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ABSTRACTS

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Research Article

ABSTRACTS

1. *Factors Influencing Immunologic Response to Non-Infectious Poliomyelitis Virus Antigens.* JONAS E. SALK,* Pittsburgh, Pa.

The immunologic response to poliomyelitis virus antigen in man is evidenced by two measurable reactions. One is the antibody response itself and the other is the state of hyper-reactivity elicited upon subsequent antigenic stimulation. The hyper-reactive state is characterized by a shorter interval between provocation and secondary antibody response, and also by the very much greater degree of the secondary as compared with the primary response. The degree of this secondary effect is influenced by a number of factors; some of these are: intensity of primary stimulation, intensity of secondary stimulation, and interval between primary and secondary stimulation. The full secondary effect, in man, cannot be elicited unless the secondary stimulation is given several months after the primary. These factors influence the degree to which the serum antibody level tends to persist. Thus, immunologic response in man, following injection of non-infectious poliomyelitis virus vaccine, is affected by previous immunologic experience. The quantitation of experience-induced host factors, and the quantitation of response to different degrees of stimulation, has provided insight into the dynamics of secondary antibody formation and into the dynamics of the immunologic mechanism of defense against paralysis. It now appears that the presence of measurable amounts of circulating antibody may not be the minimal essential prerequisite for immunity to paralysis; but, the minimal requirement seems to be the existence of a hyper-reactive state, of the immunologic mechanism, of such degree that the initiation of viral multiplication after invasion through the natural portal of entry will stimulate promptly the formation of antibody and thereby limit deeper invasion or further multiplication.

2. *Cardiac Lesions in Rabbits After Pharyngeal Infections with Group A Streptococci.* ROBERT J. GLASER,* STEPHEN I. MORSE, JAMES E. DARNELL, JR., and WILBUR A. THOMAS, St. Louis, Mo.

Although the existence of a relationship between group A streptococcal infections and rheumatic fever is generally accepted, the pathogenesis of the latter disease remains unsolved. Recently in this laboratory a method has been devised whereby streptococcal pharyngeal infections can be induced in rabbits, and it has thus become possible to simulate more closely than previously the conditions which obtain in man.

Of eight infected rabbits that constituted the initial experimental group, seven developed striking myocardial lesions, some extending to the endocardium and epicardium. The histopathologic findings which included (a) muscle necrosis; (b) cellular infiltration with histiocytes, lymphocytes, multinucleated giant cells and Anitschkow myocytes; and (c) disruption of collagen and fibrosis closely resembled those of rheumatic carditis in man. Bacteria, viral inclusion bodies and parasites were not demonstrable in the lesions.

In contrast the hearts of four "sham" controls, subjected to identical procedures except that sterile broth rather than streptococcal culture was used as the inoculum, were free of comparable alterations, as were thirty-nine of forty comparative controls. One animal in the latter group had only a single small area of myocardial necrosis.

Because myocardial lesions were observed in two animals after single infections, other experiments were directed toward defining the shortest period of time necessary for the development of changes such as described, and it was found that they occurred in all of five animals sacrificed within seven days after induction of infection.

Thus the production in rabbits of streptococcal infections in the pharynx—the site of predilection of streptococcal infections in man—results in myocardial lesions closely resembling those of human rheumatic fever in almost 100 per cent of animals. This incidence is considerably greater than has been reported in experimental animals by previous investigations using other methods, and attests to the significance of the observations.

3. *Successful Homovital Transplantation in Agammaglobulinemia.* ROBERT A. GOOD and RICHARD L. VARCO, Minneapolis, Minn. (Introduced by Irvine McQuarrie).

During the past year we have discovered and studied eight patients with agammaglobulinemia. These patients have isolated gamma globulin deficiency associated with failure of antibody production. The studies to be reported represent an attempt to interpret the fascinating "experiment of nature" posed by these patients. An associated inadequacy of the hematopoietic reticulum expressed as either neutropenia, lymphopenia, eosinopenia, hyperplasia of the fixed reticulum, and in each patient failure of plasma cell formation in response to antigenic stimulation is described.

In an attempt to elucidate the basis for homotransplantation failure in man, agammaglobulinemic subjects have been provided with homotransplants of skin from unrelated donors. Successful homotransplantation in two children with congenital agammaglobulinemia and prolonged survival of a homotransplant in a patient with acquired agammaglobulinemia resulted. Skin from the agammaglobulinemic patients transplanted to immunologically normal recipients sloughed after 4 to 7 weeks. Rejection of skin homotransplants is associated with the development of local plasmacytosis.

Agammaglobulinemic patients did not have bacterial type hypersensitivity. However, injection of intact white

blood cells from sensitive donors produced this type of reactivity against tuberculin and streptococcal products which lasted at least six months.

Transplantation of lymph nodes from a normal donor to a patient with acquired agammaglobulinemia introduced immunological reactivity, a capacity which was lost when the transplanted lymph nodes sloughed. Acute-phase reactants including C-reactive protein, mucoprotein, fibrinogen, heparin-precipitable protein, erythrocyte sedimentation rates, respond as in normal persons to the stimuli provided by disease and other noxious agents. Observations on gamma globulin concentration, immunological reactivity response of bone marrow made periodically throughout pregnancy on an agammaglobulinemic female and during the neonatal period on her offspring will be described. The implications of these observations on immunological theory will be discussed.

4. *The Relation of Circulating Endogenous Pyrogen to the Cause of Experimental Fever.* ELISHA ATKINS and W. BARRY WOOD, JR.,* St. Louis, Mo.

Although many of the physiologic factors involved in the control of body temperature have been defined, the stimuli which act upon the thermoregulatory centers of the brain to cause the fevers of disease are unknown. The demonstration of a pyrogenic substance extractable from leucocytes has added support to the hypothesis that, in diseases involving inflammation, cell injury results in the release of an endogenous pyrogen which acts as the real cause of the fever. No such pyrogenic substance circulating in the blood during fever, however, has hitherto been identified. Its presence in the blood of rabbits made febrile by the injection of exogenous bacterial pyrogen has been demonstrated in the present study.

By means of a method of passive transfer, the serum of donors sensitized by one or two prior injections, but non-tolerant to typhoid vaccine, was found to contain a rapidly acting pyrogen similar to that obtained by other investigators from leucocytes and their extracts. This pyrogen appeared after clearance of the originally injected vaccine, and its fever-producing activity was unaffected by the inhibitors in tolerant serum. In contrast, the pyrogenic effect of the uncleared vaccine present in serum obtained 5 minutes after injection was markedly inhibited.

These results indicate that a second pyrogen of endogenous origin, apparently arising from damage of host cells, eventually appears in the blood after inoculation of exogenous bacterial pyrogen. The rapid onset of fever following injection of the endogenous factor suggests that it may well be the essential factor which acts upon the hypothalamus to cause fever.

5. *The Enzymatic Formation of Porphobilinogen from δ-Aminolevulinic Acid and its Conversion to Haem.* RUDI SCHMID and DAVID SHEMIN, New York, N. Y. (Introduced by John V. Taggart).

It has previously been demonstrated in this laboratory that δ-aminolevulinic acid is an intermediate in the biosynthesis of porphyrins and of haem, and also that haem synthesis from δ-aminolevulinic acid occurs in a cell-free extract of duck erythrocytes. It has now been possible to isolate by fractional ammonium sulfate precipitation from duck erythrocytes a highly purified protein fraction which converts δ-aminolevulinic acid to the monopyrrole, porphobilinogen.

In an experiment with anaerobic incubation this protein fraction (15 mg. protein-N) with 230 mg. of δ-aminolevulinic acid-5-C^{14} and 30 mg. of glutathione yielded approximately 110 mg. of porphobilinogen; 58 mg. of crystalline porphobilinogen were isolated. The product was identified by elementary analysis, by its conversion to porphyrins on heating in acid, by ascending paper chromatography, and by a positive Ehrlich reaction. The molar radioactivity of the porphobilinogen was found to be twice that of the substrate, demonstrating that 2 moles of δ-aminolevulinic acid combine to form porphobilinogen.

Equal volumes of a cell-free extract of duck erythrocytes were incubated with equimolar amounts of δ-aminolevulinic acid-5-C^{14} (0.018 mc per mM) or with enzymatically synthesized radioactive porphobilinogen (0.036 mc per mM). The hemin samples synthesized from these substrates exhibited the same radioactivity, indicating that porphobilinogen is an obligatory intermediate in the biosynthesis of haem.

Mature human erythrocytes are incapable of forming haem from δ-aminolevulinic acid *in vitro*, but they were found to contain the enzymes capable of converting δ-aminolevulinic acid to porphobilinogen. On the other hand, haem synthesis from δ-aminolevulinic acid occurred in cell-free extracts obtained from erythrocytes of sickle cell anemia, thalassemia major and human cord blood (1.4 per cent reticulocytes, no normoblasts), whereas haem synthesis was insignificant in a case of acquired hemolytic anemia, exhibiting 22 per cent reticulocytes and occasional normoblasts.

6. *Life Span, Glucose Metabolism and Osmotic Fragility of Erythrocytes in Hereditary Spherocytosis.* A. G. MOTULSKY, E. GIBLETT, D. COLEMAN, B. GABRIO, and C. A. FINCH,* Seattle, Wash.

Investigations were performed to define the mechanism of hemolysis in hereditary spherocytosis. *Quantitative aspects:* Erythrocyte destruction rates were increased six to eight times normal. Non-anemic "carriers" were shown to have compensated hemolytic disease. Destruction rates were determined in a patient with an extremely small spleen and in several splenectomized cases. Significantly increased, although compensated, erythrocyte destruction could be demonstrated in three such patients. *Qualitative aspects:* Studies of erythrocyte intermediate glucose metabolism showed a defect characterized by high specific activity of inorganic P 32 phosphate and low specific activity of organic phosphate when P 32 labelled intermediates were separated by paper electrophoresis and chromatography. This metabolic lesion was reversible with glucose and adenosine. Increased osmotic fra-

gility produced by 24 hours' incubation at 37 degrees was partially reversible with these compounds.

Transfusion studies demonstrated that tagged spherocytes from a splenectomized patient became more osmotically fragile when exposed to splenic circulation. Conversely, when spherocytes from an untreated patient were transfused to a splenectomized recipient, their fragility reverted toward normal and approximated that of the recipient's spherocytes. Serial determinations of osmotic resistance by radioactive techniques showed no correlation between cell age and osmotic fragility.

These findings indicate: 1) The bone marrow delivers spherocytes of uniform fragility before and after splenectomy; 2) increased osmotic resistance after splenectomy is related to the absence of splenic stasis; 3) destruction of hereditary spherocytes is unrelated to cell aging and occurs as a random event in the spleen; 4) a reversible metabolic defect associated with glucose metabolism may be demonstrable in the erythrocytes before and after splenectomy; 5) occasionally, the basic defect may be severe enough to produce premature destruction even in the absence of significant splenic tissue.

7. *Sequestration by the Spleen of Red Cells Sensitized with Incomplete Antibody and with Metallo-protein Complexes.* JAMES H. JANDL, Boston, Mass. (Introduced by W. B. Castle).

Studies in patients using $Na^{2}Cr^{51}O_{4}$-labelled red cells and body surface scanning showed that moderately agglutinated red cells are removed chiefly by the liver, and coarsely agglutinated cells by lung and liver, with subsequent release within minutes of hemoglobin into the plasma. In sharp contrast, globulin-coated but unagglutinated red cells (anti-D sensitized) were rapidly and almost quantitatively sequestered by the spleen, in both D-positive and D-negative subjects; hemoglobinemia did not appear. D-negative red cells treated with anti-D serum survived normally.

Dilute saline solutions of numerous trivalent and bivalent metallic cations caused agglutination of washed normal red cells. Chromic and ferric ions, in the presence either of autologous, homologous, or heterologous sera, or of serum fractions containing globulins, can in addition bind globulins to the red cell surface without producing agglutination. This induces a positive antiglobulin (Coombs) test characterized by a prozone and by agglutination in high dilutions of antiglobulin serum. Red cells so treated were also sequestered preferentially by the spleen, whereas when agglutination was produced by metals in the absence of protein, the lung and liver were the chief sites of destruction.

Red cells sensitized with anti-D serum, or nonimmunologically with a metallo-protein complex, agglutinated when suspended in autologous serum which had been concentrated as little as 1.2 times. Studies on normal dogs, in which splenic blood hematocrits of over 90 per cent were observed, revealed total protein concentrations in the splenic serum of up to 1.5 times that in the arterial serum. This suggests that concentration both of red cells and of plasma proteins is concerned in a physical mechanism resulting in the splenic sequestration of globulin-coated red cells.

8. *The Requirement for the Properdin System in the Hemolysis of Human Erythrocytes Treated with Tannic Acid.* CARL F. HINZ, JR. and LOUIS PILLEMER, Cleveland, O. (Introduced by Thomas Hale Ham).

Normal human group O erythrocytes treated with weak solutions of tannic acid (TA cells) are hemolyzed at 37° C. by normal human serum. The properdin system is required for the hemolysis as evident from the following: 1) Serum from which only properdin has been removed fails to hemolyze TA cells; 2) Addition of properdin to such serum restores the lytic property; 3) Serum from which any component of complement is lacking, serum heated to 52° C., and resin-treated serum are not lytic, and, furthermore, the addition of properdin to such sera does not restore their lytic property. However, the addition of Mg^{++} to resin-treated serum restores lysis. These results indicate that hemolysis of TA cells requires the properdin system.

Previous studies showed that properdin, a recently described serum globulin, is essential to the hemolysis *in vitro* of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria (PNH). Thus, TA cells and PNH cells behave similarly in the properdin system, and are the only human erythrocytes so far studied that have a specific requirement for properdin in hemolysis.

Tannic acid is believed to combine with and alter the stroma of the erythrocyte, rendering it susceptible to hemolysis by normal serum. The similarities reported here between the susceptibility of PNH and TA cells to lysis by the properdin system allows further exploration into the basic cellular defect in both the PNH and TA cells.

Work on the possible use of TA cells in the assay for properdin is under way and will be discussed.

9. *The Effect of a Blocking Analogue of Cystine on Leukemia.* AUSTIN S. WEISBERGER* and LEIF G. SUHRLAND, Cleveland, O.

Previous studies indicate that L-cysteine or L-cystine may have an important role in leukocyte metabolism. Cystine deficiency in animals results in leukopenia and decreased incidence of spontaneous or induced leukemia. Cystine deficiency in marrow cultures results in granulocytopenia. Furthermore, leukemic leukocytes exhibit a rapid turnover of L-cystine. These observations suggest that substances inhibiting the incorporation of L-cystine by leukocytes might influence the course of leukemia. Accordingly, the effect of various compounds on the incorporation of radioactive L-cystine by leukemic leukocytes was studied *in vitro*.

Only compounds with certain structural characteristics decreased the uptake of L-cystine. Selenium cystine, in which selenium replaces sulfur in the cystine molecule, was effective in low concentrations in decreasing the incorporation of both radioactive L-cystine and L-methionine.

Other organic and inorganic selenium compounds were ineffective. Selenium cystine decreased the incorporation of S^{35} L-cystine by lymphosarcoma cells in rats. It also inhibited the growth of this tumor whereas other selenium compounds were ineffective.

The effect of selenium cystine was also studied in two patients with chronic myeloid leukemia and in one with acute leukemia. A rapid decrease in leukocyte count and in spleen size occurred in each patient. Initially the daily rate of fall ranged from 25,000 to 50,000 leukocytes per cubic mm. Immature granulocytes disappeared more rapidly than mature granulocytes in the two patients with chronic myeloid leukemia. In the patient with acute leukemia the leukocyte count fell from 505,000 to 25,000 cells per cubic mm. Diphenylselenide, another selenium compound, was ineffective. During therapy the amount of radioactive cystine incorporated by these leukocytes *in vitro* was markedly decreased. Toxic manifestations included nausea, vomiting, anorexia, and alopecia. No hepatic or renal damage was encountered.

These striking effects of a blocking analogue of cystine tend to confirm the importance of cystine in leukocyte metabolism.

10. *On the Mechanism of Secretion of the Sodium-Retaining Hormone (Aldosterone) Within the Body.* JOHN H. LARAGH and HERBERT C. STOERK, New York, N. Y. and Rahway, N. J. (Introduced by Robert F. Loeb).

Aldosterone, a powerful sodium-retaining hormone has been isolated from adrenal glands and chemically characterized. The physiological factors governing its elaboration within the body are not established. Considerable evidence has been presented to indicate that secretion of aldosterone is not under the control of anterior pituitary hormones, and it has been reported that restriction of dietary sodium increases urinary aldosterone.

The authors have previously demonstrated that potassium administration can produce a rise in serum sodium, that sodium depleted subjects may develop sustained hyperkalemia after feeding relatively small amounts of potassium and that hypertrophy of the rat zona glomerulosa can be correlated with level of serum potassium.

The present study was designed to determine effects of changes in sodium and potassium intake and blood concentration on aldosterone secretion. Careful metabolic balance studies were carried out on normal and diabetes-insipidus dogs receiving constant daily food and water. Serum sodium and potassium concentrations were varied over wide ranges and kept abnormal for sustained periods by methods previously reported. Steroid extracts of urine were bioassayed in rats for sodium-retaining property.

Restriction of dietary sodium even when combined with reduction in serum sodium produced only slight to negligible activity of urine extracts unless potassium was provided in the diet. When potassium was given in excess, marked increases to seven-fold biological activity occurred.

The results indicate that potassium is a powerful direct or indirect stimulus to aldosterone secretion. Changes in serum sodium appear excluded as a hormone stimulus. Dietary sodium restriction may exert its effect by modifying serum potassium or Na/K relationships within body water. However, the data best support the hypothesis that change in serum potassium is a regulator, and possibly the primary regulator, of hormone activity.

Similar studies carried out in man will also be reported.

11. *The Metabolic Effects of Metacortandracin and Metacortandralone in Man: A New Series of $\Delta^{1,4}$ Diene Steroids.* MAURICE M. PECHET, Bethesda, Md. (Introduced by Mark Altschule).

A new series of steroids, $\Delta^{1,4}$ dienes, containing two double bonds in ring A of the sterol nucleus were studied in normal subjects and their metabolic effects were compared with those of cortisone and hydrocortisone. Metacortandralone (meticortelone:ΔF), 70 mgm. daily, produced sodium loss as compared with marked sodium retention with hydrocortisone, 150 mgm. daily. The sodium loss with 30 mgm. meticortelone daily was less than with 70 mgm. The endogenous creatinine clearance, which was raised markedly with metacortandracin (meticorten:ΔE) and meticortelone in Addisonian patients, was raised only slightly in the normal subjects. Both meticorten and meticortelone produced the characteristic transitory potassium diuresis on starting and retention on stopping medication as did cortisone and hydrocortisone. Meticortelone is about five times more effective than hydrocortisone in inducing this phenomenon. Seventy mgm. of meticortelone daily produced marked nitrogen loss; this was considerably greater than with 30 mgm. daily. The effect on nitrogen of 30 mgm. daily was comparable to that of 150 mgm. hydrocortisone daily. The eosinopenic effect of meticortelone was five to ten times that of hydrocortisone. These steroids were considerably more active than cortisone and hydrocortisone in producing lymphopenia and leukocytosis. The glucose tolerance curve, normal to begin with, was of a mild diabetic form after the administration of 590 mgm. meticortelone over a nine-day period, but fasting blood sugars remained normal. Inhibition of normal adrenal function occurred with these steroids, as evidenced by diminished 17 ketosteroid excretion. Seventy mgm. meticortelone produced about 50 per cent decrease in 17 ketosteroid excretion and a concomitant six-fold increase in urinary Porter Silber chromogens.

The actions of meticorten and meticortelone are thus qualitatively similar to, albeit quantitatively greater than, cortisone and hydrocortisone, with the notable exception that sodium retention has not been observed with them in man.

12. *The Plasma Protein-Thyroid Hormone Complex in Thyrotoxicosis vs. Euthyroidism in Man.* MILTON W. HAMOLSKY, Boston, Mass. (Introduced by A. Stone Freedberg).

Previous studies from our laboratory have shown that radioactivity of endogenously and *in vitro* labelled thyroid hormone-plasma protein complex of hyperthyroid patients (diffuse toxic goitre) (1) disappeared more rapidly from circulation after intravenous infusion into dogs and (2) was incorporated in greater amounts *in vitro* by rat diaphragm than similarly labelled euthyroid plasma. A method has been developed to study *in vitro* "uptake" of I-131, I-131-1-thyroxine (TX), I-131-1-triiodothyronine (TRI) by human red blood cells from whole blood or blood reconstituted by addition of R.b.c. of 1 donor to plasma or serum from another compatible donor. Under established conditions, euthyroid red cell radioactivity (corrected to 100 hematocrit) was low with I-131 and I-131-1-TX (< 1.0 per cent) but strikingly greater with I-131-1-TRI (13.3 ± 1.9 per cent in 53 runs with bath concentrations of 6 to 149 μgm. $\times 10^{-4}$ per cc.). R.b.c. "uptake" of I-131-1-TRI was consistently greater in hyperthyroid blood. In 54 paired runs at equal radioactivity concentrations, ratios of uptake (hyperthyroid: euthyroid) ranged from 1.3 to 2.7, averaged 1.7 ± 0.3. Uptake was unchanged by (1) prior addition of stable 1-TX or 1-TRI (.006 to 0.26 μgm. per cc. blood) or by (2) separation and reconstitution of R.b.c. and plasma of same donor. However, euthyroid R.b.c. (with or without prior saline washing), showed hyperthyroid uptakes when incubated in hyperthyroid plasma or serum. Conversely, hyperthyroid R.b.c., reconstituted with euthyroid plasma or serum, had euthyroid uptakes. Comparable striking and consistent differences were observed in the two thyroid states under various experimental modifications—(1) pre-heating of plasma before reconstitution, (2) dilution of blood or plasma with saline, (3) admixture of euthyroid and hyperthyroid plasmas in varying proportions. These results are consistent with previous differences observed and indicate (1) a qualitative difference in plasma protein-thyroid hormone complex or (2) plasma factor(s) differentially affecting tissue uptake in the patient with diffuse toxic goitre *vs.* the euthyroid state.

13. *An Abnormality of the Peripheral Metabolism of Thyroxine in Patients with Treated Graves' Disease: The Syndrome of Euthyroidism Associated with Thyroidal Hyperfunction.* SIDNEY H. INGBAR and NORBERT FREINKEL, Boston, Mass. (Introduced by Maxwell Finland).

An abnormally rapid rate of turnover of synthetic radio-1-thyroxine has been found in 5 patients with untreated Graves' disease (half-time, 2.2 to 4.3 days; mean, 3.1). Contrasting values of thyroxine half-time were observed in 11 euthyroid (5.8 to 7.8 days; mean, 6.6), 8 untreated myxedematous (5.9 to 9.0 days; mean, 7.5), 9 treated myxedematous (5.9 to 8.3 days; mean, 6.9), and 3 panhypopituitary patients (8.0 to 9.7 days; mean, 9.0).

In order to ascertain whether this accelerated turnover of thyroxine was the consequence of increased PBI or of the hypermetabolic state, patients were studied during antithyroid and following surgical therapy. In 5 patients, despite clinical and laboratory evidence of restoration of euthyroidism for as long as one year, accelerated degradation of thyroxine persisted (half-time, 3.6 to 4.6 days; mean, 4.2). The data reveal an abnormality of the peripheral degradation of thyroxine which may persist despite amelioration of other stigmata of thyrotoxicity, including elevation of PBI.

Evidence that the rapid turnover of thyroxine observed under these circumstances does not reflect solely nonspecific detoxification or deiodination was obtained in 3 of the latter 5 subjects. In these patients, supranormal thyroidal incorporation of I^{131} was associated with subnormal PBI's (1.2 to 2.8 μg. per cent). Persistently accelerated turnover of thyroxine was sufficient, however, to produce normal values for the total degradation of thyroxine (20.7 to 72.0 μg. iodine per day), which were reflected in clinical euthyroidism (BMR's, -3.5 to $+8.0$ per cent). These findings suggest that at least a large portion of the rapidly degraded thyroxine must undergo true metabolic utilization. An analogous mechanism (*i.e.*, persistently rapid turnover of thyroxine) may contribute to the syndrome of clinical euthyroidism despite augmented thyroidal accumulation of I^{131}, which others have observed in patients with Graves' disease following treatment by surgery or radioactive iodine.

14. *Studies on Indole Metabolism in Patients with Malignant Carcinoid (Argentaffinoma).* ALBERT SJOERDSMA and SIDNEY UDENFRIEND, Bethesda, Md. (Introduced by James A. Shannon).

A syndrome characterized by unusual cutaneous flushing, cyanosis and valvular disease of the right heart has recently been described in association with metastatic carcinoid. Large amounts of 5-hydroxytryptamine (5HTA, serotonin) have been found in carcinoid tumors and in the blood of patients with metastatic carcinoid. Therefore, it has been suggested that the syndrome may be related to the excessive production of 5HTA by the tumor.

5HTA and its metabolic product, 5-hydroxyindole acetic acid (5HIAA), were measured in the blood and urine of normals and in patients with the carcinoid syndrome. Carcinoid patients showed markedly elevated levels and measurement of urinary 5HIAA has served as a simple diagnostic test for the condition.

Detailed metabolic studies were done on a 38-year-old housewife with the typical syndrome. The blood 5HTA level was 4.0 μgm. per ml. (normal 0.2 to 0.4 μgm. per ml.) and the average daily output of 5HIAA was 330 mgm. (normal 3 to 8 mgm.). The concept that tryptophan is a precursor of 5HTA and 5HIAA was confirmed in the human through the administration of C-14 labelled

tryptophan to this patient. Tryptophan loading caused no change in the output of 5HIAA; however, 5HIAA excretion diminished during two days of tryptophan depletion. It is concluded from these studies that the predominant pathway of tryptophan metabolism in this condition is through 5HTA.

This syndrome affords an opportunity to study what is normally a minor pathway of tryptophan metabolism. The idea that 5HTA and tryptophan metabolism might be related to the development of the cardiac lesions is highly speculative but if true would constitute a unique variety of metabolic heart disease. The diversion of tryptophan from normal pathways leading to protein and niacin may be a factor in the nutritional disturbance and in the occurrence of signs of pellagra in some of these patients.

15. *Left Heart Catheterization in Aortic and Mitral Disease.* TRUMAN G. SCHNABEL, JR., WILLIAM S. BLAKEMORE, PETER T. KUO, and STEPHEN B. LANGFELD, Philadelphia, Pa. (Introduced by Calvin F. Kay).

A technique for the simultaneous recording of pressures in the left atrium and left ventricle, or the left ventricle and aorta has been developed. This procedure has been used in nine patients severely ill with aortic or mitral lesions in whom an accurate evaluation of the nature and severity of the valvular lesion was required before recommending a high mortality operation. A number 18 needle was introduced paravertebrally into the left atrium. Two polyvinyl catheters were inserted through the needle. One was left in the atrium, the other was successfully advanced into the left ventricle in six of the patients. Concomitantly a third catheter was inserted percutaneously into the femoral artery and passed into the central aorta. By measuring the pressure gradient across the valve an immediate index of the degree of stenosis or insufficiency is obtained. When combined with estimates of cardiac output, cardiac work, and valvular orifice, size may be estimated, especially in aortic stenosis, with greater accuracy than previously possible. In addition analysis of simultaneous pressure curves provides a means for study of some of the auscultatory findings associated with rheumatic heart disease. In tight mitral stenosis several phenomena were noted: 1) There is a relatively constant pressure gradient between the atrium and ventricle during diastole in patients with auricular fibrillation. This gradient is increased in presystole when normal sinus rhythm exists; 2) the diastolic pressure gradient between the atrium and ventricle persists for an appreciable interval after the onset of ventricular contraction; 3) equalization of atrioventricular pressures occurs late and on the steepest part of the ventricular slope. These findings form in part a basis for interpreting the delay in onset and the loud and snappy character of the first sound in mitral stenosis. One fatality due to hemorrhage occurred in this group of patients.

16. *Influence of Acetyl Strophanthidin on Myocardial Electrolyte Exchange.* HARPER K. HELLEMS, TIMOTHY J. REGAN, and FREDERICK N. TALMERS, Detroit, Mich. (Introduced by Gordon B. Myers).

In vitro studies have suggested that the action of digitalis analogues is principally effected by altered cell membrane permeability to cation. To determine the rate and magnitude of electrolyte changes induced by acetyl strophanthidin, a study was undertaken employing A-V difference of sodium and potassium as a measure of their myocardial exchange after the I.V. infusion of .05 to .10 mgm. per kgm. to 10 anesthetized dogs. Simultaneous blood samples were taken from the femoral artery (A) and catheterized coronary sinus (CS) at approximately 5-minute intervals for periods up to 120 minutes.

Control A-V differences were not significant (K_A 4.05 $\pm$ .16 mEq. per L., K_{CS} 4.01 $\pm$ 1.8 mEq. per L.) (Na_A 150 $\pm$ 1.6 mEq. per L., Na_{CS} 154.2 $\pm$ 2.1 mEq. per L.). After infusion, there was abrupt loss of K from the myocardial cell within one minute, reaching, in 6 minutes, a maximum negative A-V difference of 0.86 $\pm$ 0.15 mEq. per L. (K_A 4.87 $\pm$ 0.19 mEq. per L., K_{CS} 5.73 $\pm$ 0.29 mEq. per L.) ($p < .001$). Myocardial uptake of Na occurred simultaneously with a maximum positive A-V difference of 5.6 $\pm$ 1.4 mEq. per L. ($p < .001$) (Na_A 153.5 $\pm$ 1.86 mEq. per L., Na_{CS} 147.9 $\pm$ 2.72 mEq. per L.). Since the exchange of Na was greater than K and the pH of CS blood decreased, it is probable that H^+ was also released. Significant exchanges occurred with minimal ECG alteration, but the larger exchanges were associated with the more abnormal ECG. After 25 minutes $K_{(A-CS)}$ became positive and $Na_{(A-CS)}$ negative, this phase continuing for 60 minutes. Alterations in cardiac output, respiration, and arterial pH could not account for the electrolyte changes.

As measured by stroke volume, no positive inotropic effect was observed. If this ionic exchange pattern occurs also in the failing heart, it is suggested that altered muscle protein is required for its participation in positive inotropism or that it is not a major factor in this phenomenon.

17. *Dynamics of Ventricular Function in Atrial Fibrillation.* HAROLD T. DODGE and FREDERIC T. KIRKHAM, JR., Bethesda, Md. (Introduced by Robert P. Grant).

The ventricular rate and amplitude of peripheral pulse vary from beat to beat in atrial fibrillation. This is a study of the effects of the beat to beat variations in ventricular filling and aortic pressure on ventricular function in human subjects with atrial fibrillation.

Simultaneously recorded brachial artery pressures and EKGs show that the pulse pressure amplitude may vary widely for a given R-R interval. This is because the amplitude of pulse pressure bears a direct relationship to duration of the preceding R-R interval, but at the same time bears an inverse relationship to pulse pressure amplitude of the preceding beat.

Excursions of the left heart border recorded by electrokymography reflect beat to beat variations in left ventric-

ular end-diastolic and end-systolic volumes. It was found that: (1) there are marked beat to beat variations in both end-systolic volume and end-diastolic volume, (2) end-diastolic volume is a function not only of the duration of ventricular filling, but also of the end-systolic volume from which filling begins, (3) arterial pulse pressure is directly related to end-diastolic volume, (4) beats with larger end-diastolic volumes tend to have smaller end-systolic volumes. These findings account for the inverse relationship of arterial pulse pressure for a given R-R interval to the pulse pressure of the preceding beat.

By using EKY systolic excursions as an index of stroke volume and mean arterial pressure during ejection, a relative index of work per beat has been calculated. "Work" per beat proves to be directly related to the end-diastolic volume. This is the first time "Starling curves" have been plotted for the human heart and provides a new approach to the study of heart function in man.

18. *A Cardiopulmonary Syndrome Associated with Extreme Obesity.* H. O. Sieker, E. H. Estes, Jr., G. A. Kelser, and H. D. McIntosh, Durham, N. C. (Introduced by J. V. Warren).

Four patients have been observed with a combination of extreme obesity, somnolence, Cheyne-Stokes respiration, intermittent cyanosis, polycythemia and rightward electrical axis. Three had signs and symptoms of congestive heart failure. The following data suggest the direct etiological role of obesity in this unusual syndrome.

Respiratory studies were performed on two of these patients, one without heart failure and one after compensation. Both fell asleep easily when undisturbed; and while asleep Cheyne-Stokes respiration, with 15 to 30 seconds of apnea or shallow breathing, was observed. Arterial oxygen saturation continuously cycled from 90 to 95 per cent at onset of apnea to 68 to 74 per cent at onset of breathing, with a corresponding decrease in blood pH of .05 units. Breath-holding produced a 10 to 20 per cent fall in arterial oxygen saturation in 30 to 45 seconds with immediate return after one breath.

The only abnormalities noted in ventilatory function were a 20 per cent decrease in total lung volume and a 50 per cent decrease in expiratory reserve which decreased with recumbency to 150 cc. or 17 per cent of the average normal value. This small functional residual volume may be inadequate to prevent marked swings in arterial oxygenation with shallow breathing.

Right ventricular and pulmonary arterial pressures were moderately elevated, pulmonary capillary pressure was normal. Cardiac output was within normal limits. Ferrokinetic studies indicated a secondary type of polycythemia.

It is postulated that extreme obesity markedly reduces the functional residual capacity. This plus somnolence and decreased sensitivity of the respiratory center leads to periodic hypoventilation and hypoxia, followed by polycythemia, increased pulmonary vascular resistance, and occasionally congestive heart failure. Correction of these abnormalities by weight reduction indicates that this syndrome is reversible. These data implicate marked obesity as the primary factor in this syndrome.

19. *Characteristics of the Human Placenta for Oxygen Transfer.* James Metcalfe, Seymour L. Romney, and Duncan E. Reid, Boston, Mass. (Introduced by C. Sidney Burwell).

A method based upon the nitrous oxide principle has been devised for the estimation of maternal uterine blood flow. This method has been used in the study of a small series of normal humans at term Caesarean section. Uterine blood flow so estimated averaged 500 cc. per minute. The arterio-venous difference for oxygen across the uterus has been determined, which, together with the uterine blood flow, permits calculation of uterine oxygen consumption. The fetal arterio-venous oxygen difference in the umbilical vessels has also been determined in these studies. If the assumption is made that all oxygen consumed by the term uterus is taken up by the fetus, the fetal umbilical blood flow can be estimated.

The oxygen dissociation curve of human infants at birth has been determined, as has the adult oxygen dissociation curve. With these data, plus those derived as described above, certain characteristics of the human placenta with regard to oxygen exchange can be defined. The most interesting of these, from a physiological standpoint, is the mean oxygen pressure gradient across the placenta. This is defined as the average gradient which would have to exist over the total area for gas exchange to satisfy the observations.

Similar data are available in other species. Comparison of the observations in man with those in the rabbit and sheep enables one to compare the efficiency of several placental types in regard to oxygen exchange.

20. *Measurement of Organ Blood Flow Without Blood Sampling.* Hadley L. Conn, Jr., Brookhaven, N. Y., and Philadelphia, Pa. (Introduced by F. D. W. Lukens).

The rapid diffusion equilibrium of inert gases between tissues and blood makes the rate of loss of such gases from tissue dependent upon blood flow when arterial influx of gas is negligible. The use of a radioisotopic inert gas, radioxenon, which is almost entirely cleared from the blood stream with a single passage through the lung, theoretically makes possible organ blood flow determination by external (gamma) counting over the organ in which flow is being measured. The rate of loss of gas thus determined during the period of its exhalation gives blood flow per unit tissue per unit time, when corrected for the tissue-blood gas partition coefficient. No sampling of blood is required. Initially *in vitro* partition coefficients between blood and various dog tissues were determined. Then, with the utilization of an external scintillation counter and collimator, cerebral blood flow was measured in six normal dogs. The results were compared with the Kety-Schmidt method. Muscle and hepatic blood flows were also measured in these dogs,

under normal conditions and after drug administrations, and compared with results obtained by others. The radioxenon and Kety-Schmidt methods for cerebral blood flow agreed well (41 versus 43 ml. per 100 gm. per min.) and the values for muscle and hepatic blood flow were reproducible and within the expected range. However, due to a marked xenon-hemoglobin affinity, large partition corrections were necessary except in the case of the brain in which a high xenon uptake by white matter, nearly twice that by grey, served as a compensating factor. As these corrections were somewhat variable a high order of accuracy for blood flow measurement may not be possible. These findings indicate that further evaluation of inert gas blood flow techniques may be desirable.

21. *A Single Breath Measurement of Pulmonary Diffusing Capacity.* R. E. FORSTER, C. M. OGILVIE, J. W. MORTON, and W. S. BLAKEMORE, Philadelphia, Pa. (Introduced by J. H. Comroe, Jr.).

The modified Krogh single breath test of pulmonary diffusing capacity for CO (D_{CO}) has been simplified for clinical use. The patient, starting from residual volume, has only to make a maximal inspiration of a standard gas mixture containing an innocuous amount of CO (0.3 per cent) and 10 per cent helium, hold this breath for 10 seconds and breathe out; a sample of alveolar gas is analyzed for CO (infra red meter) and for helium (catharometer). The test (1) requires less than two minutes of the patient's time, (2) requires no blood samples, (3) can be repeated in several minutes, (4) has a reproducibility of approximately 10 per cent, (5) is not invalidated by uneven ventilation, since the simultaneous inspiration of helium and CO permits correction for this, and (6) can be performed at rest or on exercise.

D_{CO} measured by this method in resting normal subjects varied from 15 to 45 ml. per min. per mm. Hg (positively correlated with body surface area). It increases markedly during and for two minutes after exercise and varies less than 10 per cent with changes in posture and lung volume. The test is being used for the routine measurement of diffusing capacity in patients with cardio pulmonary disease and has proved to be useful in identifying those patients with impaired function. None of 20 patients with a variety of severe pulmonary diseases experienced any difficulty in performing the test although many of them had vital capacities of less than 1300 ml. Abnormal values as low as 6 ml. per min. per mm. Hg have been encountered. The method has also been applied to individual lungs, using bronchial catheterization.

22. *A Comparative Study of Intrapulmonary Gas Mixing and Functional Residual Capacity in Pulmonary Emphysema, Using Helium and Nitrogen as the Test Gases.* JOHN HICKAM,* and REGINA FRAYSER, Durham, N. C.

Mixing of inspired gas throughout the lungs is somewhat uneven in normal persons and may be markedly uneven in patients with lung disease. A measure of intrapulmonary gas mixing can be derived from following the rate at which a test gas is washed from the lungs of a subject who is first equilibrated with the gas and then breathes a mixture which does not contain it. From the latter part of the washout time-concentration curve it is possible to calculate the volume and ventilation rate of a space (the "slow space") which, if homogeneously ventilated, would produce the same excretion curve. Using helium as the test gas, very large slowly ventilated spaces are often found in cases of lung disease, particularly emphysema. In addition, the functional residual capacity (FRC) can be measured from the amount of test gas washed out of the lung in a given time plus that which is estimated to remain in the slow space.

In 10 patients with moderate to severe emphysema comparative measurements of gas mixing and FRC have been made using both nitrogen and helium as test gases. Nitrogen was measured with a nitrogen meter and helium with a katharometer. FRC measurements agreed well. For the group as a whole, nitrogen and helium slow spaces were not significantly different in size or ventilation rate. In a few subjects nitrogen spaces were consistently larger and slower than helium spaces. As demonstrated by studies on a model, this effect can be caused by a combination of the greater diffusibility of helium and a tendency of the slowest space to rebreathe into a somewhat faster space.

In general, intrapulmonary mixing measurements using nitrogen and helium agree well. Occasional, consistent differences may be useful in understanding some of the mechanics of intrapulmonary gas mixing.

23. *Clinical and Biochemical Changes in Patients with Phenylketonuria on Restricted Phenylalanine Intake.* FRANK H. TYLER,* JAMES F. BOSMA, and MARVIN D. ARMSTONG, Salt Lake City, Utah.

In the hereditary disorder, phenylketonuria, mental retardation is associated with an anomaly which interferes with the normal oxidation of phenylalanine. As a result of this enzymatic deficiency, the plasma phenylalanine level is abnormally high on a normal dietary intake, and a variety of abnormal metabolites appear in the plasma and urine. Clinically, convulsions, spasticity and dermatitis are common manifestations in addition to the mental deficiency.

During the past three years, five phenylketonuric children have been fed synthetic diets free of phenylalanine. Institution of this diet resulted in prompt lowering of serum phenylalanine and disappearance of the abnormal metabolites. By the return of small amounts of normal dietary protein to the diet, approximately normal levels of serum phenylalanine were maintained for periods of three to twelve months.

All of the known biochemical abnormalities have been reversed consistently. The dermatitis has disappeared. The other clinical effects have occurred less rapidly and are more difficult to interpret. Convulsive disorders have

ceased without anticonvulsant medication. Electroencephalographic abnormalities have diminished progressively. Improvement in mental and motor performance, although variable in degree, has been observed in each child. In general, the younger the patient, the shorter the period and the less the degree of retardation, the better has been the magnitude of improvement noted. In two infants, addition of phenylalanine to the diet has resulted in clinical and biochemical relapse. Subsequent depletion has restored the previously noted improvement.

Our observations suggest that some toxic effect results from the prolonged high phenylalanine level or the biochemical abnormalities which this induces. This impairs mental and neurologic functioning in a fashion which is only partially reversible. It should be emphasized that it is impossible to determine at present whether or not prolonged phenylalanine restriction will permit normal intellectual development in certain of these patients.

24. *Effects of Dietary Fats on the Serum Lipides of Human Subjects.* E. H. AHRENS, JR.,* T. T. TSALTAS, JULES HIRSCH, and WM. INSULL, JR., New York, N. Y.

Previous clinical studies have demonstrated that isocaloric substitution of plant fat for animal fat in the diet causes a prompt and striking decrease in the concentrations of cholesterol and phospholipides in the serum. To explore this phenomenon, current experiments have tested the effects of feeding single well-defined fats of plant or animal origin to three normocholesteremic and four hypercholesteremic patients in successive five-week periods. Use of orally fed formulas as the sole source of nutrients (Am. J. Clin. Nutr., 2: 336, 1954) assured precision in control of all intakes. The basic formula consisted of fat, protein, and carbohydrate in caloric proportions of 40–15–45 per cent. Body weights were maintained constant throughout the 20 to 50 weeks of each patient's study. Measurements of free and total cholesterol, phospholipides, and triglycerides were made weekly.

The lowest serum lipide concentrations occurred during intake of corn oil formula. These levels were lower than those obtained during ingestion of olive, lard, or coconut oil formulas, or during the feeding of an isocaloric solid diet with similar P-F-C composition and with all fat of plant origin. Incremental additions of cholesterol to the corn oil formula caused no elevation of serum cholesterol until very large amounts (4 gm. per day) were given. When the proportion of calories fed as corn oil was varied from 40 to 70 to 10 per cent, holding protein and caloric intakes constant, one patient showed a cholesterol rise on 10 per cent and prompt decline on 70 per cent intake, while in a second patient lipide levels were unaffected.

Conclusion: The reduction of lipide levels following the feeding of plant fats is dependent upon the specific fat fed, is not due to the decreased intake of cholesterol *per se,* and may be more pronounced with a high intake of fat.

25. *Inhibition of HCl Formation in the Human Stomach by Diamox®; The Role of Carbonic Anhydrase in Gastric Secretion.* HENRY D. JANOWITZ, DAVID A. DREILING, and FRANKLIN HOLLANDER, New York, N. Y. (Introduced by Louis J. Soffer).

The effect of large doses of the carbonic anhydrase inhibitor Diamox® on histamine-stimulated gastric secretion was studied in 15 human subjects and on basal unstimulated secretion in 10 subjects, before and after the intravenous administration of the sodium salt in the dose range 32 to 144 mg. per kg. body weight, given over the course of one hour. Disturbing side-effects were not elicited with doses of 100 mg. per kg. or less. Significant reversible inhibition of acid secretion occurred when doses of 73 mg. per kg. or higher were used. The reduction in HCl output following histamine stimulation ranged from 67 per cent to 100 per cent (mean = 82 per cent) of control values; a similar reduction of basal secretion was also demonstrated. The 100 per cent inhibition is in contrast to a maximum of 97 per cent in the pouch dog; this difference is ascribed to intragastric neutralization. Previous animal studies have shown that the patterns of secretion of sodium and potassium in gastric juice are unaffected by this compound. Studies based on the topical application of this compound to the mucosa of canine pouches have indicated that these effects are due to a local action on the carbonic anhydrase of the stomach, and not necessarily related to circulating plasma levels of drug. They are also independent of alterations in the plasma electrolytes. The present evidence is in keeping with the hypothesis that carbonic anhydrase activity during acid secretion serves to neutralize alkali formed within the parietal cell, simultaneously with HCl, and furnishes bicarbonate as the major (but not sole) anion for exchange with chloride of the plasma. Without this enzyme activity the rate of formation of HCl is markedly depressed.

26. *The Constant Relationship Between Extracellular and Intracellular Buffering with Varying Degrees of Metabolic Acidosis.* WILLIAM B. SCHWARTZ,* KARL J. ØRNING, and RICHARD PORTER, Boston, Mass.

It is known that with large mineral acid loads a significant fraction of retained hydrogen ions is buffered in sites other than extracellular fluid or blood, but the severity of intracellular acidosis with varying degrees of extracellular acidosis is unknown. To explore this relationship, distribution of hydrogen ions among body buffers was calculated during intravenous hydrochloric acid administration in 21 intact unanesthetized dogs.

In 13 experiments HCl was administered continuously at approximately 80 microequivalents per Kg. per min. An initial large fall in extracellular [HCO_3] was followed by progressively smaller reductions. Calculated hydrogen ion distribution and loss of cellular potassium suggested that in mild acidosis extracellular and blood buffers are utilized first and that intracellular buffering occurs chiefly with severe acidosis.

Because of the possibility that this pattern might have resulted from delayed extracellular mixing or slow

transcellular cation exchanges, eight additional experiments were performed in which equilibrium was allowed to occur between short successive acid infusions. [HCO_3] fell sharply during acid administration but more than 50 per cent of this deficit was restored in two hours of equilibration. Comparable [HCO_3] depression occurred with each acid infusion regardless of the absolute bicarbonate level. A curve defining successive bicarbonate levels at equilibrium was roughly linear, and the calculated contributions of extra- and intracellular buffers with progressive acid increments was approximately constant. Intracellular potassium loss was roughly constant in all but the first period, in contrast to the apparent late shift during continuous acid infusions. Urinary ammonium and titratable acid increments were relatively negligible.

The data indicate that the apparent, large, early extracellular and blood buffer utilization in continuous acid infusions is an artefact. Actually, extracellular and intracellular buffering have a fairly constant relationship at all degrees of metabolic acidosis. Serum [HCO_3] appears to reflect linearly whole body buffer stores in acidosis produced by mineral acids.

27. The Effect of Respiratory Alterations of pH on the Internal Equilibrium of Potassium. BELDING H. SCRIBNER and JAMES M. BURNELL, Seattle, Wash. (Introduced by Robert S. Evans).

Studies of acid-base disorders have not separated changes in the internal equilibrium of potassium from changes in total body potassium. In fact, the observation that muscle potassium is low in chronic metabolic alkalosis has led to the assumption that, even in the absence of renal regulation of body potassium, alkalosis causes transfer of potassium out of cells and acidosis causes transfer into cells. We are presenting evidence that the transfer actually occurs in the opposite direction.

Plasma potassium levels were measured during alterations in blood pH in dogs with ligated ureters under Pentothal® anesthesia. Respiratory acidosis (pH 7.0) was induced in five dogs by inhalation of 30 per cent CO_2-70 per cent O_2, and the mean plasma potassium level rose from 4.2 mEq. per L. to 7.5 mEq. per L. in four hours, eight times that of control dogs. Respiratory alkalosis (pH 7.8) was induced in five dogs by mechanical hyperventilation and the mean plasma potassium level fell from 4.5 mEq. per L. to 2.9 mEq. per L. in four hours. Altered equilibrium was maintained for several hours and only after restoration of normal pH did the plasma potassium gradually return to control levels.

These data suggest that intracellular buffering involves transfer of potassium and when pH changes are abrupt buffering may take several hours to reach equilibrium.

We present the hypothesis that acidosis lowers and alkalosis raises the intracellular-extracellular potassium concentration ratio. This alteration of the ratio persists as long as the pH change persists. The low muscle potassium in chronic alkalosis must be due solely to increased renal excretion of potassium.

The influence of pH on the internal equilibrium of potassium must be considered when interpreting the serum potassium level of patients with acid-base imbalance.

ABSTRACTS TO BE READ BY TITLE

Arterial Unsaturation, Venous Admixture, and Porto-Pulmonary Anastomoses in Patients with Cirrhosis of the Liver. WALTER H. ABELMANN, PAUL CALABRESI, GERTRUDE KRAMER, WILLIAM F. MCNEELY, and MICHAEL A. GRAVALLESE, JR., Boston, Mass. (Introduced by Laurence B. Ellis).

Arterial blood of patients with hepatic cirrhosis without ascites may be unsaturated for oxygen. The mean saturation of 25 patients was 92.6 per cent (S.D. 2.1 per cent). In 19 of these the arterial oxygen partial pressure was measured (mean 74 mm. Hg; S.D. 10 mm. Hg) and found significantly decreased (normal mean 92 mm. Hg; S.D. 8 mm. Hg). Resting alveolar-arterial oxygen pressure gradients at two levels of inspired oxygen were determined in 9 patients. All room air gradients (mean 27 mm. Hg) were above normal limits. Gradients became normal (mean 8 mm. Hg) during low oxygen breathing. In 5 of these patients the state at the two levels of inspired oxygen appeared sufficiently comparable to permit quantitation of distribution and diffusion factors according to Riley and Cournand. These 5 patients showed normal lung volumes, ventilation, and diffusion capacity for oxygen. Dead space ventilation was normal in 1 and elevated in 4 (28 per cent to 42 per cent). Venous admixture was increased (range 8 per cent to 20 per cent; mean 13 per cent). These findings suggest an abnormal ventilation-perfusion relationship; they do not differentiate between perfusion of underventilated alveoli and true venous admixture.

Post-mortem portal venograms performed in 9 cirrhotics, after ligation of the venae cavae, suggested that portopulmonary anastomoses might function as a channel for such venous admixture. In all 9 cases the injection mass filled paraesophageal veins, which were dilated and tortuous, and extended upward beyond the level of the carina. The paraesophageal veins anastomosed freely with mediastinal veins, and through these with pericardial and pleural veins. These venous plexi were in communication with the azygous and hemiazygous veins. Occasionally, anastomoses could be seen between mediastinal veins and peribronchial veins at the tracheal bifurcation. In 2 cases the injection mass was found in the pulmonary veins, in the left auricle, and in the left ventricle, while the pulmonary artery and aorta were free of mass.

The Osmotic Adjustment of the Erythrocyte in States of Altered Serum Sodium Concentration. JAMES W. AGNA, and HARVEY C. KNOWLES, JR., Cincinnati, O. (Introduced by Richard W. Vilter).

Preliminary studies have shown a relation between the concentrations of serum sodium and erythrocyte potassium per whole cell. This relation was considered a result of

osmotic adjustment of the erythrocyte to alterations of extracellular fluid tonicity. Investigations were carried out to substantiate this relation and to elucidate the mechanism of erythrocyte osmotic adjustment.

Studies were made on 10 normal subjects; 11 patients with adaption hyponatremia (absent or unsatisfactory response to salt therapy); 6 patients with depletion hyponatremia (beneficial response to salt therapy); and 6 patients with hypernatremia. Patients were classified by serum sodium concentration regardless of other electrolyte derangement.

The relations of the concentrations per liter of water of serum sodium to erythrocyte potassium and to erythrocyte potassium plus sodium were linear and highly significant. In adaption hyponatremia, expressed per solids, there was a gain of cell water with no change in potassium or sodium content. In depletion hyponatremia there was both a gain of cell water and a decrease of potassium and sodium. In hypernatremia the cells gained potassium, probably lost water, and showed no change in sodium content. In no instance was abnormally high sodium content found. No correlation was noted between the erythrocyte concentration and the clinically estimated total body content of potassium.

The findings suggest that osmotic activity of the erythrocyte occurs *in vivo* in a manner similar to that found *in vitro*, and that osmotic adjustment occurs by both water and electrolyte shifts.

Effect of Potassium on Intracellular Bicarbonate of the Renal Cortex. HELEN M. ANDERSON and GILBERT H. MUDGE,* New York City, N. Y.

Extracellular alkalosis of potassium depletion is associated with increased bicarbonate reabsorption and aciduria. Cooke and others have shown that this condition is characterized by an intracellular acidosis of skeletal muscle. It has been previously proposed that the stimulus for the elaboration of an acid urine might be an intracellular acidosis of the renal tubules. Reported efforts to demonstrate this in intact rats have been unsuccessful.

Tissue slices from rabbit kidney cortex lost K^+ and gained Na^+ when prepared in isotonic NaCl. They were incubated in Warburg flasks in buffered bicarbonate with substrate. Paired slices were incubated with and without the addition of K^+ (5 to 10 mEq. per L.) to the external medium; total osmolarity was kept constant by adjusting NaCl. The gas phase was 5 per cent CO_2 to 95 per cent O_2. After incubation tissues were analyzed for acid labile bicarbonate (Conway) and other electrolytes.

Final tissue concentrations were: (without K^+ added) —K^+, 32; HCO_3^-, 12.5; (with K^+ added)—K^+, 71; HCO_3^-, 16.6 (all values mM per kg. wet wt.). These differences are statistically highly significant. With K^+, the higher tissue HCO_3^- was associated with a correspondingly lower tissue chloride. The sum of Na^+ and K^+ did not vary significantly. Similar changes were noted when chloride of the medium was replaced by nitrate.

Assuming the free diffusion of CO_2, the results indicate an intracellular acidosis in K^+ depleted tissues. The effect of added K^+ is due to its cellular accumulation since no changes in tissue bicarbonate were observed when K^+ uptake was inhibited by dinitrophenol or anaerobic incubation.

Intracellular acidosis has been produced by K^+ depletion of kidney slices in an *in vitro* system. Direct extrapolation to the intact animal is complicated by the possibility that the tubular cells may not be homogeneous in terms of this phenomenon.

Forearm Metabolism in Man. Inadequacy of Carbohydrate Oxidation to Account for Resting Oxygen Consumption. REUBIN ANDRES, GORDON CADER, and KENNETH L. ZIERLER,* Baltimore, Md.

A dominant concept concerning skeletal muscle is that it depends for its energy upon carbohydrate consumption. This concept has been tested and found wanting by the following experiment.

Blood flow to the forearm (hand occluded) and arteriovenous concentration differences (brachial artery-deep antecubital vein) were measured. The product of these values is the rate of removal of a metabolite from or of its addition to forearm blood, largely by skeletal muscle. In 11 subjects at rest and after approximately 16 hours' fasting, lactate production accounted for about one-third the glucose removal. In the steady state, glucose removal minus lactate production defines the rate of oxidation of glucose. Under the above conditions, glucose oxidation accounted for only a minor fraction of forearm oxygen consumption (mean, 23 per cent; median, 16 per cent).

This appraisal depends upon the assumption that during the period of study—16 hours after the last meal—carbohydrate stores in muscle were not consumed. Even if this assumption be false, it is possible to estimate the magnitude of carbohydrate storage and assess its role as substrate. To this end heavy oral glucose loading was imposed. Glucose uptake then transiently exceeded immediate oxidative substrate requirements of the forearm. This stored excess carbohydrate, if oxidized exhaustively, could account for continuing oxygen consumption for only a few hours.

It is concluded that oxygen consumption in human skeletal muscle at rest serves mainly the metabolism of non-carbohydrate substrates. That these substrates are chiefly lipid is suggested by the respiratory quotient of forearm blood which was 0.77 ± 0.04 (S.E.M.) in the 11 subjects.

Some Biologic Properties of Doubly Encapsulated Pneumococci. ROBERT AUSTRIAN* and HARRIET P. BERNHEIMER, New York, N. Y.

From pneumococcal transformation reactions, doubly encapsulated organisms forming simultaneously polysaccharides types I and III or types III and V have been isolated. These cellular types result from the interaction of desoxyribonucleates from pneumococci of type I or type V with the cells of a pneumococcus derived from

capsular type III. In each instance, the introduction of the genetic unit for the production of a new capsular polysaccharide into cells of the strain derived from type III is followed by a significant increase in the production of type III polysaccharide as well as by the formation of polysaccharide of a different serologic type.

Cells of doubly encapsulated variants give a positive quellung reaction with type specific antiserum against each of their capsular components. Exposure to the soil bacillus enzyme which specifically hydrolyzes type III polysaccharide results in loss by the cell of the positive quellung reaction with type III antiserum, but reactivity with the antiserum to the second capsular polysaccharide is retained. When doubly encapsulated strains are grown in the presence of antiserum against either capsular component, the cells are agglutinated. In mice, both varieties of doubly encapsulated pneumococci are highly virulent. Mice can be protected against a hundred lethal infecting doses of such organisms, however, by antiserum against either of their capsular components. Such protection is not afforded by a single antiserum when infection is induced with mixtures of two pneumococcal strains each producing but one of the capsular polysaccharides of the doubly encapsulated variant.

The experiments provide another basis for explaining serologic cross reactions in pneumococcus and demonstrate that antigen-antibody reactions involving either of two surface components of the cell may result in protection against lethal infection.

Demonstration of Humoral Factor (or Factors) in Experimental Hypersplenism by the Lactating Rat Technique. MARIO BALDINI, Boston, Mass. (Introduced by William Dameshek).

Splenomegaly and the "macromolecular syndrome" (Huepner) were induced in rats by the injection of methyl cellulose intraperitoneally. The rats developed pancytopenia in association with a hyperplastic bone marrow, *i.e.*, possible "hypersplenism." Pancytopenia did not develop in previously splenectomized rats. In "hypersplenic" rats, splenectomy resulted in normalization of the blood picture.

Whether the pancytopenia of "hypersplenism" is due to increased destruction of the various cellular components or to humoral factors resulting in marrow inhibition is not clear. The question of a humoral factor was studied with the lactating rat technique.

Rats born of "hypersplenic" mothers but lactated by normal lactating females beginning with the first 48 hours of life showed normal body weights and blood pictures.

Rats born of normal mothers but fed from "hypersplenic" mothers soon after birth, showed a slower body growth and developed anemia after 13 days of lactation. The leukocyte values varied greatly, while thrombocytopenia appeared more frequently. The reticulocytes were slightly increased.

Normal baby rats fed from "hypersplenic" mothers splenectomized soon after parturition did not develop anemia. Rats fed by lactating female rats with anemia due to repeated bleeding showed no abnormality of growth or anemia.

The organs of the anemic baby rats showed no distinct pathologic changes and no methyl cellulose could be demonstrated in the reticulo-endothelial cells throughout the body.

Conclusion: The anemia induced in baby rats fed by "hypersplenic" lactating females is due to the presence of a humoral factor residing in the enlarged, pathologic spleen of the mothers. The nature of the humoral factor has not been elucidated.

Acute Effects of Mercurial Diuretics on the Normal Human Kidney. EARL S. BARKER and JOHN KAPP CLARK,* Philadelphia, Pa.

Renal hemodynamics, oxygen consumption, electrolyte excretion and tubular function were measured in 26 normal men by clearance techniques (usually including renal vein catheterization) before and for approximately two hours after 2 cc. of meralluride (Mercuhydrin®) or mersalyl (Salyrgan®) intravenously.

Decreased PAH transport (E_{PAH} or Tm_{PAH}) was evident within 10 minutes, maximal by 30 to 45 minutes and, with mersalyl, appeared recovering late in the study. Depression of E_{PAH} averaged 4.5 per cent after meralluride and 22.2 per cent after mersalyl. Tm_{PAH} was depressed to 74 per cent of control values by meralluride and to 21 per cent by mersalyl.

Excess urinary chloride lost during the entire period of observation was 132 mEq. with mersalyl and 58 mEq. with meralluride. With both agents, rates of chloride excretion were greatest at the end of the experiment (long after maximal effects on PAH transport). Sodium excretion approximately paralleled chloride; potassium tended to decrease and magnesium showed a definite increase.

While these differences between the two agents are highly significant, they may indicate a more intense but briefer action for mersalyl rather than greater total activity.

Although rates of diuresis varied considerably (variable water load), progressively increasing hematocrits indicated dehydrating effect definitely greater with mersalyl, but not correlating well within the group with chloride or PAH effects.

Post-mercurial values for glomerular filtration, renal blood flow and oxygen consumption did not change significantly with either drug. While considerable decreases suggesting real drug effects occurred in a number of subjects, they did not correlate with any of the effects discussed above.

These data indicate specific mercurial blocking effect not dependent on general metabolic depression, and further suggest that effects on PAH and electrolytes involve different systems as evidenced by their magnitude and separation in time.

Acquisition of Staphylococci by Hospitalized Patients. CARL A. BERNTSEN, JR., New York, N. Y. (Introduced by Ralph Tompsett).

Several previous reports have described the replacement of drug sensitive by drug resistant strains of staphylococci in hospitalized patients. The present report describes the new acquisition of staphylococcus aureus by hospitalized patients originally free of staphylococci and demonstrates the differences in this phenomenon in the treated and untreated patients.

At Bellevue Hospital from September 1944 to March 1955, daily nose and throat cultures have been obtained from 212 patients in a general medical ward. On admission 31 per cent were carriers of staphylococcus aureus. The present report concerns the remaining 147 patients who were free of staphylococcus aureus on admission. During hospitalization and treatment of one group of 75 patients, thirty-nine or 52 per cent acquired staphylococcus aureus. Of these twenty-seven or 36 per cent of the total became consistent carriers. In the remaining 72 untreated patients, sixteen or 22 per cent acquired staphylococcus and 11 per cent became consistent carriers.

Coincident with increase in carriers of staphylococcus in the treated group there occurred a second change serving to distinguish treated from untreated groups. In the treated group initially there were twenty-three patients or 31 per cent with beta hemolytic streptococci. After treatment only six patients or 8 per cent carried beta streptococci. The majority of these again acquired beta streptococci more than a week after their treatment was stopped. In contrast 61 per cent of untreated patients carried the beta streptococcus on admission and 44 per cent retained it on discharge. The accelerated fall in beta streptococcus carrier rate in the treated group likely is a consequence of the use of tetracycline. Possibly the increase in carrier rate of staphylococcus aureus in the same group depends upon decrease in beta streptococci for there appear no other changes in nose and throat flora.

These findings suggest that both treated or untreated patients free of staphylococci on admission become carriers during hospitalization. However, the frequency of acquisition of staphylococci in the treated group is more than three times that of untreated patients.

The Control of the Circulating Leukocyte Level. H. R. BIERMAN,* K. KELLY, S. COBLENTZ, F. CORDES, and D. LEFF, Duarte, Calif.

The constancy of the leukocyte count in the peripheral blood of normal subjects is remarkable. Despite marked deviations above or below normal range due to physiological or pharmacological stimuli, the normal level is, after test agents are given intravenously, regained promptly—usually within 4 to 10 minutes.

In an effort to study production and delivery of leukocytes into the blood, together with the constancy of this inviolate level, large numbers of leukocytes were withdrawn from dogs on eight occasions by employing the Cohn blood fractionator. One and two-tenths to 6.4 billion leukocytes (12 to 36 per cent of the total circulating number) had to be removed before a significant drop in count was observed. At least 6.4 billion leukocytes had to be removed to produce a leukocyte count below 5,000 per cu. mm. The leukocytic response to epinephrine was employed to denote the availability of cells in the tissue reservoir. To obtain valid data concerning production of leukocytes, the tissue reservoir had to be depleted. At this point, there was no leukocyte response to epinephrine challenge. The return of leukocytes toward normal from the leukopenic state occurred within 2 hours approaching 160 million leukocytes delivered per minute. The bone marrow exhibited both prompt and delayed evidence of proliferation.

During the period of leukocyte withdrawal, the plasma globulins were reduced. The reappearance of the beta globulins preceded that of the alpha and gamma globulins on each occasion.

It would appear that there is a reservoir of leukocytes in the tissues divided roughly into two components—a readily available (RA) and a non-readily available portion (NRA). The NRA compartment appears to be in equilibrium with the RA component and the latter appears to be related to the peripheral circulating leukocyte level. The peripheral WBC level appears to be maintained as long as cells are available in the RA reservoir. A depleted RA reservoir is the major stimulus to marrow production.

The significance of the findings in terms of leukocyte production, delivery and homeostasis will be presented.

Immediate Changes in Renal Function during Sympatho-Adrenal Stimulation and Blockade as Estimated by a One-Minute Clearance Period Technique. WILLIAM D. BLAKE,* and AGNAR A. STRAUMFJORD, Portland, Ore.

The error inherent in calculating glomerular filtration (GFR) during a changing urine flow (V) is caused by delay in change of urine concentration due to tubular transit time. In dogs about 85 per cent of tubules have a delay of approximately one minute. (Calculated from data of Chinard.) Hence one-minute-clearance periods will give reasonable estimates of GFR if, for each value of V, the concentration obtained in the following period is used in the calculation. Studies were done on anesthetized dogs with urine collected by catheters inserted up to the renal pelves bilaterally. During "control" periods V was maintained at more than 1½ ml. per min. V was altered by administration of epinephrine or norepinephrine either intravenously or by catheter directly into the renal artery or by splanchnic stimulation or blockade. When either hormone was infused into the renal artery, there was parallel decrease in V, total solute excretion and GFR with little or no change in urinary concentration of creatinine (U_{Cr}). Concentration of sodium in urine (U_{Na}) rose only transiently whether or not sodium was the chief urinary solute.

Whenever sympatho-adrenal activity was altered by other means (direct or indirect splanchnic stimulation), there were always reciprocal changes in V and U_{Cr} with little or no change in GFR. U_{Na} changed in a direction opposite to that of V only when sodium was the chief urinary solute. During glucose diuresis V and U_{Na} changed in the same direction. Under both conditions rates of water and sodium excretion changed in the same direction. It is concluded that, during sympatho-adrenal activity, acute changes in V and sodium excretion are partially independent of change in GFR. Further, sympatho-adrenal activity is not simulated by infusion of either epinephrine or nor-epinephrine directly into the renal artery.

Changes in Body Composition and Exchanges of Water During the Course of Acute Renal Failure. L. W. BLUEMLE, JR., H. P. POTTER, and J. R. ELKINTON,* Philadelphia, Pa.

Eight adult female patients with acute renal failure were studied by balance technique. Changes in total body water (ΔW) and total body fat (ΔFat) were calculated from changes in body weight, balance of solids and the metabolic mixture; changes in total body fat-free solids (ΔFFS) by difference. Data are presented as mean values with standard deviations per 24 hours per standard 70 kg. body weight for the following three phases: oliguria (average 5 days studied), early diuresis (average 9 days), and late diuresis (average 7 days).

Changes in body composition for the three phases, respectively, were as follows (grams): ΔW -13 ± 323, -479 ± 257, -58 ± 154; ΔFat -206 ± 38, -175 ± 103, -123 ± 81; ΔFFS -32 ± 18, -71 ± 33, -85 ± 44. The corresponding rates of water exchange were as follows (ml.): exogenous water intake 1083 ± 455, 3097 ± 790, 3933 ± 825; water of oxidation (H_2O_{ox}) 319 ± 37, 358 ± 111, 324 ± 75; endogenous preformed water released by tissue catabolism (H_2O_{pf}) 130 ± 61, 145 ± 59, 120 ± 52; insensible water loss (IL) 1134 ± 138, 1275 ± 393, 1168 ± 270; sensible water loss (urine, vomitus, etc.) 313 ± 123, 2732 ± 835, 3398 ± 698.

The results indicate a decrease in total body fat and fat-free solids in all phases, the latter decrement increasing with respect to the former during the course of diuresis. The greater loss of total body water during early diuresis reflects the excretion of water accumulated from exogenous and endogenous sources in excess of the usual ratio to fat-free solids. Exogenous water required in excess of sensible loss to maintain total body water in proportion to total fat-free solids ($IL - [H_2O_{ox} + H_2O_{pf}]$) was 685 ± 133, 771 ± 307, 724 ± 215 ml. per day.

The Effects of Sodium Chloride on the Biophysical Characteristics of Sodium Hyaluronate. B. BLUMBERG, G. OSTER, and K. MEYER, New York, N. Y. (Introduced by C. Ragan).

In a previous paper, the workers reported on light scattering studies on the size and shape of the hyaluronate particle. The data suggested that the particle is a "swollen" meshwork or syncytium of the linear polymer, free draining and easily deformable, whose outline is a sphere or near sphere. Hyaluronic acid is an important constituent of the ground substance. It has been suggested that it is of importance in support, storage, ion transport repair, and other vital physiological functions. It was felt that changes in the physical nature of the hyaluronate solutions might have a profound effect on these functions and we have, therefore, studied the effect of the sodium ion on biophysical measurements of hyaluronate solutions.

Protein-free hyaluronates of analytically high purity prepared by an alcohol precipitation method from human umbilical cord and streptococcal capsule were studied. Measurements of flow birefringence, viscosity, sedimentation and light scattering were made on solutions of hyaluronate buffered with pH 5.0 acetate containing salt at various concentrations. With increase in salt concentration, flow birefringence decreased. Intrinsic viscosity, sedimentation and the amount of light scattered, all extrapolated to infinite dilutions, decreased. Moreover, the major changes in these measurements occurred with the addition of small amounts of salt to the solution, with little or no change with the addition of salt beyond a sodium ion strength of approximately .13.

Interpretation of these data in terms of physical models can be only approximate and general. However, the data extrapolated to infinite dilution suggest that the particles aggregate in a low salt concentration environment and disaggregate as salt is added. Regardless of the interpretation of the extrapolated values of the data, it is clear that solutions of hyaluronate undergo marked physical changes when salt concentration is lowered and that the material is particularly sensitive to such changes in a region of physiological interest. These physical changes may be of importance in the role of the ground substance in salt and water metabolism, edema, inflammation, and transport exchange mechanisms.

Investigation of Tolerance to Bacterial Endotoxin with Radiochromium Labelled E. Coli *Endotoxin.* A. I. BRAUDE, FRANCIS J. CAREY, and MARGARET ZALESKY, Dallas, Texas. (Introduced by Donald W. Seldin).

The nature of tolerance to endotoxin was examined by correlating the fate of injected radioactive endotoxin with degree of susceptibility to its action. The endotoxin was extracted from *Escherichia coli* and then firmly labelled by incubation with $Na_2Cr^{51}O_4$. Radioactivity proved directly proportional to toxicity.

Distribution of radioactivity was determined after injection of lethal doses of labelled endotoxin into the circulation of mice and rabbits subjected earlier to the following manipulations with non-labelled *E. coli* endotoxin or X: irradiation:

1. No manipulation.
2. Repeated injections to produce tolerance. The LD_{50} for mice was markedly elevated and the fever index of rabbits greatly reduced.

3. X: irradiation to increase susceptibility of mice.

4. Repeated injections followed by injection-free intervals to permit loss of tolerance. Circulating precipitin titers for endotoxin remained high.

5. X: irradiation before repeated injections of mice to produce moderate tolerance without circulating antibody.

Susceptibility was directly related to the speed with which endotoxin disappeared from the blood. In normals, circulating endotoxin disappeared within 2 hours and large amounts appeared in the liver. With increased resistance, 95 per cent disappeared from the circulation within 15 minutes and passed chiefly into lung and liver. In irradiated animals with increased susceptibility, by contrast, the circulating level never dropped below 50 per cent.

When induced tolerance lapsed but antibodies persisted, circulating endotoxin rapidly disappeared into the lung; after 2 hours, however, pulmonary concentrations fell almost to zero and circulating endotoxin reappeared in high concentrations. Irradiated tolerant animals, lacking precipitins, manifested little immediate pulmonary removal of endotoxin but within 30 minutes circulating concentrations fell permanently below 10 per cent.

These results indicate that tolerance depends on speedy and permanent removal of circulating endotoxin into hepatic and other cells. Although not essential for tolerance, circulating antibody may contribute to it by inducing rapid but temporary pulmonary removal which by itself cannot provide protection.

The Hydraulics of Overriding the Aorta. P. Brostoff, S. Rodbard, and J. Margolis, Chicago, Ill. (Introduced by Louis Katz).

The principles governing flow when the aorta overrides the right ventricle were studied by means of an integrated circulation model. When right ventricular pressure was less than aortic diastolic pressure, there was the expected left to right shunt across the interventricular defect but no shunt from right ventricle directly into the overriding aorta. By creating pulmonic stenosis the right ventricular pressure was increased so that for a period it was higher than aortic diastolic but less than left ventricular pressure. Under these circumstances a bidirectional shunt appeared. The right ventricular to overriding aorta shunt began as soon as right ventricular pressure exceeded aortic pressure and continued until the pressure in the aorta exceeded that in the right ventricle. This shunt took place during early systole. When left ventricular pressure exceeded right ventricular pressure a left to right shunt across the ventricular septal defect began and this occurred later than the right to left shunt. There was shunting from right ventricle directly into the overriding aorta and across the ventricular septal defect into the left ventricle during the period when right ventricular pressure was greater than aortic diastolic pressure and left ventricular pressure. As right ventricular pressure continued to increase the shunted fluid travelled preferentially from right ventricle directly into the aorta. Since model and animal experiments have demonstrated only unidirectional shunting in pure ventricular septal defect, it is concluded that overriding of the aorta is necessary for the production of bidirectional shunting.

Metabolism of the Conjugated 17-Hydroxycorticosteroids. Harold Brown and Edwin Englert, Jr., Salt Lake City, Utah. (Introduced by B. V. Jager).

Inasmuch as a considerable portion of the plasma 17-hydroxycorticosteroids (17-OH-CS) is conjugated, a study of this fraction in various clinical states should contribute to the understanding of corticosteroid metabolism.

After a standard one-half hour infusion of hydrocortisone in normal subjects, the levels of the conjugated 17-OH-CS in the plasma reached a peak in two hours and then declined more slowly than the levels of the free 17-OH-CS. In patients with cirrhosis, the peak plasma levels of the conjugated 17-OH-CS were considerably lower and the levels of the free 17-OH-CS declined more slowly than in normal subjects. In patients with uremia the plasma levels of conjugated 17-OH-CS prior to infusion were elevated. After the infusion there was a very high and sustained level of the conjugated 17-OH-CS, although the level of the free material in the plasma declined at an almost normal rate.

After standard infusions of tetrahydrocortisone (tetrahydro E), dihydrocortisone (dihydro E) and tetrahydrohydrocortisone (tetrahydro F) into normal subjects, the initial plasma levels of conjugated 17-OH-CS were at a peak which was much higher than that achieved with comparable hydrocortisone infusions.

In the patients with cirrhosis, tetrahydro E infusions produced the same pattern of conjugated 17-OH-CS levels in the plasma as in the normal subjects. After the infusion of dihydro E or tetrahydro F, however, there was a delay in the attainment of peak levels.

These studies suggest that: (1) the metabolism of hydrocortisone involves a process of reduction and subsequent conjugation; (2) in cirrhosis of the liver, there is an impairment of reduction but not of conjugation; (3) hydrocortisone is converted ultimately to tetrahydro E which is conjugated and excreted *via* the kidney; (4) in renal failure there is an accumulation of this conjugated 17-OH-CS in the plasma.

The Effects of Surgical Procedures on Simultaneously Determined Radiosodium, Radiosulphate, and Radiopotassium Spaces in Human Subjects. Belton A. Burrows,* Donald J. Davis, John F. Kelly, Anthony A. G. Lewis, and Joseph F. Ross,* Boston, Mass.

The apparent volumes of distribution, or spaces, of radiosodium, radiosulphate, and radiopotassium have been compared in human subjects before and after elective surgical procedures which did not produce gross changes in fluid and electrolyte balance. Following the simultaneous intravenous administration of radiosodium and

radiosulphate the rate of decline in plasma activity, expressed as per cent of dose corrected for renal excretion, is similar for the two substances during the first one to two hours. The radiosodium and radiosulphate spaces calculated from the plasma activity at twenty minutes correspond closely to the spaces derived from the extrapolation of subsequent plasma activities back to zero time, and are of equivalent value as an index of extracellular fluid volume.

The twenty-minute radiosodium space is approximately three-fourths the twenty-four-hour value, suggesting that one-fourth of "total exchangeable" sodium is non-extracellular and yet metabolically active. For radiopotassium the initial rate of decline in plasma activity is similar to that for radiosodium. After this period of mixing, presumably with extracellular fluid, the plasma activity of radiopotassium falls more rapidly than that of radiosodium and requires a longer period of equilibration to approach relatively constant values, at approximately twenty-four hours.

The results indicate that the response to surgical trauma does not influence the apparent rates of equilibration of radiosodium, radiosulphate, or radiopotassium; the determination of "total exchangeable" sodium and potassium; or the partition of exchangeable sodium between extracellular and non-extracellular moieties, using the twenty-minute radiosulphate or radiosodium space as an index of extracellular fluid volume. In the patients studied before and one week following elective surgical procedures there was no consistent change in these measurements.

Ventilation, Oxygen Tension and Acid-Base Adjustments During Recovery from Congestive Heart Failure. DOUGLAS CARROLL,* Baltimore, Md.

Serial studies of arterial blood oxygen and carbon dioxide tensions and body weight were made in four patients recovering from combined right and left heart failure. In three it was possible to obtain repeated ventilation and oxygen consumption determinations. During the severe edematous stage of heart failure, carbon dioxide and oxygen tensions were low, whereas ventilation and oxygen consumption were high. With cardiac compensation, there was a gradual rise in carbon dioxide and oxygen tensions, although oxygen tensions tended to remain low normal. Ventilation and oxygen consumption fell, but ventilation never returned to normal values, even with optimal cardiac compensation.

The studies indicate that during severe combined right and left ventricular failure, there are severe abnormalities in the distribution of gas to the lungs. These changes are believed to be caused by extravasation of fluid around the pulmonary capillaries and edema of the walls of bronchioles or smaller air passages.

An increased oxygen consumption at rest during the severe stages of heart failure gradually returned to normal as cardiac compensation occurred. This high oxygen intake was thought to be expended in the performance of the increased work of breathing necessitated by the excessive turgidity of the lungs associated with heart disease.

The hyperventilation present when optimal cardiac compensation had occurred suggests that some pulmonary congestion exists despite lack of signs or symptoms.

Finally, the return of ventilation to its baseline (albeit high) prior to complete loss of body weight supports the view that the fluid shifts which occur in the lesser circulation are relatively small and occur before greater circulation fluid shifts have been completed.

Further Studies on the Abnormalities in the Metabolism of Copper in Wilson's Disease. G. E. CARTWRIGHT,* J. A. BUSH, H. MARKOWITZ, J. P. MAHONEY, and C. J. GUBLER, Salt Lake City, Utah.

The ceruloplasmin level, measured by an immunologic technique, was 34 (27 to 38) mg. per 100 ml. in 10 normal subjects and 9 (2 to 19) mg. per 100 ml. in 14 patients with Wilson's disease. No correlation was observed between the ceruloplasmin level and the duration or severity of the clinical manifestations of the disease. Analyses of tissues of one control subject and one patient with Wilson's disease revealed small quantities of ceruloplasmin in the liver and kidney but none in spleen, brain, bile, or erythrocytes.

Three normal subjects, following the oral administration of Cu^{64}, excreted 66, 83, and 95 per cent of the activity, respectively, in the stools and less than 0.1 per cent in the urine. Three patients with Wilson's disease excreted 35, 57, and 82 per cent in the stools and 2, 5, and 2 per cent, respectively, in the urine.

Following the intravenous administration of Cu^{64} to three normal subjects, 11, 14, and 16 per cent of the activity was recovered in the stools and 0,3 per cent in the urine. Three patients with Wilson's disease excreted 2, 2, and 3 per cent in the stools and 4, 5, and 8 per cent in the urine. The activity in the plasma of the normal subjects decreased rapidly over the first 4 hours and then increased during the period up to 48 hours after the injection. The activity in the initial phase was not precipitable with anti-serum to ceruloplasmin, whereas that present in the plasma at 48 hours was almost completely precipitated. In the patients with Wilson's disease no secondary increase in plasma activity took place. The activity present in the plasma at 48 hours was almost entirely in the supernatant solution after precipitation of the ceruloplasmin.

The significance of these observations will be discussed.

Association of Goiter and Hypothyroidism. C. E. CASSIDY and E. B. ASTWOOD,* Boston, Mass.

If simple goiter is an expression of impaired thyroxine synthesis hypothyroidism should sometimes coexist. However, the association has seldom been recorded except in chronic thyroiditis, extreme iodine deficiency, or from known thyroid-inhibiting substances. Observation of goiter and myxedema attributed to excess iodine from Lipiodol (Raben, J. Clin. Endocrinol. & Metab., 13:

469, 1953) in one case and from KI in asthma remedy in another (VanderLaan—in preparation) prompted a survey of patients with simple goiter seen in the past two years. Of about 150 cases of non-toxic goiter, 5 were judged on clinical grounds to be hypothyroid and 4 fully myxedematous. The ages were 26 to 50; 3 were men. Estimated thyroid size ranged from 30 to 80 gm. Twenty-four hours I^{131} uptake was normal in 4 patients (14, 28, 40 and 45 per cent) and low in 4 (2.2, 3.9, 4.8, and 7 per cent). Protein-bound iodine concentrations were 0.6 to 4.0 (mean 2.3) μg. per cent. Medication with thyroid relieved the symptoms and signs in all, and caused regression of the goiter in 5 of 8 patients. In no instance were there local or systemic manifestations of an inflammatory process, or known exposure to antithyroid substances or unusual diets. The cause of the disturbance was obscure and the observation that some patients remained well when thyroid medication was stopped suggested that the etiologic factor was transitory.

The Effects of Intravenous Sodium Glutamate on Blood "Ammonia," Keto-Acids and Amino Acids in Patients with Hepatic Coma. THOMAS C. CHALMERS, FRANK L. IBER, HYMAN ROSEN, and STANLEY M. LEVENSON,* Washington, D. C.

Sodium glutamate is occasionally effective in alleviating the coma of severe hepatic disease. In an effort to elucidate the mechanisms involved, blood "ammonia," alpha-keto glutarate, pyruvate, and citrate were measured in 7 patients who eventually died in coma, and in 3 of the patients, one of whom awoke temporarily, individual amino acids were determined.

A possible rationale for glutamate therapy was demonstrated in one patient with rapidly progressing coma. "Ammonia," pyruvate, and 15 of 17 plasma amino acids rose to more than triple their pre-coma, near-normal values, but glutamate, aspartate, and alpha-keto-glutarate decreased.

Six patients in coma from 8 to 96 hours received glutamate 9 times (24 grams over 3 to 4 hours). "Ammonia" decreased on 8 occasions (mean decrease 94 μ-moles per liter, S. E. 37) and α-keto-glutarate increased 8 times (mean 36, S. E. 17). There were no consistent changes in pyruvate and citrate.

In a patient whose coma was unaffected by glutamate, alpha-amino-nitrogen rose from 2,840 to 5,470 μ-moles per liter (4.0 to 7.6 mg. per cent). Aspartate, alanine, and the amides (glutamine and asparagine) accounted for 87 per cent of the increase in amino acids, excluding glutamate which rose from 152 to 957 μ-moles per liter. The blood "ammonia" dropped from 140 to 88 micromoles per liter, alpha-keto-glutarate and pyruvate rose from 30 to 140 and 155 to 260, respectively.

In the only patient who awoke following the infusion "ammonia" dropped from 148 to 102, and α-keto-glutarate rose from 53 to 76, pyruvate dropped from 222 to 106 and α-amino nitrogen rose from 563 to 4,761 μ-moles per liter (0.8 to 6.7 mg. per cent). Aspartate and alanine accounted for 63 per cent of the increase in amino acids, excluding glutamate (17 to 3,730). In this patient the amides decreased.

It is concluded that although a drop in ammonia and rise in α-keto-glutarate following glutamate infusion suggest a beneficial effect, these changes can occur without any sign of improvement. In contrast to previous supposition the drop in ammonia is not always accompanied by a rise in amides.

The Distribution of "Nephron Delay Time" in Normal Man. A. W. CHILDS, H. O. WHEELER, B. COMINSKY, E. LEIFER, O. L. WADE, and S. E. BRADLEY,* New York, N. Y.

The dimensional differences between nephrons suggests that there is a comparable functional variation. Evidence of such a functional non-uniformity was adduced in a recent study (Trans. Assoc. Am. Phys., 65: 147, 1952) of the time required for filtrate to flow from the glomerulus to the bladder (nephron delay time). Analysis of the disparities between the amounts of inulin filtered and the amounts appearing in the urine during successive ten-minute periods following rapid intravenous injection indicated that 60 per cent of the filtrate reached the bladder in the first ten minutes, 30 per cent required twenty minutes, and 10 per cent required thirty minutes or longer.

These findings indicated a considerable range in functional capacity of the nephrons that seemed consistent with other evidence. Since the analysis requires graphic integration of the relationship between changing concentrations of inulin in plasma and urine, the derived values depart from accuracy in the extent to which finite intervals of time are substituted for infinitesimals. Because ten-minute periods might thus have introduced a serious error, nephron delay was determined in ten human subjects on the basis of two-minute collection periods. A much more restricted range of variation was detected.

On the average, 1 per cent of the filtrate required two minutes to reach the bladder, 21 per cent required four minutes, 62 per cent required six minutes, 14 per cent required eight minutes, and 1 per cent required ten minutes. In four patients with severe renal disease, mean nephron delay was prolonged and the frequency distribution widened. It is probable that still shorter periods of collection would further reduce the apparent variation. Within such a narrow range, laminar flow could be an important determinant of the pattern of delay.

Renal Excretion Patterns of Some Electrolytes and Non-Electrolytes. FRANCIS P. CHINARD* and THEODORE ENNS, Baltimore, Md.

The renal excretion patterns of various substances following a single circulation through the kidneys have been determined in dogs. The test substances, together with a glomerular substance (inulin or creatinine), are injected instantaneously into one renal artery. Urine is collected from each ureter separately for 30 periods each of 30 seconds' duration. Correction for recirculation is made by period by period subtraction of the amounts excreted by the control kidney from the amounts excreted

by the kidney into which injection was made. The fractions of the injected substances excreted in each period, corrected for recirculation, are plotted against time to give the excretion patterns; appearance, modal, and mean transit times are calculated from these data. There are no major differences in the excretion patterns or transit times of simultaneously injected glomerular substances. Appearance and transit times of Na-22 and Cl-36 are approximately equal and less by about 30 seconds than the corresponding times for creatinine. Half the eventually excreted Na-22 (and Cl-36) is in the urine when 25 per cent or less of the eventually excreted creatinine is in the urine. Substantial fractions of the urinary sodium and chloride ions appear to bypass a portion(s) of the tubular segments. The transit times of *p*-aminohippurate are greater by about 45 seconds than the corresponding times for creatinine. This delay and the rapid uptake of PAH from blood suggest that the slow process in PAH secretion is the transport across the tubular cells. Excretion patterns of K-42, and of labelled water, glucose, and urea will also be presented.

A Method for the Coupling of Protein Antigen to Erythrocytes: Use of the Method in the Diagnosis of Tuberculosis. LEON R. COLE, J. JAY MATLOFF, and VIRGINIA R. FARRELL, Boston, Mass. (Introduced by Kendall Emerson, Jr.).

A technique has been devised whereby protein antigen is coupled to formalized erythrocytes, and such antigen-coated erythrocytes used to measure antibody. This method provides a stable antigen preparation.

Human erythrocytes are treated with formalin by the method of Flick (1948). After removal of the formalin, clumps are dispersed by mixing in a Waring blender for 20 minutes. The formalized cells are then treated with tetrazotized benzidine. Tuberculin PPD, added at this point, couples onto these treated cells. The PPD-coated erythrocytes are suspended in 0.5 per cent normal guinea pig serum (N.G.P.S.). Twofold dilutions of test sera, from 1:5 to 1:640, are prepared in 1.5 per cent N.G.P.S. To each tube is added a drop of PPD-coated cells. Agglutination patterns are read after standing overnight at 3°C. The sensitized, formalized erythrocytes are stable for well over a week, but require rehomogenizing before each use. The presence of PPD on the formalized cells has been demonstrated by bioassay. Tetrazotized benzidine couples proteins mainly through linkages with their tyrosine and histidine groups. The present technique thus might be applicable to the study of antibody response to a variety of agents, including certain bacterial proteins, antibiotics and hormones, some of which have been demonstrated or suspected to have antigenic properties.

Eighty-four patients with active pulmonary tuberculosis were studied. In most the tuberculous activity was of recent onset. Serum titers of from 1:40 to 1:640 were observed in 76 per cent. Eighty-six hospital patients with other illnesses served as controls. In the control series, 67.5 per cent of patients had titers of $< 1:5$, and 90 per cent fell into a group with titers of 1:10 or less; 8 per cent were 1:20, and 2 per cent were 1:40. Certain correlations between the clinical status of the tuberculous and nontuberculous patients and their titers will be discussed.

The Distribution of Circulating Blood Within the Splanchnic Vasculature. B. COMINSKY, J. R. K. PREEDY, R. HAYS, and H. O. WHEELER, New York, N. Y. (Introduced by Franklin M. Hanger).

Changes in the size of the liver and spleen observed during circulatory adjustments suggest that the splanchnic vasculature may serve as a "reservoir" from which blood may be discharged on need. Measurements of "circulating splanchnic plasma volume" in intact dog and man by a regional dilution technique (Trans. Assoc. Am. Phys., **46**: 294, 1953) also indicate that marked changes in volume may occur. This technique does not appear to measure blood sequestered within the splenic pulp nor does it yield information regarding the behavior of the various components of the splanchnic vasculature during changes in total volume. A means of evaluating the distribution of circulating blood within the splanchnic bed based upon the measurement of "splanchnic hematocrit" is reported in this paper.

The circulating splanchnic plasma volume (SPV) was measured by the regional dilution technique, using I^{131} labelled human serum albumin and circulating splanchnic red cell volume (SRCV), using P^{32} tagged red cells in 11 intact and 8 splenectomized dogs, anesthetized with Nembutal ®. The splanchnic hematocrit was calculated by dividing the value for splanchnic red cell volume by the value of the sum of splanchnic plasma and red cell volumes (SRCV/SPV + SRCV). This figure was always less than peripheral arterial hematocrit, averaging $79.4 \pm (\sigma)$ 8.9 per cent of peripheral hematocrit in intact animals and 71.8 ± 18 per cent in splenectomized dogs, a statistically insignificant difference. This finding indicates that the circulating splanchnic blood volume is for the most part confined to very small blood vessels, presumably the hepatic sinusoids. Allen and Reeve (Am. J. Physiol., **175**: 218, 1953) have shown that the hematocrit of pooled hepatic sinusoidal blood is 69 per cent of arterial hematocrit. On the basis of this figure it may be shown that approximately 77 per cent of the circulating splanchnic blood volume lies within the sinusoids.

Measurements of the Mechanics of Respiration in Newborn Infants. C. D. COOK, J. M. SUTHERLAND, S. SEGAL, R. B. CHERRY, M. B. MCILROY, J. MEAD, and C. A. SMITH, Boston, Mass. (Introduced by C. A. Janeway).

Simultaneous and continuous recordings of respiratory volume and intraesophageal pressure (as a measure of intrapleural pressure change) were obtained 39 times in 23 normal newborn infants ranging in weight from 2.4 to 3.8 Kg. and in age from 1 hour to 7 days. Lung compliance and resistance as well as the work of respiration were calculated from these data. Five similar studies

in two infants severely ill with neonatal respiratory distress (the hyaline membrane syndrome) were carried out.

These data indicate that the mean compliance of the normal newborn infant's lung is approximately 5 ml. per cm. H_2O (range 2 to 9) and the mean resistance approximately .03 cm. H_2O per ml. per sec. (range .005 to .13). The work of respiration for an average resting 2.5 Kg. newborn infant is found to be 1600 gm. cm. per minute with approximately 70 per cent of the work done against elastic resistance and 30 per cent against viscous resistance. In contrast to these data, the observations on the two infants with respiratory distress show a marked reduction in compliance (1 and 0.6 ml. per cm. H_2O, respectively). There is also a marked increase in the work of respiration, a change primarily related to differences in elasticity and to the increased respiratory rate.

The application of a simplified formula (Work = 0.6 PV) for the calculation of the minute work of respiration proposed by one of the authors (M. B. McI.) is discussed in relation to the present data. The present data on the mechanics of respiration in normal newborn infants will be correlated with similar data for adults.

The Volume of Distribution of High Molecular Weight Dextran and Its Relation to Plasma Volume in Man. ALBERT B. CRAIG, JR. and CHRISTINE WATERHOUSE,* Rochester, N. Y.

The fractionation of dextran has permitted studies with a polysaccharide which has molecular weights much greater than most serum proteins. Intravascular retention of dextran is responsible for its usefulness as a plasma expander. Theoretically, if such a substance were given in small quantities, and if low concentrations of dextran could be accurately determined, one might be able to estimate the volume of circulating plasma by the dilution principle.

Suitable modifications of the anthrone determination of dextran as described by Bloom and Wilcox have been made to permit the measurement of serum concentrations below 100 mg. per cent with an accuracy of ± 2 per cent. Five normal male subjects were given 2.0 to 2.5 grams of a 6 per cent solution of dextran (N.R.C. Fraction No. 6, average mol. wt. 195,000) intravenously, and serum concentrations were determined at appropriate intervals from one minute to four hours. Rapid mixing was apparent, and the serum concentration fell at a slow exponential rate (1.8 per cent per hour). Renal excretion of this fraction was found to be negligible. The procedure was repeated two to four times on each individual, and the standard deviation of the variation from the individual's mean volume was ± 1.9 per cent (range ± .6 to ± 3.8 per cent). The average volume of distribution of dextran was 36.8 ml. per kg. of body weight.

High molecular weight dextran is distributed in a reproducible space. This space seems to be lower than plasma volume found with T-1824 or I^{131} albumin. However, dextran volume is comparable to the plasma volume found by using tagged cells and the venous hematocrit.

The Influence of Dietary Fructose on Intravenous Glucose Tolerance in Man. JAMES W. CRAIG, MAX MILLER,* and WILLIAM R. DRUCKER, Cleveland, O.

Hill, Baker, and Chaikoff (J. Biol. Chem., 1954, **209**, 705) concluded that the type as well as the quantity of dietary carbohydrate may influence the rate of utilization of subsequently administered glucose. Rats fasted for one day and then fed a diet whose sole carbohydrate was fructose for three days showed almost as great a diminution in tolerance for orally administered glucose as did rats fasted for four days; rats fed a diet whose sole carbohydrate was glucose served as controls. The altered tolerance was attributed to an impaired liver glucokinase activity, a manifestation of enzymatic adaptation to diet.

The present experiments were designed to determine whether or not dietary fructose altered glucose tolerance in man. Four normal young male subjects were fed a diet whose sole carbohydrate was 200 or 250 grams of fructose daily for three days, after which an intravenous glucose tolerance test was performed. Tolerance tests were performed in the same subjects after three days on a diet whose sole carbohydrate was the same quantity of glucose. Control tolerance tests were also obtained in the same subjects on a normal diet containing the same quantity of carbohydrate in mixed form. In these four subjects, fructose feeding produced no significant alteration in the intravenous glucose tolerance test.

In other human experiments it has been found that the increase in the glucose concentration of portal anastomotic vein blood after oral fructose administration is only a fraction of that found after equal amounts of oral glucose. The failure to find an altered glucose tolerance after fructose feeding suggests that liver glucokinase activity *in man* is not decreased by a lowered concentration of glucose in the portal vein blood. The explanation for starvation diabetes *in man*, therefore, awaits further experimentation.

A Procedure for Study of the Visceral Circulation in Man. JAMES W. CULBERTSON,* JOHN W. ECKSTEIN, and WALTER M. KIRKENDALL, Iowa City, Ia.

The physiopathology of a number of clinical circulatory disturbances remains incompletely understood because recorded clinical physiological observations have been fragmentary. Investigators have tended to concentrate on measurements of blood flow rate and pressure in a single regional circuit, often without concomitant estimation of total systemic blood flow rate (= MVCO). Previously reported combined procedures have related respiratory and circulatory phenomena.

Taking MVCO to represent pulmonary regional as well as total systemic blood flow, we have designed a practical procedure for measuring also the abdominal visceral (= renal + hepatic) blood flow rates separately but simultaneously during the same experimental session. Intracardiac and pulmonary arterial and "capillary" pressures are recorded before MVCO is determined (Fick principle). Then, with the venous catheter in the liver and a urethral catheter in the bladder, control estimates

of hepatic and renal plasma flow and splanchnic oxygen consumption are made by clearance methods. The constant intravenous infusion contains Bromsulfalein, sodium para-aminohippurate and inulin in a pharmaceutically and clinically compatible combination. These procedures require 90 minutes, a team of three physicians and < 150 ml. of blood for analysis. Provision is made for securing blood and urine samples for blank analysis, and adequate physiochemical equilibration time is allowed. In order to make estimates of renal and hepatic blood flow coincide the schedule conveniently staggers the collection times of blood and urine samples.

After completion of control observations acute physiological or pharmacodynamic changes may be induced deliberately and the experiment continued for another hour, with the initial series of observations repeated in reverse order.

Illustrative examples of the application of this procedure are available, depicting the acute hemodynamic changes seen following intravenous atropine in one instance and oral whiskey in another. The procedure is adaptable in various ways to suit individual investigators' needs.

Renal Conservation of Potassium during Electrolyte Restriction and the Effects of Sodium, Cold, and ACTH. T. S. DANOWSKI,* M. BLACK, R. MURTHA, and P. WIRTH, Pittsburgh, Pa.

Studies herein reported indicate that during potassium deprivation, in contradistinction to a widely held belief to the contrary, the kidney is capable of potassium conservation as precise as its conservation of sodium on sodium restricted regimens.

Transfer of grown rats to a diet free of electrolytes or containing only chloride resulted in a prompt and virtually parallel decrease in urinary sodium and potassium. The replacement of distilled drinking water with 0.5 or 1.0 per cent saline resulted in a 3 to 5-fold increase in urinary potassium excretion. Exposure of the rats to 5° C. temperatures also partially interfered with the renal conservation of potassium in response to a low electrolyte intake. Similar results were obtained with ACTH. The combination of cold and 1 per cent sodium chloride in the drinking water, or ACTH and oral 1 per cent sodium chloride, further increased urinary potassium output. In all instances, however, the potassium conservation response was still evident though less pronounced. These data indicate that in the rat deprived of electrolytes potassium conservation can be as effective as sodium conservation. Exposure to cold, administration of ACTH or the presence of sodium chloride in the intake interferes only partially with this response. The sodium and cold effects, and the sodium and ACTH effects, appear additive.

Assuming a similarity of response in rat and human kidneys, these findings suggest that the continued urinary loss of potassium during inanition which is one of the chief causes of potassium depletion in clinical situations is not attributable to an inherent inability of the kidney to conserve potassium. It is ascribable however to modifications in a fundamentally precise conservation response as a result of stress, sodium intake, and presumably other unidentified factors attendant upon illness.

Application of "The Doctrine of Original Antigenic Sin" to Immunization Against Influenza. FRED M. DAVENPORT and ALBERT V. HENNESSY, Ann Arbor, Mich. (Introduced by Thomas Francis, Jr.)

The "doctrine of original antigenic sin" epitomizes the discovery that, throughout life, antibody response to infection with influenza viruses is oriented to the dominant antigens of strains encountered in childhood. Three successive shifts in dominance of the antigens that characterize group A viruses have occurred. Consequently, antibody response to influenza A-prime in three successive cohorts of the population is predominantly to A-prime, A, or swine strains. Specific antibody increase to viruses encountered after childhood diminishes, yet the antibody spectrum broadens with age owing to repeated exposures. Concurrently, the attack rate falls. One objective of vaccination against influenza is to induce at all ages that composite of antibodies characteristic of older age groups. To ascertain an efficient method of achieving this goal, groups of 25 children, military recruits, or persons over 30 were vaccinated serially at two-week intervals with monovalent vaccines containing A-prime, A, or swine influenza viruses. Administration of these vaccines was alternated in "checker board" fashion. More than 500 individuals were studied. Antibody response was measured by hemagglutination inhibition in sera obtained before and two weeks after each vaccination. The results obtained demonstrate that reinforcement of the primary antibody characteristic of each age group is independent of the vaccine given. However, to achieve the maximal composite of antibodies, children require vaccination with swine and A, recruits with swine and A-prime, and persons over 30 with A and A-prime strains. A single vaccine composed of swine, A and A-prime viruses was tested and found to yield satisfactory antibody levels at all ages. These findings illustrate a cogent method for ascertaining the essential antigenic components of influenza vaccines, and provide a rational basis for alteration of current vaccine formulae.

An Electron Microscope Study of Sectioned Platelets and Megakaryocytes. QUIN B. DEMARSH, JEAN KAUTZ, and ARNO G. MOTULSKY, Seattle, Wash. (Introduced by H. S. Bennett).

Megakaryocytes and platelets obtained from human marrow biopsies, and platelets concentrated from peripheral blood have been fixed and sectioned sufficiently thin for electron microscope study. The preparations reveal many new structural features not seen in unsectioned material, and afford a promising approach for the study of platelet-megakaryocyte problems in health and disease.

The endoplasm of sectioned mature megakaryocytes displays numerous future platelet units, separated from each other by a three-dimensional array of platelet demarcation membranes, here described in the human for the first time. These membranes form from precursor structures, the platelet demarcation vesicles, found in the endoplasm of younger megakaryocytes. The vesicles line up and coalesce to form double layered plates. These fuse further to form the demarcation membranes, which come to enclose pockets of cytoplasm, each of which contains a few granules and constitutes a future platelet unit. Numerous additional granules can be seen elsewhere in the cytoplasm, along with mitochondria, Golgi material, large irregular vacuoles and endoplasmic reticulum. The ectoplasm is free of platelet demarcation membranes and other organelles.

Numerous small round or oval granules are also seen scattered throughout the hyaloplasm in sectioned platelets. The hyaloplasm is otherwise homogeneous, and is bounded by a relatively thick and sturdy platelet membrane. Most platelets are round or ovoid, but a few display spicules or agranular blebs at their borders.

Normal and abnormal megakaryocytes and platelets from sternal biopsy have been examined, as well as platelets from the peripheral blood of both normal patients and those with various bleeding disorders and blood dyscrasias. An attempt is being made to correlate submicroscopic morphology with clinical findings, in order to clarify especially the mechanism of platelet membrane formation in thrombocytopenic and thrombasthenic patients.

Studies on the Survival and Metabolic Activity of Platelets in Humans, Utilizing Radioactive Phosphorus. R. G. DESAI, WALTER SMALL, and IRMA MEDNICOFF, Boston, Mass. (Introduced by Samuel H. Proger).

Studies have been made demonstrating the feasibility in humans of tagging platelets *in vivo* by the use of radioactive phosphorus.

Using sterile, siliconized apparatus, polycythemic platelet-rich plasma tagged *in vitro* with P-32 was injected into normal people, leukemic patients and the same polycythemic donor. Daily samples of blood from the recipients were collected, and platelet-rich plasma, platelet free plasma and washed platelets were prepared. By measuring the number of platelets and radioactivity in each sample, it was possible to calculate the radioactivity per million platelets. The apparent half survival time of platelets from polycythemic individuals injected in normals was 35 to 50 hours, while the same platelets in leukemics survived for 18 to 28 hours. The advantage of this method has been the requirement of smaller numbers of injected platelets, accuracy and reproducibility of measurements, and its applicability in various hematological states, *e.g.*, polycythemia, thrombocytosis, etc. It may also prove to be a method of studying *in vitro* the *in vivo* viability of platelets.

The results of the experiments have shown the uptake of P-32 by the platelets to be a function of temperature, incubation time, the amount of radioactivity and the tagging medium. The distribution of radioactivity in whole blood has been studied and indicates a direct relationship between metabolic activity of the cells and the phosphorus uptake. The integrity of the tagged platelets has been demonstrated by the clot reaction, thromboplastin generation, morphology and the Warburg respiration technique.

Kinetics of Human Adrenal Cortical Activity. V. DI-RAIMONDO, R. H. ORR, D. ISLAND, and P. H. FORSHAM,* San Francisco, Calif.

When ACTH is given as an 8-hour i.v. infusion and hourly 17-hydroxycorticoids are determined, two phases in steroid excretion are noted: one of increasing excretion during the infusion and another of decreasing excretion following it. The effects of dose, time of day, and nature of ACTH on these phases of 17-hydroxycorticoid excretion were studied. For doses of ACTH of 1 USP Unit and above the acceleration in urinary excretion of 17-hydroxycorticoids was maximal. With smaller doses the acceleration varied with the dose. The duration of enhanced steroidogenesis was proportional to the dose. From minimal effective dose data the daily basal output of ACTH by the human pituitary is probably less than 1 USP Unit. Both acceleration and deceleration in urinary 17-hydroxycorticoid excretion were altered when infusions of ACTH were begun at 8:00 p.m. instead of 8:00 a.m. A diurnal variation in response to ACTH paralleling the spontaneous diurnal variation in adrenal cortical activity was demonstrated. This suggests that the diurnal variation is not controlled by endogenous ACTH secretion. Whereas sheep and hog ACTH accelerate steroidogenesis in the same manner the duration of enhanced steroidogenesis was more prolonged for the latter when comparing doses of equal ascorbic acid depleting capacity. This appears to be due to species differences. It is now possible, by studying the kinetics of steroidogenesis, to characterize any type of ACTH accurately in terms of steroidogenic potency, duration of action, and i.v./i.m. activity.

Effects of Sitosterol on Serum Lipids of Hypercholesterolemic Subjects. CHARLES H. DUNCAN and MAURICE M. BEST, Louisville, Ky. (Introduced by J. Murray Kinsman).

During the past two years the plant sterol, beta-sitosterol, has been administered to a number of human subjects, and its effect on the various serum lipid fractions noted. Of these, 10 meet the following criteria for inclusion in this report: control serum total cholesterol (Abell) of 260 mg. per 100 ml. or higher, and absence of those conditions in which elevation of serum cholesterol commonly occurs, diabetes mellitus, hypothyroidism, biliary obstruction, the nephrotic state, and essential hyperlipemia. Six to 8 grams of beta-sitosterol was administered immediately before the ingestion of food, the usual daily dosage being 20 to 25 grams. There was no

restriction as to type or amount of diet. The mean total period of observation was 45 weeks, including control (placebo) and treatment periods.

The administration of sitosterol was accompanied by a decrease in serum total cholesterol from a mean control level of 289 mg. per 100 ml. to 242 mg. per 100 ml. The decrease in cholesterol ranged from 21 to 104 mg. per 100 ml. The mean levels of the other serum lipid fractions were also decreased by sitosterol; total lipid from 967 to 832 mg. per 100 ml. (range minus 39 to minus 365); phospholipid from 356 to 325 mg. per 100 ml. (range plus 20 to minus 90); neutral fat from 324 to 260 mg. per 100 ml. (range minus 9 to minus 174). In 7 of the 10 patients lipoproteins as determined by the ultracentrifuge have also been studied, a trend toward reduction of S_f 3–100 classes being noted. The effects of sitosterol were noted by the end of one week, increased for the next several weeks, and were sustained throughout periods of administration up to one year. With the substitution of a placebo serum lipids returned to the pre-treatment range.

Natriuretic-Diuretic Effect of Angiotonin in Essential Hypertension. HARRIET DUSTAN, CARLOS NIJENSON, and A. C. CORCORAN,* Cleveland, O.

Observations on renal effects of angiotonin infusion were made during osmotic diuresis and hydropenia in 10 patients with essential hypertension and 1 normal subject. Angiotonin (hypertension) and renin induce natriuresis and diuresis in rats and rabbits, as renin does also in dogs with sectioned carotid sinus and aortic depressor (buffer) nerves, while renin does not in normal dogs (del Greco and Corcoran, 1954) and angiotonin does not in hydrated normal humans (Nickel *et al.*, 1954).

Angiotonin caused decreases in C_{PAH} and increases in filtration fraction in all, and associated decreases in $C_{Mannitol}$ in 3 of the patients. Urine flow and Na output were depressed in 5 patients and in the 1 normal subject; these include the 3 patients with decreased $C_{Mannitol}$. However, urine flow and Na output were increased in 5 of the patients, reaching nearly twice control rates in 1. The proportion of Na calculated as reabsorbed was decreased in these 5, while calculations of Tc_{H_2O} (free water reabsorption) demonstrate increases (0.2 to 1.4 ml. per min.). The diuretic effect of angiotonin in these patients is therefore osmotic and attributable to decreased Na reabsorption.

Two patients under treatment with ganglion-blockers at the time of test showed negative control rates of Tc_{H_2O} which became slightly positive after angiotonin. Abnormal water transport in these two presumably relates to a depressing effect of hexamethonium on maximum water reabsorption previously observed in hydropenic, osmotic diuretic dogs (del Greco and Corcoran, 1954).

The disparate effects of angiotonin on Na reabsorption in hypertensive patients may reflect differences similar to those mirrored by renal responses to renin in normal and buffer-nerve sectioned (neurogenic hypertensive) dogs.

An Evaluation of $Na_2Cr^{51}O_4$ as an Agent for the Determination of the Erythrocyte Life Span In Vivo *in Various Hemolytic States.* FRANKLIN G. EBAUGH, JR., CHARLES P. EMERSON,* and GERALD P. RODNAN, Bethesda, Md., and Boston, Mass.

Since there is a loss of Cr^{51} from labeled circulating erythrocytes in normal individuals, the validity of this technique has been studied in the hemolytic anemias by selective agglutination and radioactive Cr^{51} counting. The rate of elution of Cr^{51} from circulating normal donor cells in recipients with hemolytic disease and from abnormal red cells (sickle cell anemia and congenital spherocytosis) transfused into normal recipients was no faster than from normal donor cells in normal recipients, *i.e.*, 52 to 97 days—for half the Cr^{51} to disappear from circulating RBC. It was not possible to estimate red cell survival by external monitoring of the gamma radiation emitted from the patient because Cr^{51} which left the blood on any given day had a tissue phase before eventual urinary excretion. During the first 24 to 48 hours after intravenous injection of *in vitro* tagged RBC or aqueous $Na_2Cr^{51}O_4$, the radioactivity of the blood dropped 2 to 10 per cent, a decrease greater than that solely due to RBC destruction in the period. Because this decrease is observed for RBC tagged *in vivo* as well as *in vitro*, for non-chromium labeled donor cells as measured by selective agglutination, and since no such increase is seen in the elution rate of Cr^{51} from donor cells immediately following transfusion, this rapid initial fall may not be due to cell damage or loss of Cr^{51} from circulating cells but due to failure of donor cells or *in vivo* tagged cells to fully equilibrate with the entire red cell mass during this period of time. Whole blood should be used for Cr^{51} survival studies since RBC washed in saline had a mean survival time (corrected for elution of Cr^{51}) of 93 days in normal men compared with 127 days for unwashed cells.

Basic Amino Acids in Experimental Potassium Deficiency. ROBERT E. ECKEL and JAMES E. C. NORRIS, Cleveland, O. (Introduced by Reginald A. Shipley).

Since Christensen *et al.* have shown that basic amino acids affect the distribution of ions between cells and the extracellular fluid, the role of these acids in the electrolyte disturbance of K depletion was studied. In 100 gm. rats where growth was arrested by four weeks of a K deficiency diet, mean data for groups of six rats showed total basic amino acids (BAA) and 1(+)lysine attained concentrations of 9.89 and 7.74 mM per Kg. intracellular water (ICW), respectively, compared to levels of 0.82 and 0.73 mM per Kg. ICW in paired weighed controls and 1.52 and 1.68 mM per Kg. ICW in growing controls. Lysine also accumulates to the same degree in DOCA produced K deficiency despite liberal K intake and normal growth.

The feeding of 1(+)lysine HCl accelerates the development of K deficiency on a K deficient diet and further increases intracellular lysine to 25 mM per Kg. ICW. In pair fed animals on the same diet containing K, the

intracellular Na increases significantly from 15.7 to 22.2 mM per Kg. ICW and lysine increases to 10.5 mM per Kg. ICW. Total cations are elevated above normal in both cases.

Because of the very small changes in serum BAA and lysine concentrations, the lysine gradient across the muscle cell membrane increases in K deficiency. About half the increased hydrogen and ammonium ion excretion during recovery from K deficiency observed by Cooke *et al.* would occur if the BAA present in K deficiency were metabolized in the recovery process. The known metabolism of 1(+) lysine HCl to ammonia in acidosis and to urea in the absence of acidosis furnishes an explanation of the urinary ammonia findings of Cooke. Lysine thus apparently has a role as a labile muscle cation analogous to the role of ammonia, bicarbonate, and citrate in the urine.

The Metabolism of Plasma Lipoproteins. HOWARD A. EDER * and DANIEL STEINBERG, Bethesda, Md.

The metabolism of plasma lipoproteins in the rabbit was studied by labeling the proteins with alanine-1-C^{14} and the phospholipids with P^{32}. Plasma lipoproteins were fractionated by ultracentrifugal flotation into lipoproteins with densities less than 1.063, lipoproteins with densities between 1.063 and 1.21 and residual proteins with densities greater than 1.21.

After injection of alanine-1-C^{14} the $D < 1.063$ lipoproteins attained maximal specific activity at 6 hours. Activity then fell precipitously so that by 20 hours it was less than 25 per cent of the maximal activity; thereafter it decreased more slowly. The D 1.063–1.21 lipoproteins attained specific activities only one-third as great and the maximal activity was found after 6 hours. The initial rate of decrease in activity was considerably slower and the $t_{1/2}$ of the slow phase was about 12 days. The curve for the $D < 1.21$ fraction, the bulk of the plasma proteins, was very similar.

In other experiments the various fractions were labeled by alanine administration and each of the three fractions was administered to a different recipient rabbit. The labeled $D < 1.063$ lipoproteins disappeared with great rapidity while the labeled $D < 1.063$–1.21 lipoproteins disappeared more slowly. The data do not permit conclusions regarding the interconversion of the proteins of one class to another. Similar experiments with protein of higher activity are in progress.

That the rapid disappearance of the $D < 1.063$ lipoproteins was not due to the presence in this fraction of chylomicra with high metabolic activity was demonstrated by experiments in which the very low density components were analyzed separately.

Phospholipid reached maximal activity by 15 hours. The specific activities in the different fractions were virtually identical. This can be explained by the observation that phospholipid rapidly transfers from one lipoprotein fraction to another even *in vitro*.

These experiments demonstrate that the various lipoprotein proteins differ in their metabolic patterns.

The Effects of Steroid Hormones on Anabolism and Catabolism of Normal Tissues and Lymphoid Tumors in Humans on Protein-Free Diets. LEONARD P. ELIEL * and ROBERT P. HEANEY, Oklahoma City, Okla.

Maximal reduction of protein anabolism, by administration of protein-free, calorically adequate diets has been employed to assess the effects of steroid hormones on anabolism and catabolism of normal and neoplastic tissues in man. In three studies, after achievement of stable, minimum nitrogen excretion, cortisone acetate, 200 to 400 mg. per day p.o., or Testosterone propionate, 50 mg. per day i.m., was administered for one to three weeks. The balances of nitrogen and electrolytes were determined. One patient had no lymphoid tumors, the other had chronic lymphatic leukemia with generalized adenopathy and splenomegaly.

Amino acid deprivation was invariably associated with losses of nitrogen, phosphorus, and potassium in proportions consistent with catabolism of normal tissue. The leukemic patient failed, however, to demonstrate significant changes in lymphoid tumor mass, either by clinical measurement or by altered ratios of the phosphorus to nitrogen balances.

The patient without lymphoid tumors demonstrated, upon exhibition of cortisone, a minimal rise in nitrogen excretion, averaging 0.1 gm. per day. The patient with leukemia, however, demonstrated almost complete disappearance of enlarged lymph nodes and moderate spleen shrinkage. There was a rise in nitrogen excretion averaging 1.36 gm. per day, 60 per cent of which was calculated to arise from loss of lymphoid tumors. Administration of Testosterone to the same patient, during a second study under identical conditions, failed to produce any reduction in the negative nitrogen balance.

The data suggest that cortisone acetate does not under these conditions accelerate catabolism appreciably, since it increases nitrogen excretion from normal tissues but slightly when anabolism is reduced markedly. The data also suggest that Testosterone propionate does not act by inhibiting catabolism since it failed to diminish nitrogen excretion when anabolism was maximally reduced. Continued anabolism of lymphoid tumors during protein deprivation is suggested by their failure to shrink and their usual response to administration of cortisone.

Studies on the Physiologic Role of Glucagon (Hyperglycemic Factor). H. ELRICK, C. J. HLAD, JR., T. WITTEN, T. M. BOW, and A. SMITH, Denver, Colo. (Introduced by G. S. Gordan).

Glucagon causes hyperglycemia and a decrease in liver glycogen, apparently by accelerating liver glycogenolysis. Beyond this, little is known about its action or its function in the body. The purpose of this paper is to describe some experiments designed to elucidate the action of glucagon on glucose utilization and to propose a theory as to its physiologic role.

Glucose was administered intravenously by constant infusion pump for periods of two hours to 38 normal

adults under controlled basal conditions. The effects of glucagon and/or insulin on arterial (capillary) and venous blood sugar were studied. It was found that glucagon consistently increased A-V glucose differences markedly, whereas insulin did not. Both hormones together (1) increased A-V glucose differences beyond what glucagon alone did and (2) maintained blood sugar levels between the elevated or depressed levels resulting from each alone. These results have been confirmed in the pancreatectomized dog.

The increases in A-V glucose differences were far greater than those predicted or actually obtained by elevated glucose levels *per se*. Since the experimental technique used minimizes changes in blood flow, it is felt that the A-V differences observed were proportional to peripheral glucose utilization.

The theory is proposed that glucagon and insulin function as a team to enhance peripheral glucose utilization and, simultaneously, maintain relative constancy of blood glucose and liver glycogen levels. Since glucagon action is rapid and short-lived, the postulated action probably serves as an emergency function. Because insulin and glucagon have opposing effects on blood glucose and liver glycogen, each is ideally suited to complement the action of the other in maintaining constancy of these two reservoirs of body glucose in the face of accelerated glucose utilization. The observed effects of glucagon are not due to hyperglycemia *per se* or to a secondary release of insulin.

The Relationship of Serum Myeloma Proteins and Bence-Jones Proteins, Particularly with Respect to Their Methionine Content. RALPH L. ENGLE, JR., LILA A. WALLIS, and BERNARD UDIN, New York, N. Y. (Introduced by Paul Reznikoff).

The relation of Bence-Jones protein to the abnormal serum protein component in patients with multiple myeloma remains unknown. Bence-Jones proteins have been variously reported to contain either no methionine or amounts corresponding to less than two residues per molecule of protein. Accordingly, the aminoacid composition of the serum myeloma proteins and Bence-Jones proteins occurring simultaneously in two patients was determined. In addition, similar analyses were made of five other Bence-Jones proteins.

The proteins were isolated by thick filter paper and starch block zone electrophoresis, and were examined for aminoacid composition by two dimensional paper chromatography. The sensitivity of the technic used was sufficient to detect one residue of methionine per Bence-Jones protein molecule of 44,000 molecular weight.

The only qualitative difference in the aminoacid content of the proteins studied was the absence of detectable amounts of methionine in five of the seven Bence-Jones proteins. In one of the two patients having both serum myeloma and Bence-Jones proteins, methionine was found in both proteins in about the same concentration as in normal gamma globulin. However, chemical differences in these two proteins were found by N-terminal aminoacid analysis (method of Edman). Aspartic acid was the predominant end residue for the serum protein, while there was no N-terminal aminoacid for the urinary protein by this technic. In the other patient, the Bence-Jones protein contained no detectable methionine; yet the serum myeloma protein contained the usual amount of methionine.

The results suggest that most Bence-Jones proteins contain only trace amounts of methionine. Other Bence-Jones proteins, however, contain about the same per cent methionine as normal gamma globulin. The chemical differences between the Bence-Jones and the serum myeloma proteins of the same patient suggest that these proteins are not directly related.

Folic Acid and Citrovorum Factor Metabolism in Gout. WILLIAM W. FALOON and SHIRLEY FEIGEL, Syracuse, N. Y. (Introduced by Eugene L. Lozner).

Citrovorum factor has been demonstrated to function as a transformylating medium in purine synthesis and in direct uric acid synthesis. The conversion of pteroylglutamic acid to citrovorum factor and the urinary excretion of folic acid (Strep. Fecalis growth stimulating factors) and citrovorum factor have been studied in gouty patients.

Urinary excretion of citrovorum factor (CF) and folic acid (FA) was studied during four-day periods of oral administration of 10 mgm. of pteroylglutamic acid (PGA) daily. The average daily urinary excretion of CF during these periods in eight gouty patients was 18 gamma (range 6 to 29); in 10 healthy normals 38 gamma (range 18 to 65); and on four hospitalized non-gouty individuals 36 gamma (range 17 to 60). The differences in CF excretion by gouty and normal individuals were statistically significant, (p less than 0.01). Blood ascorbic acid levels were lower in gouty patients (average 0.42 mgm. per cent) than in normals (0.94 mgm. per cent) or non-gouty patients (0.71 mgm. per cent). No correlation was found, however, between blood ascorbic acid and CF excretion in patients or in normals. Continued administration of PGA for periods longer than four days resulted in increasing CF excretion to levels comparable with normals. In contrast to the CF excretion, the average FA excretion was nearly identical in gouty patients and healthy normals, being 4500 gamma and 4300 gamma per day, respectively. PGA administration produced no consistent change in serum uric acid or urate excretion.

Ascorbic acid given orally during PGA administration yielded increased CF excretion in the three gouty patients so studied. Salicylates, phenylbutazone, and colchicine produced no consistent change in CF excretion.

The decreased excretion of CF during administration of FA in gouty patients may be explained by several hypotheses which will be discussed. The most probable of these are decreased body stores or increased utilization of CF.

Studies of The Influence of Adrenal Steroids on Pyruvate Metabolism in Man. THOMAS F. FRAWLEY, Albany, N. Y. (Introduced by Walter S. McClellan).

Blood glucose determinations and glucose tolerance tests may not reveal early alterations in carbohydrate metabolism due to adrenal steroids. Studies were undertaken to ascertain the effect of so-called carbohydrate-active adrenal steroids on certain intermediaries of carbohydrate metabolism. Consequently some of the metabolic reactions involving glucose, pyruvate, lactate, phosphorus, and potassium have been studied.

Ingle's studies in animals using constant intravenous injections of adrenal steroids prompted an adaptation of this technique to clinical studies in man. Constant infusions of hydrocortisone were administered intravenously (12 to 25 mg. per hour) over a seven-hour period—as this simulates probably the manner in which the adrenal cortices secrete their hormones. At the third hour, glucose (0.5 Gm. per Kg. body weight as a 20 per cent solution) was administered intravenously for twenty-five minutes. In normals the glucose curve obtained during intravenous hydrocortisone did not differ significantly from control curves. Pyruvate was slightly more elevated and prolonged. Superimposing a stressor (pyromen) elevated and prolonged the pyruvic acid response without significantly changing the glucose tolerance. During the stress of hypoglycemia the pyruvic acid response to hydrocortisone (25 mg. per hour) was observed to be greater than with corticosterone administered similarly. Patients receiving corticotropin or corticosteroid therapy showed an elevated fasting blood pyruvic acid prior to a change in blood glucose. The tolerance for fructose was measured since fructose phosphorylation is independent of insulin and presumably the anti-insulin effect of adrenal steroids. Intravenous fructose (0.5 Gm. per Kg. in 10 per cent solution) during intravenous hydrocortisone (12.5 mg. per hour) gave normal tolerance values and the normal rise in pyruvic acid was somewhat prolonged.

Adrenal steroids can produce an alteration in blood pyruvic acid independently without any appreciable change in blood glucose. This effect like that occurring with potassium and phosphorus suggests certain fundamental differences between so-called "steroid-diabetes" and diabetes mellitus.

The Effect of Inflammation on the Utilization of Erythrocyte and Transferrin Iron for Hemoglobin Synthesis. E. J. FREIREICH, A. MILLER, C. P. EMERSON,* and J. F. Ross,* Boston, Mass. and Los Angeles, Calif.

The pathogenesis of the anemia associated with inflammatory disease remains an enigma. Although inflammation produces marked changes in iron metabolism, none of the demonstrated changes have explained the development of anemia. As an approach to this problem, we have studied the re-utilization for hemoglobin formation of radioiron released from transfused nonviable radioiron labelled donor erythrocytes in normal dogs and in dogs in whom sterile abscesses were produced by injections of turpentine. Similar observations were made following intravenous injection of tracer amounts (5 micrograms) of transferrin-bound radioiron. Although both control and "abscessed" animals rapidly incorporated 75 per cent to 85 per cent of transferrin-bound iron into newly formed hemoglobin within 10 days the re-utilization of "erythrocyte iron" was markedly decreased in rate and amount in the "abscessed" animals (only 10 to 20 per cent in 5 days, and 45 to 50 per cent in 30 days), in contrast to efficient and rapid utilization by the controls (45 to 55 per cent in 5 days and 70 to 80 per cent in 30 days).

These studies indicate that inflammation does not impair the utilization for hemoglobin formation of very small (? physiological) tracer doses of transferrin-bound plasma iron, but that inflammation does markedly interfere with the normal mechanism of catabolism and reutilization of erythrocyte iron. Since hemoglobin synthesis normally is dependent upon re-utilization of "erythrocyte iron," relative decreased delivery of this "building stone" to the plasma would result in a decrease of serum iron concentration and would contribute to the development of anemia.

Study of Hepatic Lymph in the Intact Animal. MEYER FRIEDMAN,* SANFORD O. BYERS, and RAY H. ROSENMAN, San Francisco, Calif.

Collection and analysis of hepatic lymph offers an unique method by which the intercellular fluid of the liver may be studied because of the probable chemical identity of the two fluids. By means of a new method, hepatic lymph was collected from normal rats and from those exposed to various experimental procedures.

The average lymph flow of six normal rats was found to be approximately 0.4 cc. per hour with an increase of 300 per cent after ligation of the bile duct. The average cholesterol, protein and cholic acid concentrations were always less than those of plasma, being 52, 75, and 74 per cent, respectively, of the plasma values. The average lymph glucose, however, was found to be about twice that found in plasma. The concentration of the latter, however, appeared to vary with the rate of flow.

The cholesterol content of lymph unlike that of bile appears to be independent of the rate of hepatic synthesis of cholesterol for when the latter is increased by thyroid administration, no change occurs in lymph cholesterol despite the marked increase observed in biliary cholesterol. Conversely, experimental elevation of plasma cholesterol in the normal rat by intravenous administration of hypercholesteremic serum is followed by a marked increase in lymph but not in bile cholesterol.

It is probable that with the possible exception of glucose, the constituents of hepatic lymph are chiefly derived from and their concentration determined by the plasma content of these same constituents. Analysis of this fluid, therefore, may offer a new means of studying the diffusion pattern of various plasma solutes from the hepatic vasculature.

The Effects of Increased Ventilation on the Work of Breathing in Patients with Pulmonary Emphysema and in Normals. MORTON GALDSTON* and JACK GELLER, New York, N. Y.

The effects of increased ventilation induced by Diamox® (2.2 to 6.3 mgm. per Kg. every 6 hours) and/or single 0.5 G. intravenous Aminophylline doses in emphysematous patients with and without carbon dioxide retention, and in normals, were analyzed by plotting alveolar pCO_2 mm. Hg and oxygen consumption (ml. per Min.) against ventilation (L. per Min.) and CO_2 output (ml. per Min.) against alveolar ventilation (L. per Min.).

In two advanced emphysematous patients alveolar pCO_2 fell from 54 to 44 mm. Hg and from 62 to 45 mm. Hg, respectively, when ventilation increased 2.0 to 2.7 L. per Min. In the former, this was accompanied by increased CO_2 output and oxygen consumption. Further ventilatory increase, accomplished only in the latter (4.0 L. per Min. maximum attained), failed to decrease alveolar pCO_2 because increased rate of CO_2 production due to increased work of breathing reached the maximum which the alveoli could ventilate effectively.

Two moderately emphysematous patients attained minimal alveolar pCO_2 levels (32 and 28 mm. Hg) with 3 to 4 liters ventilatory increase, without a change in metabolic rate. No further alveolar pCO_2 fall occurred, even with a 5½ to 9½ L. per Min. increase in ventilation. In one, with a markedly restricted maximum breathing capacity, this was accompanied by dyspnea, increased oxygen consumption and carbon dioxide output.

One of three normals attained a minimal alveolar pCO_2 of 35 mm. Hg with a ventilatory rise of only 2 liters. Additional ventilatory increase of 1 L. per Min. was accompanied by a greater metabolic rate and no change in alveolar pCO_2. The remaining two, whose alveolar pCO_2 fell to 34 and 24 mm. Hg, with 3.5 to 5 L. per Min. ventilatory increases, did not exhibit a minimum pCO_2 value or an elevation in metabolism.

These studies demonstrate that increase in ventilation beyond an optimal point no longer results in more effective alveolar ventilation because of increased work of breathing and may be detrimental.

The Mechanism of Cardiac Changes Observed in Uremia. M. M. GERTLER, J. KREAM, and J. W. HYLIN, New York, N. Y. (Introduced by B. S. Oppenheimer).

The concomitant and sequential cardiac changes in the early and terminal stages of uremia as manifested by the electrocardiogram closely parallel the cardiac changes often observed with high levels of serum potassium and/or stimulation of the cardiac vagi. These changes cannot be attributed solely to changes in intracardiac serum electrolytes for the relationship between intracardiac electrolytes and electrocardiographic measurements has not been firmly established. Because of the known association between vagal stimulation and potassium concentration another possible mechanism for these changes in uremia was considered, namely, a study of the cholinesterase system in cardiac musculature.

Uremia was produced in eight male dogs by bilateral ligation of the ureters. Three animals were treated actively with ionic exchange resins while five were permitted to continue the usual uremic course. Electrocardiograms were taken daily on the animals and when death appeared imminent, the animals were sacrificed.

The hearts were quickly removed and frozen. Homogenates of ventricular muscle were made and analyses were performed for cholinesterase activity by Ammon's method. It was found that the uremic animals had an average cholinesterase activity of 2.8 units (range 2.1 to 3.3) while in the normal animals the average was 4.4 units (range 3.5 to 5.6) as indicated by Q CO_2 values.

The significance of these findings as a possible explanation for the cardiac changes observed in uremia will be presented.

Myoalbumin, Plasma Albumin and Interstitial Fluid in Human and Rabbit Muscles. DAVID GITLIN, DORIS NAKASATO, and WILLIAM R. RICHARDSON, Boston, Mass. (Introduced by James L. Gamble).

The functional significance and biochemical properties of myoalbumin, a protein found in aqueous extracts of muscle, are largely unknown. Myoalbumin has never been isolated in its native state and has been characterized solely by solubility in aqueous solutions, resistance to denaturation and electrophoretic mobility. The superficial resemblance of these properties to those of plasma albumin prompted us to investigate the relationship between myoalbumin and plasma albumin in human and rabbit muscles employing radioactive and immunochemical methods in association with electrophoretic fractionation.

It is possible to demonstrate that: 1) The concentration of plasma albumin in the interstitial fluid of normal human and rabbit muscles is, respectively, about one-fifth and one-fourth that in the circulation; and 2) the mass of albumin in the interstitial fluid is approximately equal to that in the vascular system. These relationships are not specific for albumin but are apparently true for other plasma proteins, such as γ-globulin, which we have been able to estimate. The extravascular presence of such relatively large amounts of plasma protein was predicted by Drinker's hypothesis that the protein content of lymph fluid is representative of that in the interstitial fluid and is in accord with Starling's hypothesis of the capillary.

It has also been found, in correlation with immunohistochemical data, that myoalbumin for the most part is not derived from the muscle fiber and is simply plasma albumin originally present in the interstitial fluid and residual blood plasma in the muscle sample. The paradoxical increase in myoalbumin which accompanies denervation atrophy is due primarily to a relative increase in the extracellular mass of plasma albumin.