and perhaps aldosterone, aggravate this condition. Muscle spasm, not "tendon contractures," is observed.

Serum Cholesterol Esterase (Sperry Enzyme) in Idiopathic Hypercholesteremia, Idiopathic Hyperlipemia, and in Coronary Artery Disease. CHUN-I WANG and DAVID ADLERSBERG, New York, N. Y. (Introduced by Milton Mendlowitz).

The activity of serum cholesterol esterase (Sperry Enzyme) was studied in four groups of patients: (I) 14 normocholesteremic controls, (II) 33 patients with idiopathic hypercholesteremia, (III) 10 with idiopathic hyperlipemia and (IV) 14 normocholesteremic patients with proven coronary artery disease.

The average values were as follows: Group I, total serum cholesterol 225 ± 35, free cholesterol 59 ± 11 mg. per cent, cholesterol esterase 41 ± 10 units; Group II, total cholesterol 372 ± 92, free cholesterol 101 ± 31 mg. per cent, cholesterol esterase 26 ± 11 units; Group III, total cholesterol 533 ± 148, free cholesterol 181 ± 48 mg. per cent, cholesterol esterase 25 ± 8 units; Group IV, total cholesterol 204 ± 35, free cholesterol 54 ± 10 mg. per cent, cholesterol esterase 32 ± 8 units. There was no straight relationship between the activity of cholesterol esterase and the levels of free serum cholesterol. None of the patients presented evidence of hepatic damage.

The difference in cholesterol esterase activity between groups I and IV was significant at the 0.03 level, that between groups I + IV and groups II + III was significant at the 0.0001 level. Serum cholesterol esterase activity was diminished in groups II, III and IV although the ratio between free and total serum cholesterol was normal (approximately 30 per cent). Difference in esterification of serum cholesterol in the various errors of lipid metabolism was suggested in previous studies performed with labelled cholesterol and acetate (J. Clin. Invest., 34: 48, 1955).

Agewise Standard Value for C_{In}, C_{PAH}, and Tm_{PAH} in Adult Males. DONALD M. WATKIN and NATHAN W. SHOCK, Bethesda and Baltimore, Md. (Introduced by Charles G. Zubrod).

Standard values per 1.73 M^2 surface area for inulin clearance and for effective renal plasma flow and Tm as determined with Diodrast® have been reported in a series of 70 subjects by Davies and Shock. In the present study, C_{In}, C_{PAH}, and Tm_{PAH} were measured under basal conditions in 110 male subjects ranging in age from 19 to 92 years. From each of six age categories subjects were selected for study on the basis of history, physical findings, and urine analysis. All were free from manifest renal disease, diastolic hypertension or myocardial failure. All were ambulatory, cooperative, and afebrile.

Standard C_{In}, C_{PAH} and Tm_{PAH} declined in a linear fashion with age from the fourth through the ninth decades. Filtration fraction rose significantly, C_{In}/Tm_{PAH} showed no significant change, and C_{PAH}/Tm_{PAH} declined significantly with age.

The following regression equations (X = age in years) were computed by the method of least squares for the functions measured per 1.73 M^2 surface area and the ratios derived:

$$C_{In} = -1.163X + 157.0\ *$$
$$C_{PAH} = -6.753X + 820.2\ *$$
$$Tm_{PAH} = -.8648X + 120.6\ *$$
$$ff. = +.0715X + 17.51\ *$$
$$C_{In}/Tm_{PAH} = -.00158X + 1.382$$
$$C_{PAH}/Tm_{PAH} = -.0278X + 7.710\ *$$

* Regression significant, $P < .001$

Approximations of standard values for age may be quickly computed by assuming a decrement of one ml. per min. per year of age from a level of 130 ml. per min. at age 30 for C_{In} and one mg. per min. per year of age from a level of 100 mg. per min. at age 30 for Tm_{PAH}.

The Production of Impending Hepatic Coma in Alcoholics with Cirrhosis by a Carbonic Anhydrase Inhibitor (Diamox®). LESLIE T. WEBSTER, JR., Boston, Mass. (Introduced by Charles S. Davidson).

Impending hepatic coma, characterized by confusion and the typical "flapping" tremor, previously observed in cirrhotic patients given oral nitrogenous substances—ammonium exchange resins, ammonium salts, urea, and increments of dietary protein—is here described following oral administration of a carbonic anhydrase inhibitor, Diamox® ($C_4H_6N_4O_3S_2$).

Of twelve alcoholics with cirrhosis given from 500 mg. of Diamox® on alternate days to 1000 mg. daily for periods varying from three to twenty-seven days, four developed mild to marked confusion (one during three separate periods). One had the "flapping" tremor previously; two developed it, one during three separate periods. All four had longstanding severe liver disease and became confused within forty-eight hours of the initial dose (two after 500 mg.). Three had a history of confusion or coma related to liver disease. Measurements of venous blood "ammonia" concentrations showed increases to abnormal levels during three of four periods. Serum potassium decreased during five of six periods, significantly during three. Potassium chloride orally, during three periods, did not prevent or alleviate confusion although serum potassium concentrations remained normal or dropped slightly.

Of the eight remaining patients, without history of confusion or coma, none developed confusion or tremor; three developed drowsiness. Five had liver disease comparable to that of the group developing confusion. One developed a slightly elevated blood "ammonia" concentration of questionable significance; one, a low serum potassium concentration. Most of the twelve patients observed showed a rise in serum carbon dioxide and chloride concentrations but no significant change in sodium.

Five hundred milligrams of Diamox® produced the syndrome of impending hepatic coma in susceptible patients with cirrhosis, but presumably by a different mechanism from that of the previously implicated nitrogenous substances because it contains little or no available nitrogen. Potassium chloride neither prevented nor improved

the syndrome although decreased serum potassium concentrations often occurred.

The Mechanism of Cardiac Failure in Tachycardia. RENÉ WÉGRIA,* H. H. WANG, and V. V. GLAVIANO, New York City, N. Y.

In dogs anesthetized with chloralose, left ventricular output and coronary sinus outflow were continuously measured with rotameters and mean arterial blood pressure continuously recorded, the oxygen contents of arterial and coronary sinus bloods were determined at appropriate intervals. Auricular tachycardia of progressively increasing rates was induced by electrical stimulation of the left auricular appendage. It was observed that within the range of rates studied, each increment of heart rate resulted in a decrease of cardiac output and arterial blood pressure, an increase in coronary blood flow and oxygen consumption, and a decrease in cardiac work and efficiency. Whether the decrease in cardiac efficiency is caused by a relative decrease in coronary flow, which seems improbable, or whether it is due to the direct effect of the tachycardia on the myocardium, will be discussed.

The Role of Connective Tissue in Body Fluid Physiology. WILLIAM B. WEIL, JR. and WILLIAM M. WALLACE,* Cleveland, O.

Extracellular fluid (E.C.F.) electrolyte is, by generally accepted definition, in diffusion equilibrium with plasma. Such is not considered to be the case for intracellular fluid (I.C.F.) electrolyte. The present study was designed to determine, by direct analysis, the nature of the diffusion equilibrium between plasma and connective tissue.

In vitro diffusion experiments on rabbit skin and tendon, as examples of loose and dense connective tissue, indicate that the diffusible sodium and chloride of connective tissue are distributed in about 90 per cent of the tissue water, that less than 10 mEq. per kg. of each ion may be intracellular or bound to a tissue protein, and that in the connective tissue of the normal rabbit, as in other animals studied, the volume of distribution of chloride is greater than that of sodium.

In rabbits with a 20 per cent weight loss due to simple water deprivation, water and its contained electrolyte were lost in such a manner that usual ion ratios for connective tissue were maintained.

Adrenal insufficiency produced the expected increase in E.C.F. potassium and a decrease in E.C.F. sodium concentration but no change in chloride concentration. The connective tissue showed a relatively greater loss of chloride than sodium with consequent reversal of the usual sodium to chloride relationship. It can be inferred that water and sodium shifted "intracellularly." A similar reversal was noted in the connective tissue of two infants who died of severe hypertonic dehydration and in whom plasma chloride was elevated more than was the sodium concentration.

These data support the concept that connective tissue fluid and electrolyte are primarily extracellular in nature, but they also indicate that the equilibrium with plasma is not always one of simple diffusion and that only to a very limited degree can the connective tissue be a reservoir for "extra" anion or cation.

The Pathogenesis of Acute Peripheral Arterial Occlusion. STANFORD WESSLER and MARVIN GILBERT, Boston, Mass. (Introduced by Herrman L. Blumgart).

Some of the contradictory therapy recommended for the management of acute peripheral arterial occlusion rests on inadequate knowledge of the underlying pathologic physiology.

Utilizing SPCA-rich serum fractions and vascular stasis, an experimental method was developed in dogs for the routine induction of non-adherent femoral artery thromboses of known size and age which could be released to the periphery. Postmortem arteriography with radiopaque media that filled the arterial tree without crossing the capillary or venous beds, facilitated localization of the emboli and evaluation of the collateral circulation.

These studies have demonstrated that fresh 4 cm. clots within minutes after their release are routinely broken into multiple fragments at arterial bifurcations and after 72 hours are so reduced in size (lysis) that they cannot be recovered in the visualized arterial tree. A demonstrable preformed interarterial network 40 to 500μ in diameter effectively bypasses these emboli.

A similar injection study of human legs has shown that although fresh occlusions are present in one-half of extremities amputated for gangrene, they rarely produce severe ischemia in the absence of old occlusive disease. Without previous obstruction, fresh clots less than 25 cm. in length have not caused gangrene. Shorter occlusions are compatible with viability because the preformed collateral circulation provides an adequate bypass: hypertrophy of these anastomatic vessels can subsequently progress to permit return of pulsatile flow distal to an occlusion.

The fragmentation, dissolution, and ultimate length of the fresh arterial clot, the extent of prior occlusion, the response of the collateral circulation to the residual obstruction and systemic factors affecting ischemia (shock, anemia) can readily explain, without invoking the concept of "spasm," the phenomena associated with acute arterial obstruction. These observations permit formulation of a rational program for the management of acute peripheral arterial occlusion.

Thrombocytosis. K. J. R. WIGHTMAN, Toronto, Ont. (Introduced by R. F. Farquharson).

Patients with an excessive number of platelets may present various clinical pictures. This fact, together with the difficulty of doing platelet counts, seems to have obscured the diagnosis in many cases, and made the condition appear less common than it actually is.

A group of eleven such patients has been studied in the past few years. The manifestations of their disorder may be grouped as follows:

Thrombotic and embolic,
Hemorrhagic,
Constitutional and hematologic.

The severity of symptoms has ranged from mild to extremely grave. Two of the patients have died. Treatment of several cases with radio-active phosphorus has given generally satisfactory results, although the severest cases have been resistant to some extent.

The condition may be apparently primary, or secondary to a wide variety of diseases. The primary cases appear to have a natural history very like polycythemia vera, with a slightly elevated blood volume and a tendency to be associated with such conditions as hypertension, peptic ulcer, and Raynaud's phenomenon. However, frank polycythemia has not developed over a prolonged period of observation in any of these cases. A slight leukocytosis is not uncommon. In two cases the condition has developed after splenectomy, and this operation is contraindicated in persons who show evidence of the disease. Splenomegaly has been present in about half the patients. The marrow shows an increased number of megakaryocytes.

The bleeding which occurs resembles that seen in hemophilia or other defects of the coagulation mechanism. We have found no evidence to substantiate the view that the platelets lose their functional capacity as they increase in numbers. The exact mechanism of the hemorrhage has not been elucidated.

The condition probably occupies a position in the group of so-called myeloproliferative disorders, but in view of its distinctive features, deserves consideration as a separate entity.

Left Atrial Pressure Pulse Waves in Patients with Predominant Mitral Incompetence From Healed Rheumatic Endocarditis. JOHN B. WILD, GEORGE N. BEDELL, and JOHANN L. EHRENHAFT, Iowa City, Ia. (Introduced by Elmer L. DeGowin).

Others have described methods for obtaining pressure pulse waves by preoperative left atrial needling in attempts to distinguish predominant mitral stenosis from predominant incompetence. During cardiotomy we have recorded directly left atrial waves from eleven patients with preponderant mitral incompetence and simultaneous left ventricular waves from two of them.

Analytical observations: 1) Left ventricular end-diastolic pressure is significantly lower than simultaneous left atrial pressure; 2) The commonest left atrial waves in predominant mitral incompetence have sharp, jagged upstrokes, climbing to peaks and falling rapidly. Very similar tracings are recorded from some patients with pure mitral stenosis; 3) Two patients with predominant incompetence had greater pulse pressure (associated with low diastolic pressure) than any case of preponderant stenosis; 4) Low diastolic pressure is found in some symptomatic patients with pure mitral stenosis; 5) Plateau-type tracings may be found in either predominant stenosis or incompetence; 6) One patient with predominant incompetence had a sharp upstroke, a relatively flat top and a sharp downstroke. No similar tracing was found in 95 patients with preponderant stenosis; 7) No patient with predominant incompetence showed a well-marked sharp "o" wave following the fall of the "v" wave; 8) One patient with stenosis, who developed severe incompetence during operation immediately after valvuloplasty (which proved fatal 10 days later) showed striking acute changes in the left atrial waves; but, considered on its own merits, this tracing was not diagnostic of predominant mitral incompetence; 9) "A" waves in preponderant incompetence ranged 0 to 4 mm. Hg and in predominant stenosis, 0 to 13.

We have concluded that it is impossible to diagnose predominant mitral incompetence with assurance from analysis of left atrial pressure pulse waves. Therefore, we believe that the clinical risk of blind preoperative needle puncture of the left atrium, by whatever route, is unjustified.

A Study of the Osmometric Behavior of the Human Red Blood Cell. T. F. WILLIAMS, C. C. FORDHAM, and W. HOLLANDER, JR., Chapel Hill, N. C. (Introduced by C. H. Burnett).

Aliquots of whole defibrinated blood were either untreated or treated with 1) addition of solutions of different concentrations of salt or sucrose, and 2) addition of different amounts of dry urea. The osmotic activity of these specimens was estimated by freezing point depression initially and after lysis of the cells induced by freezing.

The osmotic activity (mOsm. per L.) of the hemolyzed specimen (C_H) was less than the unlysed (C_S). The decrease in activity after lysis was least with untreated blood, intermediate in the specimens treated with urea, and greatest in those to which solutions of NaCl or sucrose had been added. In the salt and sucrose studies the loss of activity increased as C_S increased. These data fit the equation: $C_H = 0.9728\ C_S + 4.06$.

The relationship between (C_S–C_H) and the proportion of the total water content of the lysed specimen derived from cells is a curve. These data should have described a straight line if the loss in activity resulted from the admixture of hypotonic cell water with serum water, or the liberation of "bound" water associated with lysis.

The anticipated and observed values for C_S in specimens of blood with known water content and initial activity to which solutions whose water content and activities were known had been added agree within 1 to 2 per cent, but deviations are unidirectional at the various levels and are significant.

The directions of these deviations and the loss of activity with lysis are consistent with Adair's data concerning the relationship between osmotic activity and concentration of hemoglobin. Moreover, when that relationship is applied to these data the discrepancies are considerably diminished or eliminated. The data, therefore, appear to be consistent with the hypothesis that red cells behave as perfect osmometers in a range extending from 210 to 750 mOsm. per L.

Vasodilators in Plasma from Patients with Infections and Altered Nutritional States. CHARLES L. WISSEMAN, JR. and SHELDON E. GREISMAN, Baltimore, Md. (Introduced by Joseph E. Smadel).

Changes in vascular physiology contributing to plasma leakage, hemo-concentration and shock are important in

the pathogenesis of spotted fever and hemorrhagic fever (HF) and also occur in experimental animals inoculated with certain microbial products. The possible occurrence of humoral vasoreactive factors which contribute to the markedly dilated and excessively permeable capillaries of patients with HF was investigated in Korea. Plasma (1.5 to 2 ml.) from acutely ill HF patients, upon intravenous injection into rabbits, caused delayed (1 to 3 minutes) but intense and persistent (5 to 20 minutes) dilatation of the small conjunctival vessels. Similar effects were not usually obtained with convalescent plasma or random plasmas taken from normal persons. The active component was non-dialyzable, unstable on storage at 4° or −70° C. and promptly destroyed by freezing with ether.

Plasmas from normal persons taken post-prandially and also after 48 hours' starvation, but not after 24 hours, exhibited identical vasodilator action. Similar effects were also obtained with some plasmas from patients with a variety of acute febrile illnesses. Thus, the reaction appears dependent upon endogenous material occurring under a number of circumstances. The active component in post-prandial and starvation plasmas possessed properties similar to those listed for HF plasmas. Although certain of its properties suggested a lipoprotein, the factor appeared with the alpha globulins on fractionation and was clearly separated from those lipoproteins which rise at d = 1.063 upon ultracentrifugation.

The nature and normal function of the vasodilator substance under discussion remains obscure, but it may be related to the homologous capillary permeability factors of Mackay *et al.* or the heterologous capillary damaging factor of Lake *et al.* The probable importance of this newly recognized phenomenon in man in infectious and nutritional states deserves additional investigation.

The Stressful Interview as an Experimental Tool. STEWART WOLF,* Oklahoma City, Okla.

A stressful life experience is a stimulus which, like contact with bacteria, is not strictly quantifiable in Man. Since the force of the stress derives from the significance of the event and, since significance varies with the shading of attitudes, values, and experiences of each individual, it is unlikely that such stress will ever be described in quantitative terms.

The stressful interview is a prototype of the stressful event which can be introduced artificially in such a way as to satisfy most of Koch's postulates. Its subject matter is selected on the basis of previous knowledge of the patient and his bodily responses during certain events in his life. By applying the stimulus of the interview at a time when the bodily indicators are being measured, and after a suitably recorded control period, the earlier evidence of coincidence can be supplemented by direct experimental testing, *i.e.*, discussion of the event which coincided in time with a flare-up of the bodily disturbance in question. Then an attempt can be made by reassurance to withdraw the stimulus and restore the control situation.

The stressful interview has a special place in the evaluation of pharmacodynamic agents which act on central nervous system and autonomic mechanisms. The stressful interview allows one to test the potency of such agents by opposing them with the kind of force which would be encountered in the patient's daily life.

Representative data from nearly 3,000 stressful interviews applied 1) to help establish the relevance of life experience to a variety of bodily disorders and 2) to evaluate the potency of pharmacodynamic agents which act on central nervous and autonomic mechanisms will be presented in support of this technic as a serviceable and reliable experimental tool.

Peripheral Venoconstriction in Human Congestive Heart Failure. J. EDWIN WOOD, JULIUS LITTER, and IRWIN H. FRIEDMAN, Boston, Mass. (Introduced by Robert W. Wilkins).

The purpose of this study was to measure peripheral venous distensibility in congestive heart failure. The measurement was repeated after recompensation. Venous distensibility was also measured in a group of patients with no cardiovascular disease.

Forearm venous distensibility was measured with a water-filled, variable depth volume recording plethysmograph. Prior to each experiment, the *effective* local venous pressure (internal pressure minus external pressure) and the venous volume of the forearm segment were reduced to a low, constant, reproducible value by adjusting the external water pressure on the forearm to a level which exceeded the previously measured natural local venous pressure. With this as a starting point, local effective venous pressure was increased by 30 mm. Hg with a proximal cuff. The resultant forearm volume increase was recorded and converted to cc. per 100 cc. of forearm tissue. This figure was the venous distensibility. A low value indicated peripheral venoconstriction. Results obtained were dependent upon the resistance of the local vein walls to a standard differential pressure. The results were not influenced by the level of central venous pressure *per se*.

Eighteen patients with congestive heart failure associated with elevated venous pressure had a mean venous distensibility of 2.9 cc. per 100 cc., S.D. 0.5 cc. Twelve patients with congestive heart failure but normal venous pressure had a mean venous distensibility of 3.7 cc. per 100 cc., S.D. 0.8 cc. Ten patients with no cardiovascular disease had a mean venous distensibility of 4.5 cc. per 100 cc., S.D. 0.5 cc. Five patients with congestive heart failure were restudied after compensation occurred and venous distensibilities had increased from 2.3 to 3.5 cc., 3.2 to 4.3 cc., 2.1 to 4.4 cc., 2.5 to 3.3 cc. and 2.4 to 3.1 cc. per 100 cc., respectively. These studies indicated that peripheral venoconstriction was present in patients with congestive heart failure. The venoconstriction tended to remit as the patient improved.

Symptomatic Treatment of Subjects with Presumably C.N.S. Arteriosclerosis by the Intravenous Administration of Histamine. JANET WORRELL, Rochester, Minn. (Introduced by Bayard T. Horton).

Twenty-one subjects with a presumptive diagnosis of C.N.S. arteriosclerosis were selected for this special

study. Ages ranged from 57 to 85 years. The average age was 65.4 years. Histamine was administered intravenously in either a 1:250,000 dilution or a 1:500,000 dilution for a period of one and one-half hours daily.

Thirteen of the 21 patients (62 per cent) noted marked symptomatic improvement. This group received a mean of 42 daily histamine treatments with the range from 3 to 134. Eight patients (38 per cent) noted no subjective improvement in their symptoms. This group received a mean of 10.5 daily treatments with the range of 2 to 26. Six patients returned for further therapy and three were given treatment at home. Of these nine patients, five again received symptomatic relief.

A patient was considered to have obtained marked symptomatic improvement when vertigo or ataxia had disappeared after treatment and the patient was able to walk unassisted, or when the patient had complete relief from dizziness and headache and improvement in memory or mental alertness. If the patient was only partially relieved of these symptoms and admitted to 50 to 60 per cent improvement, he was considered not improved along with those who had no change in symptoms. Before treatment, eleven (52 per cent) of the patients had vertigo or ataxia so that they could not walk alone; seven (63 per cent) of these had relief of symptoms at the time of discharge.

The significant difference in the mean number of intravenous histamine treatments given the group with marked symptomatic improvement as compared with the group with no improvement would seem to indicate that only prolonged intravenous histamine may be effective in symptomatic treatment of these patients.

Serum Glutamic Oxalacetic Transaminase as an Index of Liver Cell Injury. FELIX WROBLEWSKI and JOHN S. LADUE, New York City, N. Y. (Introduced by Robert F. Watson).

Glutamic oxalacetic transaminase has been found in all human sera studied. This enzyme is present in high concentration in liver as well as other tissues such as heart, kidney, muscle, and brain. Variations in serum transaminase in more than 100 patients with various types of liver diseases will be presented and correlated with liver function tests.

In human as well as experimental animals, injury to liver tissue by carbon tetrachloride results in elevation of serum transaminase activity of 50 to 1000 times normal. The levels remain high for variable periods and fall with or before clinical improvement. The enzyme activity in infectious or homologous serum hepatitis rises 10 to 80 times normal in all patients studied during the first 7 to 10 days of their disease. Transaminase activity was moderately increased (15 per cent to 600 per cent) in patients with metastatic cancer of the liver and this elevation appears to be a fairly sensitive sign of metastatic liver disease. Cirrhotics have variable levels which appear to rise during activation of their disease. The height of transaminase activity and the pattern of serial determinations appear to be worthy of further study in the evaluation of liver disease.

Pathways of Urate Synthesis in Gout. JAMES B. WYNGAARDEN, Bethesda, Md. (Introduced by William P. Chapman).

The present study was designed to compare rates and extents of glycine incorporation into purines of normal and gouty persons, to gain information about biosynthetic intermediates of normal and abnormal urate production. One control (osteoarthritic) and two gouty men were given 5 μc of glycine-1-C^{14} (.13 mg.) orally while on a purine free, low protein diet. Urinary purines were isolated as copper salts, dissolved in HCl, and uric acid separated by reduction of volume and chilling. Residual purines were further purified *via* copper and ammoniacal silver precipitations, and placed on Dowex-50-H^+ column (200 to 400 M, 2.5×12 cm. bed) as hydrochlorides. Separation was achieved by gradient elution with HCl ($0.15 \rightarrow 2.65$ N). Purines were recovered, recrystallized, and counted. In the control, specific activity of urinary urate (day 1) was 151 c.p.m. per mM, whereas in gouty patients values were 488 and 618 c.p.m. per mM. The first gouty patient was clearly an "overproducer," excreting 822 mg. of urate daily, whereas the second had a normal daily output of 400 mg. In the control, specific activities of xanthine were slightly but not significantly lower than those of urate on the first two days, whereas in the gouty subjects initial xanthine specific activities were only $\frac{1}{3}$ to $\frac{1}{7}$ those of corresponding urate samples. These findings suggest that gouty subjects produce urates by two pathways, one of which does not proceed *via* xanthine, and that this latter pathway is responsible for excessive urate production. In the control subject, two routes may also have been present, but findings suggest that the pathway by-passing xanthine may be of minor importance. Specific activity determinations of hypoxanthine, adenine, and 7-methylguanine are currently being made; the presence of isotope in 7-methylguanine establishes for the first time its participation in human purine metabolism.

The Demonstration of Antileukocytic Complement-Fixing Antibodies in the Serum of Patients with Disseminated Lupus Erythematosus. HYMAN J. ZIMMERMAN, PAUL HELLER, and VINCENT YAKULIS, Chicago, Ill. (Introduced by Granville A. Bennett).

The demonstration of the L.E. phenomenon in some patients with penicillin hypersensitivity and of the ability of rabbit antileukocytic serum to induce nucleophagocytosis, to a degree simulating L.E. cells, led to the hypothesis that the L.E. plasma factor might be an antileukocytic antibody. The present study was undertaken in the attempt to test this possibility.

An extract of human leukocytes, from granulocytic leukemia or normal blood was used as antigen. Using a modified Kolmer Technique with this antigen, 400 sera were tested for complement-fixation.

Positive reactions were obtained in 70 per cent of (7 out of 10) patients with disseminated lupus erythematosus and in 80 per cent of (4 out of 5) patients who had received multiple transfusions, and in guinea pigs sensitized to this antigen. Complement-fixation also was

demonstrated in individual patients with chronic agranulocytosis, rheumatoid arthritis, drug hypersensitivity and in two patients receiving penicillin for pneumonia.

All of the patients with disseminated lupus erythematosus, who had positive complement-fixation showed the L.E. cell phenomenon, but there was a poor correlation between the intensity of the L.E. cell phenomenon and of complement-fixation. A positive correlation was demonstrated between the production of nucleophagocytosis by these sera and the sera of patients with the other diseases cited and the ability to fix complement in the presence of the antigen.

In the remainder of the 400 sera tested, the reaction was negative. Sera high in content of anti-A, anti-B and anti-RH antibodies produced no fixation of complement.

These data suggest that antileukocytic antibodies occur in most patients with disseminated lupus erythematosus, in patients who have received multiple blood transfusions, and in a few patients with other diseases.

Effects of Sympathomimetic Amines on Cardiac Rhythmicity and Conduction in Man. PAUL M. ZOLL, ARTHUR J. LINENTHAL, LEONA R. NORMAN, MILTON H. PAUL, and WILLIAM GIBSON, Boston, Mass. (Introduced by S. L. Gargill).

Quantitative comparisons have been made in man of certain specific cardiac effects of several sympathomimetic amines: arousal, acceleration and maintenance of idioventricular pacemakers; excitation of multifocal ventricular activity; and improvement of atrioventricular conduction. Little precise clinical information on these actions is available although these drugs are widely used as cardiac stimulants and vasopressor agents.

Unique opportunities for controlled observations were presented by six patients with complete atrioventricular block and persistent absence of spontaneous ventricular activity. While life was maintained for hours or days by an external electric cardiac pacemaker, these drugs were administered intravenously at graded rates. The relative magnitude, duration, and dose-response relations of these effects were determined by close electrocardiographic observation and were related to blood pressure changes.

Epinephrine, norepinephrine, and isopropylnorepinephrine differed only quantitatively. Epinephrine appeared most effective for the arousal, acceleration, and maintenance of an idioventricular pacemaker; these responses often occurred promptly with doses (4 to 10 micrograms per minute) which did not excite ectopic ventricular activity and which did not affect the blood pressure significantly even in patients with hypertension. The small doses of norepinephrine required to elevate the blood pressure sometimes excited ectopic ventricular activity, which terminated at once when the drug was stopped. Isopropylnorepinephrine sometimes excited multifocal ventricular activity in the same dose required to arouse an idioventricular pacemaker; the effect on the blood pressure, if any, was a depressor one. Careful comparison of the effects of these and other sympathomimetic amines delineated their beneficial and untoward actions and indicated the agent of choice for each of a variety of specific therapeutic purposes.

All these agents often restored some degree of atrioventricular conduction. This effect indicates a functional, in addition to the known structural, basis for atrioventricular block and supports a pharmacologic approach to its treatment.

An Analysis of the Chemical Events and their Interrelationships with Alterations in the ECG During Respiratory Acidosis and Alkalosis. S. B. JOYNER, D. A. DAVIS, D. T. YOUNG, E. CRAIGE and L. G. WELT,* Chapel Hill, North Carolina.

The chemical alterations induced by respiring high concentrations of CO_2, and hyperventilation with room air have been studied in three groups of dogs as follows: 1) simultaneous arterial and central venous blood, 2) simultaneous arterial, peripheral and hepatic venous blood, and 3) arterial blood samples in experiments conducted 48–72 hours following nephrectomy and injection with inulin.

The effects of the initial induction of hypercapnia from the control state are: an increase in pCO_2 with the associated decrease in pH, an increase in the inulin space, and an accession of sodium, potassium, glucose, and phosphorus to the extracellular fluid. The increment of potassium appeared to be derived from the muscles, glucose from the liver (splanchnic area), and phosphorus from both.

Hypocapnia following a period of hypercapnia is associated with: an increase in pH which accompanies the decrease in pCO_2, a further increase in the volume of distribution of inulin, and a further accession of potassium, glucose, and phosphorus to the inulin space. Potassium is lost from the liver but gained by muscle. The liver releases glucose and both liver and muscle release phosphorus.

The alterations associated with the change from hypocapnia to hypercapnia contrast with those which accompany the initial induction of hypercapnia and are as follows: a decrease in the inulin space, and losses of sodium, potassium, glucose, and phosphorus from the extracellular fluid.

The electrocardiographic alterations observed in these studies resemble those seen with hyperkalemia. However, the attempt to correlate the independent chemical events with the degree of electrocardiographic deterioration was unsuccessful, and serves to emphasize the fact that alterations in the ECG, like most other physiologic events, must be a net result which represents a vector derived from many diverse influences.