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ABSTRACTS

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Research Article

The Role of α-Globulins as Insulin-Carrying Proteins in Human Serum. THADDEUS E. PROUT, VASANT V. ODAK AND GEORGE J. DENDRINOS, Baltimore, Md. (introduced by Samuel P. Asper, Jr.).

Electrophoretic migration of radioinsulin (I^{131}-insulin), either alone or in serum and in various supporting media, is similar to that of α_1-globulin. Thus, it has not been possible to determine whether insulin added to serum remains free or is bound to protein. Electrophoresis on agar gel offers distinct advantages over other media for the study of this problem: *a*) albumin and α-globulins migrate toward the anode while β- and γ-globulins are separated by endosmotic flow in the direction of the cathode; and *b*) insulin alone moves to a position well ahead of the fastest moving albumin fraction, whereas insulin added to serum migrates with α-globulins. Upon addition of large quantities of insulin to normal serum (10 U per ml), insulin migrates not only with the α-globulins but also into the area ahead of albumin. Thus, saturation of binding proteins allows further migration of the excess free insulin. Serum, containing 50 mU I^{131}-insulin per ml, was partitioned by agar electrophoresis and challenged with antihuman rabbit serum by diffusion from a lateral reservoir. Radioautography demonstrated the presence of radioactivity in the precipitin arcs formed by the α-globulins. These observations provide evidence that insulin in normal serum is bound to the α-globulins. The capacity of α-globulin to bind insulin appears to be approximately 10 mU per ml of normal serum. Insulin resistance in some diabetic patients is related to an acquired insulin-binding γ-globulin. The γ-globulin in serum of an insulin-resistant diabetic was capable of binding approximately 300 mU of insulin per ml and removed insulin from the carrying protein of normal serum. The affinity for insulin by the acquired insulin-binding globulin of resistant patients is far greater than that of their insulin-carrying proteins. This suggests that the presence of insulin on the normal carrying protein is necessary for clinical control of diabetes.

Direct Effects of Ethylenediamine Tetraacetate on Renal Excretory Function. THEODORE N. PULLMAN,* A. R. LAVENDER AND IMPI AHO, Chicago, Ill.

Ethylenediamine tetraacetate (EDTA) binds polyvalent metal ions in a ring complex thereby inactivating the chelated ion. To maximize renal effects of EDTA while minimizing its systemic effects we have infused it slowly in dilute solution directly into one renal artery in the dog. The experiments were performed on fasting hydropenic anaesthetized animals. A renal artery was cannulated and normal saline slowly infused into it. The ureters were separately catheterized. After several control periods EDTA was added to the arterial infusate. All animals showed prompt predominantly unilateral natriuresis, chloruresis, and diuresis. Differential sodium excretion, negligible during pre-EDTA periods, increased to 100 to 200 μEq per minute during EDTA administration. Much smaller increases in potassium excretion also occurred. The natriuresis was not accountable for by small initial increases in filtration rate. EDTA also induced markedly increased differential calcium excretion which tended to parallel EDTA excretion mole for mole. Intrarenal infusion of calcium-EDTA chelate (CaEDTA) produced similar effects of much smaller magnitude, in some instances almost negligible. The effects of CaEDTA on calcium and EDTA excretion, however, were indistinguishable from those occurring with free ligand (EDTA). Preliminary experiments with ZnEDTA (of higher stability than CaEDTA, zinc being bound preferentially to calcium) indicate that this chelate produces slight natriuresis and negligible calciuresis but with EDTA excretions as high as with free ligand. MgEDTA effects (lower stability constant than CaEDTA) resembled those due to free ligand. These data establish that EDTA exerts a saluretic and diuretic effect by an action directly on the renal tubule. We have not established a mechanism for these effects, but suggest the possibility that it may involve chelation of an ion critical to the operation of a sodium transport system.

Pyrimidine Biosynthesis in Man: Suppression In Vivo by Anti-neoplastic Agents. MITCHELL RABKIN, ELIZABETH FREDERICK, MYRON LOTZ AND LLOYD H. SMITH, JR.,* Boston, Mass.

Many *in vivo* studies have been carried out on purine metabolism in man because of the presence of a readily available end-product, uric acid. In the absence of a comparable unique end-product, no previous over-all measurements of pyrimidine metabolism in the intact organism have been possible. In the biosynthetic sequence of pyrimidine nucleotide formation, an irreversible decarboxylation occurs in the conversion of orotidine-5′-phosphate to uridine-5′-phosphate. The release of CO_2-C^{14} from carboxyl-labeled pyrimidine precursors should therefore reflect *in vivo* pyrimidine nucleotide formation. Experiments were designed to test this hypothesis and the usefulness of this procedure in studying pyrimidine biosynthesis *in vivo* and its suppression by selected anti-neoplastic agents. Following intravenous administration of carboxyl-labeled orotic acid in the rat, approximately 50 per cent of the isotope appeared as respiratory CO_2-C^{14} within 2 hours. An additional 35 per cent appeared as unaltered urinary orotic acid. In sharp contrast, ring-labeled orotic acid released minimal CO_2-C^{14}, probably reflecting nucleotide or nucleic acid catabolism. The administration of carbamylaspartic acid, the earliest unique pyrimidine precursor, demonstrated a similar dichotomy in rate of CO_2-C^{14} evolution from carboxyl- and noncarboxyl-labeled substrates. Studies with competitive acceptors of 5-phosphoribosylpyrophosphate suggested that the availability of this reactant was not limiting. In the rat, administration of 6-azauridine, 6-azauracil, or 5-fluoro-orotic acid markedly inhibited pyrimidine nucleotide synthesis, as indicated by the diminished appearance of respiratory CO_2-C^{14} from carboxyl-labeled orotic acid. Corresponding studies in man have

demonstrated the usefulness of this procedure in estimating over-all pyrimidine biosynthesis. In patients receiving certain anti-neoplastic agents, it has allowed an *in vivo* measurement of the degree of suppression of this metabolic sequence.

The Alveolar Diffusing Volume and the Water Space of the Pericapillary Tissues of the Lungs as Measured by Multiple Indicator Dilution Curves. LLOYD RAMSEY, W. PUCKETT, A. JOSE AND W. LACY, Nashville, Tenn. (introduced by C. R. Park).

When a solution containing albumin-I^{131}, tritiated water, and nitrous oxide is injected into the right atrium, each substance will be distributed into a different physiological compartment during its first transit through the pulmonary capillaries. Albumin is confined to the vascular compartment; tritiated water mixes in an additional pericapillary tissue space; the nitrous oxide compartment includes the tritiated water space plus the alveolar gas volume which is available to the pulmonary capillaries for gaseous diffusion. Blood samples are collected anaerobically from a systemic artery at 1-second intervals following simultaneous injection of the three indicators. Albumin-I^{131} and tritiated water in each sample are determined by scintillation counting, and nitrous oxide by gas chromatography. Since flow is the same for all three indicators, the difference in shape of the three dilution curves is caused by the different volumes into which the indicators distribute. The difference between the distribution volumes of tritiated water and nitrous oxide (corrected for the partition coefficient of nitrous oxide between pulmonary tissue and air) is the alveolar diffusing volume. The difference between albumin-I^{131} and tritiated water volumes (corrected for the ratio of water content in whole blood and pulmonary tissue) is the pericapillary tissue volume of the lung. The alveolar diffusing volume and pericapillary tissue volume in dogs are 17 and 3 ml per kg, respectively, whether calculated by mean transit time or slope volume differences. The values appear to be smaller in humans. Patients with mitral valve abnormalities showed a twofold elevation of pericapillary tissue volume at rest which increased with exercise. Experimental pulmonary edema in dogs caused a fourfold increase in this measurement. Unilateral pneumothorax caused striking decrease in alveolar diffusing volume without change in pericapillary tissue volume.

Urinary Excretion of Calcium in Wilson's Disease (Hepatico-lenticular Degeneration). RAYMOND V. RANDALL, NORMAN P. GOLDSTEIN, JOHN B. GROSS, JOHN W. ROSEVEAR AND WARREN F. MCGUCKIN, Rochester, Minn. (introduced by Randall G. Sprague).

It has been reported that some patients with Wilson's disease have excreted excessive amounts of calcium in the urine and have had renal stones or nephrocalcinosis. Since it is not known whether the hypercalciuria represents another of the renal tubular defects associated with this disease, we have investigated the urinary excretion of calcium in each of 3 patients with Wilson's disease while varying widely the dietary intake of calcium. The first patient's urinary excretion of calcium averaged 106, 335, and 413 mg per day with a daily intake of calcium measuring 120, 1,538, and 2,352 mg, respectively. The second patient's urinary calcium averaged 121, 299, 348, and 475 mg per 24 hours with a daily intake of 100, 648, 1,229, and 2,347 mg of calcium, respectively. The third patient's urinary values for calcium averaged 238, 468, and 629 mg on intakes of 113, 592, and 1,620 mg of calcium, respectively. With each change in the dietary intake of calcium there was an equally abrupt change in the urinary excretion of calcium. Other investigators have shown repeatedly that as the intake of calcium varies there is relatively little change in the urinary excretion of calcium by normal persons. By contrast, our patients with Wilson's disease showed a three- to fourfold increase in the urinary excretion of calcium as the intake increased. The range as well as the abruptness of the change in the urinary excretion of calcium suggests that this phenomenon primarily reflects increased intestinal absorption. The relationship of this to the increased absorption of copper from the intestine in Wilson's disease is not known.

The Activation of Christmas Factor by Activated Plasma Thromboplastin Antecedent. OSCAR D. RATNOFF,* Cleveland, Ohio.

This study confirms the hypothesis that plasma thromboplastin antecedent (PTA), activated "spontaneously" or by glass-activated Hageman factor, converts Christmas factor from an inactive to an active form. This study demonstrates, then, that the initial steps in clotting are the successive activations of Hageman factor, PTA and Christmas factor. Previously, Biggs and Bidwell found that only after a preliminary incubation period would mixtures of PTA-deficient and Christmas factor-deficient serums behave like normal serum in the thromboplastin generation test, supporting this view. Christmas factor-like activity was measured specifically, using the partial thromboplastin time. Platelet-deficient plasma, deficient in antihemophilic factor, was recalcified and incubated in glass. At intervals, aliquots were assayed for Christmas factor. An increase of four- to eightfold in Christmas factor activity occurred within 6 minutes. Thus, antihemophilic factor was not needed for the activation of Christmas factor. Similarly, Christmas factor activity evolved in plasmas deficient in pro-SPCA (factor VII), Stuart factor or proaccelerin, but not in plasmas deficient in Hageman factor, PTA or Christmas factor. However, Christmas factor activity evolved normally in PTA-deficient plasma in which the defect was corrected by adding Hageman factor-deficient plasma (containing PTA) or crude activated PTA. Pro-SPCA-deficient plasma which had not been in contact with glass was adsorbed with aluminum hydroxide gel. An eluate of this gel, containing Christmas factor, Stuart factor and prothrombin, was incubated with

partially purified, activated PTA and calcium. Again, Christmas factor activity increased. Thus, the apparent activation of Christmas factor by activated PTA occurred in plasma or fractions deficient in pro-SPCA, Stuart factor, antihemophilic factor or proaccelerin. It required calcium and proceeded more rapidly at 37° than at 1° C. Neither platelets nor glass-like surface were required. Whether unrecognized intermediate steps are needed to activate Christmas factor is as yet undetermined.

The Mechanism of Renal Chloride Reabsorption. FLOYD C. RECTOR, JR. AND JAMES R. CLAPP, Dallas, Tex. (introduced by Donald W. Seldin).

Renal reabsorption of Cl is thought to be a passive process driven by the electromotive forces generated by active Na reabsorption. The lowest distal tubular Cl concentration ($[Cl]_L$) achievable by passive reabsorption would be determined by the transtubular potential difference (E_T), according to the Nernst equation— $E_T = -61.5 \log \text{plasma } [Cl]_P / [Cl]_L$. To determine whether Cl reabsorption in the distal tubule is an active or a passive process, the relationship between the $[Cl]_P/[Cl]_L$ ratio and distal E_T was studied in normal and Na-depleted rats during Na_2SO_4 diuresis utilizing micropuncture techniques. E_T was measured with glass microelectrodes filled with 3.0 M KCl; [Cl] in plasma and distal tubular fluid was determined with a Ramsay microchloride apparatus, type II. Mean distal E_T was −60 mv (lumen negative) in normal rats and −71 mv in Na-depleted rats. The highest E_T was −120 mv (in Na-depleted rat). The average $[Cl]_P$ was 100 mEq per L. Therefore, if Cl reabsorption were purely passive, the lowest $[Cl]_L$ attainable from the highest measured E_T would be 1.0 mEq per L. The $[Cl]_L$ was measured in 221 instances. Of these, 24 fell below the critical level of 1.0 mEq per L; 8 were between 0.5 and 1.0 mEq per L, 11 were between 0.1 and 0.5 mEq per L and 5 were between 0.01 and 0.1 mEq per L. To reduce $[Cl]_L$ to the lowest measured levels (< 0.1 mEq per L) by a passive process would require E_T ranging from −180 to −240 mv, values considerably higher than any observed E_T. It is concluded, therefore, that Cl is being reabsorbed in the distal tubule against its electrochemical gradient by a process of active ion transport.

Relation of Myocardial Carbohydrate Metabolism and Ion Transport to Contractility. TIMOTHY J. REGAN, PATRICK H. LEHAN, MARTIN J. FRANK, ERNEST N. LANDY AND HARPER K. KELLEMS,* Jersey City, N. J.

Enhanced glucose uptake and metabolism have been postulated as important to the activity of digitalis. In the intact anesthetized dog glucagon-free insulin, 0.2 U per kg, has been used to examine the time course of its effects upon myocardial glucose uptake and metabolism, and net potassium transfer. Direct injection through a left intracoronary catheter minimized changes in arterial glucose, K^+, and catechols. Sequential arteriovenous differences have been employed as a measure of substrate extraction, while the duration of isometric contraction and maximal rate of rise in left ventricular pressure served as indices of myocardial contractility. In 8 animals the maximal insulin effect on myocardial glucose and K^+ uptake, and upon the respiratory quotient occurred within 30 minutes. During this time no change in contractility was observed, implying that the digitalis influence on carbohydrate metabolism is unrelated to its inotropic activity. Although a 25 per cent increment in extracellular potassium during strophanthidin administration does not inhibit the myocardial contractile response, or the net K^+ loss elicited by the drug, insulin's ability to induce uptake of K^+ may alter the response if the ionic egress contributes to inotropism. Strophanthidin, 0.03 mg per kg, was given systemically to 8 animals during the period of insulin's metabolic activity in the myocardium. In each instance the contractile response was prevented or minimized by insulin, when compared to prior or subsequent strophanthidin response in the same animal. While this suggests a critical role of ion transport in strophanthidin's activity, the alternative explanation that insulin competes for common binding sites may be more valid. In preliminary data, *p*-chloromercuribenzoate, given in place of insulin, was found to equally inhibit the contractile response to strophanthidin. In conclusion: 1) the effects of insulin upon glucose transport and oxidation did not change myocardial contractility, so that these effects appear unlikely as essential to digitalis activity. 2) Intracoronary insulin prevents the inotropic response to strophanthidin by interfering with potassium transfer or competing for common binding sites. The latter is favored since *p*-chloromercuribenzoate exerts a similar inhibiting effect.

Effects of a Side-to-Side Portacaval Shunt on Hepatic Hemodynamics. TELFER B. REYNOLDS,* ALLAN G. REDEKER, HARRY S. YAMAHIRO AND WILLIAM P. MIKKELSEN, Los Angeles, Calif.

Pre- and postoperative evaluation of hepatic hemodynamics by hepatic vein catheterization has been performed in 9 cirrhotic patients undergoing side-to-side portacaval shunt for esophageal varices. Preoperative hepatic blood flow (HBF) ranged from 565 to 2,405 ml per minute. There was a postoperative fall in every case, averaging 59 per cent in the group as a whole. Wedged hepatic vein pressure fell 55 per cent (average) and post-sinusoidal hepatic vascular resistance did not change significantly. Splanchnic oxygen consumption fell 37 per cent in spite of consistent increases in arterial-hepatic vein oxygen difference. The fall in HBF after side-to-side shunt was significantly greater than after end-to-side shunt in a comparable group of patients studied previously. This suggests retrograde flow of hepatic arterial blood in the portal vein through the side-to-side shunt into the vena cava. Theoretically, this blood flowing in a retrograde fashion could be

perfusing hepatic tissue functionally. Direct sampling from the hepatic limb of the portal vein at the time of side-to-side shunt suggests that the volume of retrograde flow can vary from 150 to 800 ml per minute, that moderate extraction of oxygen from this blood by the liver may occur, but that sulfobromophthalein extraction is limited. Our data thus far indicate that an end-to-side portacaval shunt is less disturbing to the physiology of the liver than is a side-to-side shunt.

Pneumonia and Hemorrhagic Otitis Media in Volunteers Infected with Eaton Agent. DAVID RIFKIND, ROBERT M. CHANOCK,* HOWARD M. KRAVETZ, KARL M. JOHNSON AND VERNON KNIGHT,* Bethesda, Md.

Second monkey kidney culture passage Eaton agent, originally derived from a patient with primary atypical pneumonia, was given to 34 adult volunteers, 17 without measurable fluorescent stainable antibody and 17 with such antibody. Serologic studies indicated that all of the former group and 13 of the latter group became infected. Eaton agent was recovered in tissue culture from 9 of the 16 volunteers tested. The agent was detected in the throat for varying intervals from the fourth to the tenth day following inoculation of 64 egg infectious doses. Eight of the 17 volunteers without antibody developed illness with objective physical findings; 3 had pneumonia and 6 had hemorrhagic otitis media (one volunteer developed both otitis and pneumonia). Of the remaining 9 volunteers in this group 3 developed minor upper respiratory illness while 6 did not become ill. Otitis developed in one of the volunteers who possessed Eaton antibody prior to challenge; there was no pneumonia, 4 developed mild upper respiratory disease and 12 remained well. Aggregation of illness with physical findings (pneumonia and/or otitis) in the antibody negative group is evidence that such illness was induced by the Eaton agent. The incubation period for mild respiratory illness, otitis, and pneumonia averaged 7, 8, and 11 days, respectively. Nine of 17 without prior antibody developed cold agglutinins while none of 17 in the antibody positive group developed such agglutinins. While streptococcus MG agglutinins developed in 1 patient with pneumonia and in 1 with otitis, there was no correlation between the carrier state for this organism and the development of clinical illness. These findings corroborate a previous observation of association of Eaton agent with naturally occurring atypical pneumonia and additionally suggests that hemorrhagic, nonsuppurative otitis media may be caused by the same agent.

The Determination of Intracellular pH in Normal Human Subjects. EUGENE D. ROBIN,* PHILIP A. BROMBERG AND ROBERT J. WILSON, Pittsburgh, Pa.

There is increasing evidence that under a variety of circumstances, intracellular pH may be independent of extracellular pH. For this reason, a technique for the measurement of intracellular pH in man would be of considerable theoretical and practical interest. Following the suggestion of Waddell and Butler, such a technique has been developed, using the weak acid, 5,5-dimethyl-2,4-oxazolidinedione (DMO), as a pH indicator. From the mass action law it may be shown that $(H^+)i/(H^+)e = (DMO^-)e/(DMO^-)i$. From simultaneous measurements of plasma pH, plasma total DMO concentration, extracellular water (sucrose space), and total body water (antipyrine space), "mean" whole body intracellular pH can be calculated. Such measurements have been performed on 9 normal men. Whole body intracellular pH averaged 6.94 ± 0.09 U at a time when arterial plasma pH averaged 7.44 U and arterial CO_2 tension averaged 31 mm Hg. The reproducibility of individual determinations was 0.04 pH units. Apparent "mean" intracellular HCO_3^- concentration averaged 7.90 ± 1.10 mmoles per L water. Parallel studies have been performed in the dog under control conditions, and following CO_2, $NaHCO_3$, "Tris" buffer, and HCl administration. These studies are consistent with rapid intracellular equilibration of CO_2 and Tris, and relatively slow equilibration of H^+ and HCO_3^-. The use of this technique should be of value in clarifying the disturbances of acid-base balance in various clinical disorders.

Depletion of Thyroid Iodine Induced by Thyrotropin in the Rat. I. N. ROSENBERG* AND C. Y. SASSON, Boston, Mass.

The thyroid content of iodinated amino acids was studied in individual rats before and shortly after thyrotropin (TSH) injection. Rats, which 24 hours earlier had received tracer I^{131}, were given propylthiouracil (10 mg) subcutaneously to minimize further organic binding of iodine; 30 minutes later, right thyroid lobectomy was done (ether), TSH (3 USP U) was then injected intravenously, and 3 hours later the left lobe of the thyroid was removed. After determination of I^{131} concentration, each lobe was digested (pancreatin), and the fraction of the radioactivity present as iodothyronines (T_X), diiodotyrosine (DIT) and monoiodotyrosine (MIT) was determined by quantitive paper chromatography (butanol: acetic acid). In 18 control experiments, only small differences were recorded between right and left lobes in I^{131} concentration and in the relative proportions of the labeled amino acids. In 20 TSH-injected animals, although the average I^{131} concentration of the left lobe was 43 per cent less than that of the right, the proportions of the labeled T_X, DIT and MIT were essentially the same in both lobes. The average concentrations of labeled T_X, DIT and MIT were, respectively, 45, 43 and 43 per cent lower in the left lobe than in the right. From these data and the specific activity of selected portions of the chromatograms (13 rats) the average losses of iodine as T_X, DIT and MIT were calculated to be, respectively, 0.034, 0.065 and 0.045 μg per mg thyroid in the post-TSH period. Specific activity considerations and the propylthiouracil block make it unlikely that disappearance of iodotyrosines could be accounted for by conversion to iodothyronines. The results suggest that under these

experimental conditions a considerable fraction of the glandular iodine released as a consequence of TSH injection may be nonhormonal.

Extra-renal Production of Factor(s) Stimulating Erythropoiesis. WENDELL F. ROSSE AND THOMAS A. WALDMANN, Bethesda, Md. (introduced by Nathaniel I. Berlin).

This investigation sought to determine whether the kidney is the sole source of erythropoietic-stimulating factor(s) (erythropoietin). Forty pairs of parabiotic rats were divided into 4 groups of 10 pairs each and nephrectomy or ureter ligation was performed on 1 of the partners of each pair. They were starved and placed in divided chambers for 16-hour periods on 2 successive days so that one partner could receive a hypoxic gas mixture (9 per cent O_2) and the other, room air at 2 L per minute. Erythropoiesis was measured by determining the per cent incorporation of Fe^{59} into the circulating red blood cells 16 hours following the intraperitoneal injection of the isotope into each of the parabiotic partners. The radioactivity per ml of blood was the same in each partner of a pair. The groups used and the results of Fe^{59} incorporation follow. Group I: anoxia to neither partner, one partner nephrectomized, 7.5 per cent; Group II: anoxia to the nephrectomized partner, 12.5 per cent; Group III: anoxia to the non-nephrectomized partner, 19.7 per cent; Group IV: anoxia to the ureter-ligated partner, 20.2 per cent. The significant differences between Groups II and III ($t \pm 2.53$, p 0.02) and between Groups II and IV ($t \pm 2.17$, p 0.05) indicate that the kidney is an important source of factor(s) controlling erythropoietic response to hypoxia. However, the highly significant difference between Groups II and I ($t \pm 2.98$, $p < 0.01$) suggests that the kidney is not the sole source of this factor(s).

Systemic and Coronary Hemodynamic Effects of an Amine Oxidase Inhibitor (J. B. 516). G. G. ROWE, S. ALFONSO, C. J. CHELIUS, H. P. GURTNER AND C. W. CRUMPTON,* Madison, Wis.

An amine oxidase inhibitor, J. B. 516 (Lakeside Lab.) was tested for its systemic (Fick) and coronary hemodynamic (N_2O method) effects in a series of intact anesthetized mongrel dogs. The compound had no discernible hemodynamic effects. Therefore a series of animals pretreated with J. B. 516 was given serotonin by continuous infusion and systemic and coronary hemodynamic effects measured again. Except for slightly greater tachycardia and somewhat more hypotension in the systemic and pulmonary arteries and in the right atrium, this series showed the same general qualitative and quantitative hemodynamic effects of serotonin as did a series of animals which had not been pretreated with J. B. 516 (i.e., pulmonary arterial pressure rose, coronary vascular resistance fell and coronary blood flow increased with narrowing of the arteriocoronary sinus oxygen difference). The effect of serotonin on J. B. 516-pretreated dogs was also similar to that of serotonin alone when administered into the left ventricle or left atrium of another series of animals except that when it was given into the left side of the heart, the pulmonary arterial pressure did not rise and right atrial pressure decreased more. The series of experiments indicates that under the conditions of these experiments serotonin is not inactivated in the lung as far as its hemodynamic effects are concerned. Administration of J. B. 516 does not change significantly the hemodynamic effects of a continuous infusion of serotonin.

Response of Bronchial Blood Flow to Tissue Proliferation in the Human Lung. THOMAS J. RYAN AND WALTER H. ABELMANN,* Boston, Mass.

If pathologic tissue proliferation requires an increased regional blood flow, proliferative pulmonary disease should be accompanied by an increased bronchial flow. Bronchial flow was measured in 37 patients as left ventricular output ($\dot{Q}_{LV}$) minus right ventricular output ($\dot{Q}_{RV}$) using indicator dilution, injected into the superior vena cava and sampled simultaneously from pulmonary and brachial arteries. Differences are expressed as per cent $\dot{Q}_{LV}$. In 19 control patients without intrinsic lung disease, $\dot{Q}_{RV}$ exceeded $\dot{Q}_{LV}$ by a mean of 6.6 per cent (SD 5.7). In 12 patients with actively proliferating lung disease, $\dot{Q}_{LV}$ was uniformly greater than $\dot{Q}_{RV}$ by a mean value of 10.5 per cent (SD 7.2), which differs significantly from the control group ($p < 0.01$). Pulmonary disease studied included 6 proven primary carcinoma, 2 proven granulomatous disease, 1 lobar pneumonia and 1 lung abscess. The increment of bronchial flow was related to the extent of pulmonary tissue involved in neoplasia as well as the stage of healing in lung abscess. In contrast, 4 patients with pulmonary emphysema or fibrosis, representing chronic lung disease without active tissue proliferation, resembled the control group: $\dot{Q}_{RV}$ uniformly exceeded $\dot{Q}_{LV}$ by a mean value of 9.7 per cent. A subsequent modification of the method involved the injection of two different indicators, separately and in rapid succession, into right and left atria for the inscription of dye dilution curves from the pulmonary and brachial arteries, respectively. In 7 determinations on control subjects, $\dot{Q}_{LV}$ uniformly exceeded $\dot{Q}_{RV}$ by a mean value of 4.0 per cent (range, 0.9 to 7.5 per cent). The present method, while not requiring left heart catheterization, leads to an underestimation of $\dot{Q}_{LV}$ and, hence, of bronchial flow. This is attributed in part to the undetected early recirculation of indicator. The data indicate that active tissue proliferation within the human lung may be associated with a substantial increase in bronchial blood flow.

Reduction in Postprostatectomy Bleeding by ε-Aminocaproic Acid. EDGARDO SACK, THEODORE H. SPAET,* RALPH L. GENTILE AND PERRY B. HUDSON, New York, N. Y.

ε-Aminocaproic acid (EACA) is an inhibitor of plasminogen activation and effective against urokinase.

Its application as a hemostatic agent following prostatic surgery was first suggested by the studies of McNicol and co-workers. Unselected patients undergoing prostatectomy were studied by the double blind method. Either EACA or saline placebo was given in identical manner in random order. EACA was given at the rate of about 5 g every 6 hours by intravenous infusion, and was continued from completion of surgery for a period of about 60 hours. This dose was selected because preliminary studies in normal subjects demonstrated that less gave unreliable urokinase depression. During this period the total urine output was collected for urokinase and hemoglobin determinations. Effective suppression of urokinase activity was achieved in all patients given EACA. Little difference in hemoglobin output between the experimental and control groups was seen in the first 12 hours after operation, but thereafter the differences were striking. The total hemoglobin output was appreciably less in the EACA-treated group and the difference from the control group was highly significant ($p < 0.0001$). The average hemoglobin loss in the control group was about eightfold that of the treated. No evident EACA toxicity was found. An interesting by-product of the study was that perineal prostatectomy was an almost bloodless operation, whereas surgery by the suprapubic route led to considerable blood loss. The effects of EACA were most pronounced in the latter. The present data do not establish whether the hemostatic effect of EACA was by suppression of urokinase or blood lytic activity. If the latter mechanism is more important, EACA may prove of value as a hemostatic following other types of surgery.

Localization of the Renal Tubular Effect of Parathyroid Hormone. A. H. SAMIY, P. F. HIRSCH AND A. G. RAMSAY, Boston, Mass. (introduced by M. W. Ropes).

A series of experiments was performed to study the effect of parathyroid hormone on the excretion of phosphate in parathyroidectomized dogs, utilizing the stop flow technique of Malvin and co-workers, before and after a single intravenous injection of 200 to 400 U of a purified parathyroid extract. Injection of the extract produced a significant phosphaturia without increasing the filtered load of phosphate. The increase in phosphate excretion resulted from a decrease in proximal reabsorption. There was no evidence of a net tubular secretion of phosphate during the free flow clearances and the stop flow periods. The excretion of phosphate did not exceed the filtered load of phosphate in any of the experiments. Furthermore, U/P_{PO_4}: $U/P_{Creat.}$ ratios were less than 1.0 in all of the stop flow experiments. Further experiments were performed to study the effect of the parathyroid extract on the transtubular transport of phosphate. P^{32} was injected with inulin 1 minute before releasing the ureteral clamp during a stop flow experiment. A small net flux of P^{32} into the tubular fluid was demonstrable. The injection of parathyroid extract did not increase significantly the net transtubular flux of P^{32} despite an accompanying increase in phosphate excretion. These studies indicate that the phosphaturic action of the parathyroid extract results from an inhibition of tubular reabsorption of phosphate and not from any significant effect on the secretion of phosphate.

The Effects of "Dry" Heat on the Circulation of Man. V. Coronary Hemodynamics. SALVATORE M. SANCETTA, ELMERICE TRAKS, DONALD B. HACKEL AND BENJAMIN WITTELS, Cleveland, Ohio (introduced by Charles H. Rammelkamp, Jr.).

The deleterious influence of hot weather on cardiac patients is well known, but data relevant to the changes resulting from a short-term "dry" heat exposure in resting man are lacking. The effects of a 2 hour exposure to a relatively dry, hot atmosphere of 40 per cent humidity and 98° F on left ventricular myocardial blood flow (coronary sinus catheterization, nitrous oxide saturation-desaturation) were studied in 24 hospitalized, resting patients, equally divided into three groups: subjects with normal hearts, with enlarged left ventricles but not in failure (normal pulmonary arterial wedge pressure on cardiac catheterization), and with enlarged left ventricles in failure (elevated pulmonary arterial wedge pressure). The baseline determinations were obtained at the comfortable environment of 40 per cent humidity and 73° F. An additional 8 control subjects were studied in the comfortable environment only. The subjects in all three groups showed significant increases in the heart rate and decreases in the intrabrachial arterial pressure. Coronary blood flow and arteriovenous differences decrease variably, though not significantly, when compared with the control patients in whom no change occurred. Coronary vascular resistance and per cent myocardial oxygen extraction did not change. The decreased coronary blood flow was caused by the reduced perfusion pressure and, more importantly, by the decreased work demand of the heart, as evidenced by a significant reduction in left ventricular myocardial oxygen consumption in all three groups. The decrease noted in all groups was also significant when compared with an over-all increase that occurred in the control subjects. This demonstrates that in the absence of restlessness and motor activity, the normal, as well as the diseased, left ventricle may tolerate a short-term exposure to hot dry weather, aside from those situations where a decreased perfusion pressure may be expected to be injurious because of purely physical and hydrostatic considerations.

New Pathway of Steroid Metabolism in Man. AVERY A. SANDBERG,* W. ROY SLAUNWHITE, JR. AND M. NEEMAN, Buffalo, N. Y.

Existing concepts of the metabolism of steroidal 4-ene-3-ones in human subjects indicated that the major pathways involve the following sequential steps: reduction of the unsaturation at positions 4,5 in ring A;

reduction of the 3-keto group; and conjugation of the resulting hydroxyl group, mainly with glucuronic acid. Experiments performed in our laboratory demonstrate a new pathway of steroid metabolism for adrenal steroids: 11β-hydroxyandrostenedione (I) and its 11-keto analog adrenosterone (II) were administered orally or intravenously as tracers labeled with C^{14}, with and without large amounts (1 to 3 g) of carrier steroid. After administration of I or II, the steroid conjugates excreted in the urine were hydrolyzed by enzyme-mild acid, affording a metabolite of low polarity in about 5 per cent yield, which has been proven to be 3,5-androstadiene-11β-ol-17-one (III). Its 11-keto analog was found after administration of II. Since the new metabolite appeared in the urine as a conjugate, its precursor probably was a 4-ene-3α-ol, easily dehydrated during the enzymatic hydrolysis at pH 5. Indeed, hydrolysis of urine at neutral pH with bacterial β-glucuronidase did not afford the 3,5-diene, which was produced by warming the steroid extract with acetic anhydride. Oxidation of an aliquot of the extract with hydrated manganese dioxide produced I. These findings prove unequivocally that the precursor of III is a 4-ene-3α-ol, probably conjugated with glucuronic acid. It thus appears that some steroidal 4-ene-3-ones can first undergo reduction of the 3-ketone group, followed by conjugation, leaving the Δ^4-unsaturation intact. It is possible that this pathway plays an important role in the metabolism of other steroids, or in certain abnormal conditions, though its occurrence may not have been detected due to the instability of allylic 4-ene-3-ols in conventional hydrolytic procedures.

The Hematotoxicity of Amphotericin B. J. P. SANFORD, J. A. PRITCHARD, R. E. WINDOM, R. S. ABERNATHY AND H. G. MUCHMORE, Dallas, Tex., Little Rock, Ark. and Oklahoma City, Okla. (introduced by Gladys J. Fashena).

Amphotericin B is the most effective agent against systemic fungal infections. While its usefulness has been limited by febrile reactions and by nephrotoxicity, hematotoxicity seldom has been recorded and generally is stated not to occur. However, decreases in hemoglobin concentrations of $\geqslant 2.0$ g per cent have been observed in 24 of our 32 patients receiving amphotericin B. This anemia, which is associated with hypoferremia, has not been accompanied by leukopenia or thrombocytopenia. In patients receiving amphotericin B, the anemia is related to therapy and not to the underlying fungal infection, since it develops despite clinical improvement, occurs in individuals with superficial fungus infections who receive amphotericin B, and remits when the drug is stopped. Likewise, the anemia does not seem to be the consequence of azotemia, since it precedes and even may occur without azotemia. These studies were undertaken to ascertain the mechanism of the anemia. Hemodilution, external bleeding, or development of incomplete or drug-dependent circulating antibodies were excluded by appropriate studies. Moderate increases in RBC destruction as measured by Cr^{51}-RBC survival were observed in only 2 of 10 patients; likewise, increases in fecal or urine urobilinogen or in serum bilirubin were not observed. Ferrokinetic studies demonstrated normal or slightly accelerated disappearance of Fe^{59} from plasma and a normal rate of incorporation of transferrin-bound Fe^{59} into RBC. These ferrokinetic data exclude suppression of RBC production of the type noted with chloramphenicol but are compatible with a defect in the transfer of iron from nonviable RBC to the plasma transferrin pool. This block, coupled with unimpaired removal of iron from plasma transferrin, results in hypoferremia. A similar mechanism has been proposed for the anemia associated with turpentine-induced inflammation. Thus, the anemia associated with amphotericin B is postulated to be the consequence of defective reutilization of iron.

Kinetics of Intestinal Absorption in Man: Normal Subjects and Patients with Sprue. HAROLD P. SCHEDL AND JAMES A. CLIFTON, Iowa City, Iowa (introduced by William B. Bean).

Absorption kinetics were studied at various levels by transintestinal intubation. Solutions pumped into the oral end of the tube entered the intestine at a known site; 15 cm distally the perfusate was aspirated and collected from the anal end of the tube. Polyethylene glycol was used as a nonabsorbable indicator. In 4 normal subjects absorption rates of glucose, D(+)-xylose, and cortisol decreased from jejunum to ileum. Absorptions of xylose (jejunal 17 ± 7 and ileal 4 ± 2 per cent per hour per 15 cm gut) and cortisol (jejunal 53 ± 10 and ileal 35 ± 14 per cent per hour per 15 cm gut) were proportional to concentration. The glucose absorption-concentration relation was a rectangular hyperbola. Absorption approached a maximum: jejunum, $V_{max} = 5.6$ g per hour per 15 cm, the Michaelis constant, K_M, was 20 mmoles; ileum, $V_{max} = 3.3$ g per hour per 15 cm, $K_M = 23$ mmoles. In a patient with active sprue the absorption gradient was absent: glucose absorption rate (1 per cent solution) was 1 g per hour per 15 cm, $V_{max} = 1.7$ g per hour per 15 cm, $K_M = 29$ mmoles, in both jejunum and ileum. Cortisol (100 mg per study) restored the absorption gradient and raised the jejunal and ileal V_{max} to 5.0 and 3.7 g per hour per 15 cm, respectively. Absorption rates for 1 per cent solutions were normal: jejunal 4 and ileal 2.5 g per hour per 15 cm. K_M remained 25 to 30 mmoles. On cortisol treatment jejunal cortisol absorption rose from 21 per cent (cortisol-4-C^{14}) to 54 per cent, but ileal absorption was unchanged, 33 vs 35 per cent. Likewise, jejunal xylose absorption rose from 1 to 7 per cent but ileal absorption was unchanged, 3 and 4 per cent vs 2 per cent. After 3 months on a gluten-free diet without steroids, absorption was normal. Another patient with active sprue had jejunal absorption of 0.7 g per hour per 15 cm for 1 per cent glucose, $V_{max} = 1.8$ g per hour per 15 cm, $K_M = 35$ mmoles. A third sprue patient in spontaneous remission had normal glucose absorption.

Steroids did not affect absorption. The K_M, 90 mmoles, differed from that of all other persons studied, including a patient with Whipple's disease.

Detection of Genetic Carriers of Spherocytosis. R. F. SCHILLING,* A. A. MACKINNEY, N. STERNSCHUSS AND N. E. MORTON, Madison, Wis.

Published data concerning this presumably simple dominant hereditary defect indicate a shortage of affected individuals. Possible explanations for this include failure to diagnose mild cases, mutation, and early death of affected persons. To test the possibility that mildly affected persons might better be detected by multiple laboratory tests, we examined 50 normals and 175 members of 26 spherocytotic families. The following were determined: reticulocytes, hemoglobin, blood smear for spherocytes, bilirubin, mechanical fragility, osmotic fragility, and autochemolysis. Using an electronic computer, a discriminant was formed to maximize the intraclass correlations between the normals and 29 patients having classical hereditary spherocytosis. This discriminant was used to classify family members as normal or affected. A physician reviewed the data and diagnosed family members independently. Of 106 members studied with complete discriminant there were three disparities between the "computer classification" and that of the physician. When using a simple discriminant based on hemoglobin, reticulocytes, blood smear, and bilirubin to study 168 family members there were five disparities. Using multiple laboratory tests improves diagnostic accuracy over that of a single test and the family history. The history is a poor discriminant for classifying family members: incidence estimated by history was only two-thirds as large as by laboratory. In completely examined sibships there is no shortage of affected individuals from the expected 0.5 for a dominant trait with high penetrance. In this material there are four isolated probands having normal parents. These may represent mutants or isolated cases of unrelated hemolytic anemia.

The Kinetics of Simultaneously Administered C^{14}-bicarbonate, Na^{24} and T-1824 in Man. ROBERT SCHWARTZ, ROGER C. DEMEUTTER AND WALTON W. SHREEVE, Cleveland, Ohio and Upton, N. Y. (introduced by Frederick C. Robbins).

The metabolism of labeled bicarbonate was studied in order to obtain data on the movement and distribution of $C^{14}O_2$ within the body fluids following the metabolism of a C^{14}-labeled compound. A single injection, containing $HC^{14}O^-_3$, Na^{24} and T-1824, followed by a continuous infusion of the $HC^{14}O^-_3$ for 30 to 60 minutes, was given to each subject (adult diabetics). Arterial and venous blood samples were taken simultaneously from the forearm and breath samples were collected for $C^{14}O_2$ analysis. Circulatory mixing and plasma volumes were determined from the dye dilution curves of T-1824 in plasma. Na^{24} similarly served as an indicator of mixing in the interstitial fluid during the first hour. The time course of the specific activity (SA) of the arterial $C^{14}O_2$ was similar to that of Na^{24} and both described smooth curves which maintained plateaus between 20 and 60 minutes. Breath and arterial $C^{14}O_2$ SA's were identical. Venous Na^{24} and $C^{14}O_2$ SA's increased variably and gradually approached or reached a course parallel to the arterial curves within 20 to 60 minutes. Arteriovenous differences in SA were greater for $C^{14}O_2$ than for Na^{24} during the infusion and cannot be entirely accounted for by metabolic (nonlabeled) CO_2 production. The apparent bicarbonate pool, calculated by integration of the arterial-breath SA curves at intervals during infusion, increased significantly. About one-third of the total administered C^{14} was expired during the infusion period and one-third the following 6 hours. Turnover time ($1.44 \times T_{\frac{1}{2}}$) of bicarbonate calculated from the SA of $C^{14}O_2$ in the breath was about 60 minutes during the first hour after infusion. Thereafter, it increased progressively. These data suggest exchange of circulating bicarbonate with a large pool of organic or inorganic constituents (e.g., possibly urea or bone carbonate) which has a relatively slow turnover.

Evidence for a Renal Tubular Amino Acid Transport System Common to Glycine, L-Proline and Hydroxy-L-proline. CHARLES R. SCRIVER, IRWIN A. SCHAFER AND MARY L. EFRON, Montreal, Canada and Boston, Mass. (introduced by John C. Beck).

An operational definition of amino acid transcellular transport at present must consider factors including Schiff-base formation with pyridoxal-5-phosphate, potassium exchange, energy supply and genetic control. A transport mechanism may be selective for some, and exclusive for other, amino acids; cystinuria is the preeminent example demonstrating a selective, genetically controlled, transport system. There is now evidence for another selective amino acid transport system; it is common to glycine, L-proline and hydroxy-L-proline. The following data support this hypothesis. 1) Intravenous amino acid infusions in 7 normal subjects: Equimolar amounts of L-proline (7 subjects), glycine (3) and hydroxy-L-proline (1) were infused intravenously (3-minute period) on separate occasions. Fasting amino acid clearances were calculated in consecutive control (30 minute) and 3 postinfusion (15, 30 and 30 minute) periods. Quantitative amino acid data were obtained using cation exchange resin elution column chromatography; qualitative confirmation of results was obtained by two-dimensional paper partition chromatography. Infusion of either imino acid in the triad produced significant increments in the renal clearance of the other two amino acids, persisting while the filtered load of the infused amino acid was increased. Glycine produced proline excretion in only one subject. The remainder of the filtered amino acids was unaffected. The transport system exhibited higher affinity for the two imino acids, thus correlating with known fasting clearance rates for glycine (high), proline (low) and hydroxypro-

line (low). 2) Studies in disorders of amino acid metabolism: Urine and plasma amino acid data in *a*) adolescent osteomalacia and renal glycinuria, *b*) Hartnup disease, and *c*) familial hyperprolinemia (newly discovered by the authors) gave supporting evidence for the renal tubular amino acid transport system under discussion. There is therefore strong evidence for a renal tubular transport mechanism common to glycine, L-proline and hydroxy-L-proline; structural and biological properties of these amino acids stimulate interesting speculations about the significance of this observation.

Regulation of Galactose Metabolism in Hemolysates from Normal, Heterozygous and Galactosemic Subjects. STANTON SEGAL, T. DAVID ELDER AND YALE J. TOPPER, Bethesda, Md. (introduced by Wallace P. Rowe).

Progesterone and menthol stimulate the metabolism of a tracer dose of galactose-1-C^{14} in galactosemic subjects by an unknown mechanism. The rate of epimerization of UDP-galactose can regulate the conversion of galactose to CO_2 by normal tissues *in vitro*. The present experiments were undertaken to ascertain whether stimulation of the rate of epimerization and subsequent reactions can accelerate galactose oxidation in systems wherein P-gal-transferase is diminished. Oxidation of galactose-1-C^{14} to $C^{14}O_2$ in hemolysates fortified with excess ATP and TPN has been studied. Stimulation of endogenous epimerase by removal of DPNH, addition of purified epimerase or of phosphoglucomutase to the normal increased galactose oxidation substantially. The same per cent increase in oxidation was observed in hemolysates having a diminished capacity to oxidize galactose due to a reduced P-gal-transferase level (blood from heterozygous subjects or mixtures of normal and galactosemic bloods). This has been demonstrated even in mixtures where the capacity to oxidize galactose was less than 10 per cent of that of the normal hemolysate, and also when the systems were saturated with galactose. No galactose was oxidized to CO_2 by galactosemic hemolysates under any of these conditions. These results demonstrate that transferase present in hemolysates does not function at maximal capacity even when its level imposes a profound limitation on the rate of galactose oxidation. The turnover number of this enzyme can be increased by accelerating removal of the two products of this reaction, UDP-galactose and glucose-1-phosphate. If small amounts of transferase are present in other tissues of galactosemic subjects it is conceivable that this deficient enzyme activity may be increased as a result of stimulation of reactions subsequent to the metabolic block.

A Copper-protein of Human Liver. JAY SHAPIRO, ANATOL G. MORELL AND I. HERBERT SCHEINBERG,* New York, N. Y.

The livers of patients with Wilson's disease contain much more copper than those of control subjects. Although several investigators believe that the hepatic pathology which characterizes this disease is caused by excessive copper, little is known of the nature either of this copper or of the small amount that is normally present. Our investigation of this problem has led to the purification of a copper-protein from the livers of control subjects and of patients with Wilson's disease. Following homogenization of fresh or frozen liver, and ultracentrifugation and dialysis of the supernatant, the solution of the copper-protein is purified by column chromatography using diethylaminoethyl cellulose and hydroxyapatite as adsorbents. The yield of the protein is 2 mg per g of wet normal liver and about 2 to 8 mg per g of wet liver from patients with Wilson's disease. The protein 1) has a molecular weight of about 10,000; 2) is electrophoretically homogeneous at pH 8.6; 3) has essentially the same amino acid analysis whether isolated from patients or control subjects; 4) has a unique ultraviolet absorption spectrum resulting from its copper content; and, 5) exhibits no oxidase activity against paraphenylenediamine, ascorbic acid, dihydroxyphenylalanine or cytochrome C. Copper constitutes, by weight, 0.1 to 0.3 per cent of the protein from livers of control subjects, and up to 3 per cent of the protein from livers of patients with Wilson's disease. This variation in copper content appears to be the only definite difference between the proteins isolated from control subjects or these patients. Finally, the copper in the protein is in the uncommon cuprous form, and is reversibly bound to sulfhydryl groups.

Prevalence of Human Infection with Vesicular Stomatitis Virus. ALEXIS I. SHELOKOV, PAULINE H. PERALTA AND PEDRO GALINDO, Balboa Heights, Canal Zone and Panama City, Republic of Panama (introduced by Joseph E. Smadel).

Vesicular stomatitis (VS), an important disease of farm animals in the western hemisphere, is caused by two distinct virus types: Indiana and New Jersey. Antibodies can be demonstrated in exposed farmers and laboratory personnel. Documented laboratory infections have been associated with influenza-like symptoms. Because of seasonal occurrence of the animal disease, its natural transmission by an arthropod vector has been postulated. During the first 12 months of a 3-year project on the ecology of arthropod-borne viruses in a tropical rain forest, which is being conducted by the Gorgas Memorial Laboratory with the collaboration of the Middle America Research Unit, we isolated two strains of Indiana VS virus from phlebotomus sandflies. Examination of 490 sera from long-time residents of a nearby community disclosed neutralizing antibodies in 27 per cent. Percentage of positive reactors increased from 10 per cent under age 10 to 35 per cent at age 30 and older. Of the 130 neutralizing sera, 28 (22 per cent) were reactive with Indiana VS virus antigen by complement fixation. On sampling of several other Panamanian communities, differences were found in the local prevalence of neutralizing antibodies, ranging from none in the urban to 35 per cent in the forested areas. Prevalence of human antibodies and isolation of the virus from biting arthropods form the basis for a field and labora-

tory investigation of this infection as a public health problem.

Kinetics of Reactions Between a Prothrombin Derivative and Proteolytic Enzyme Inhibitors. N. RAPHAEL SHULMAN * AND JOHN Z. HEARON, Bethesda, Md.

Soybean trypsin inhibitor and an inhibitor separated from plasma are known to act as anticoagulants, but the mechanism and site of their action have been controversial. Kinetic analysis of the effects of varying the concentrations of inhibitor, purified prothrombin and biological activators (thromboplastin, accelerator, Ca^{++}) on the final yield of thrombin indicated that the inhibitors do not react with thrombin or with any substance present in the system before prothrombin conversion occurs. Rather, a complex is formed between inhibitor (I) and a derivative (D) in the pathway of thrombin (T) formation. The complex cannot be reversed by biological activators of prothrombin (P), but I in the D-I complex (C) can be neutralized with an equimolar amount of trypsin which, by virtue of its greater affinity for I, displaces D. The trypsin-I complex thus formed is inert, and the subsequent conversion of regenerated D to T is dependent on biological activators. The following model and equation describe the properties of this unusual form of inhibition in which an intermediate derivative of a proenzyme participates in two essentially irreversible, competitive-rate reactions:

$$P \rightleftharpoons D \begin{cases} \xrightarrow{k} T \\ \xrightarrow[rI]{} C \end{cases}; \qquad C_\infty = I_0 P_0 / [I_0 + (k/r)]$$

where C_∞ is a final concentration and I_0 and P_0 are initial concentrations of reactants, k is a first order rate constant dependent on concentration of activators, and r is a second order rate constant for combination of I and D. The system may operate to control blood coagulation, for low activator activity (low k) which may occur intravascularly would favor C formation and prevent T formation, whereas high activator activity (high k) which occurs extravascularly would favor T formation. Similar kinetics may be applicable in other systems involving activation of enzymes in the presence of inhibitors (e.g., fibrinolytic system).

Treatment of Nontoxic Nodular Goiter with Triiodothyronine: A Double Blind Study. Joseph E. Sokal, KATSUTARO SHIMAOKA, JORGE MADILLO AND FRANK C. MARCHETT, Buffalo, N. Y. (introduced by David K. Miller).

Despite extensive discussion over many years, the management of nontoxic nodular goiter remains a highly controversial subject. Among the points in major dispute is the response of such goiters to thyroid medication. One group has reported that decrease or disappearance of goiter occurred in every one of 36 patients treated with triiodothyronine. Others have stated that treatment with thyroid preparations so rarely results in decrease of goiter that it is virtually useless. In order to obtain unbiased data on this point, we are conducting a double blind study of the effect of 75 μg of triiodothyronine daily on nontoxic nodular goiters. Patients receive active drug or placebo by random allocation and are examined periodically by an internist and a surgeon. Each examiner records his observations privately and neither knows the results of periodic determinations of I^{131} uptake and serum PBI. Whenever possible, each patient serves as his own control, receiving placebo for 3 months and triiodothyronine for 3 months, with a 1-month rest period between courses. To date, 26 patients have completed both triiodothyronine and placebo courses, 10 patients have completed triiodothyronine courses only, and 5 patients have completed placebo courses only. Depression of I^{131} uptake or PBI was seen in three-fourths of the triiodothyronine courses. Side effects such as nervousness, palpitations, tremor, and heat intolerance were seen during both placebo and triiodothyronine treatment. The two examiners usually agreed on the effect of treatment on goiter size. Decrease in goiter occurred during 8 per cent of the placebo courses and 42 per cent of the triiodothyronine courses. Two goiters increased in size during triiodothyronine treatment; one of them was malignant.

The Diagnostic and Prognostic Category, Suspect Coronary Heart Disease. JEREMIAH STAMLER,* HOWARD A. LINDBERG AND DAVID M. BERKSON, Chicago, Ill.

A key problem confronting clinical investigation today is the diagnosis of disease in its early, incipient or suspect stages. This is particularly important when incidence and mortality from frank disease are high and when recent research advances offer the possibility of primary and secondary prevention. These conditions prevail conspicuously today with respect to atherosclerotic coronary heart disease (CHD). The present study, begun on January 1, 1958, involves the long-term follow-up of 1,594 men aged 40 to 59, the entire male labor force of this age employed by a Chicago utility company. Data collected at 1 to 2 year intervals demonstrate high prevalence rates for such coronary risk factors as hypercholesterolemia, hypertension, obesity, heavy smoking, positive family history (singly or in combination). Definite CHD was found in 48 per 1,000 men. The category, suspect CHD, was used to designate men with suspect (but not definite) angina pectoris, with suspect (but not proved) episodes of myocardial infarction or coronary insufficiency, with certain electrocardiographic abnormalities not pathognomonic of definite CHD. Men with suspect CHD have a 2 to 3-fold greater risk of developing frank CHD in middle age than men free of any clinical signs of CHD. The over-all prevalence rate of suspect CHD was high (111 per 1,000), principally due to suspect angina pectoris or ECG findings. The prevalence rate of suspect CHD was significantly higher in hypercholesterolemic than normocholesterolemic, hypertensive than normotensive, obese than nonobese men. In men with combinations of these abnor-

malities, suspect CHD prevalence rate was in the range 151 to 333 per 1,000, with 4 to 8-fold increase in risk compared with normal men. These findings lend support to the concept that suspect CHD is a valid diagnostic and prognostic category. It would seem advisable in men with suspect CHD to institute prompt measures in an attempt to correct risk-increasing abnormalities and to achieve possible prevention of frank CHD.

Insulin Content of Pancreas from Nondiabetic and Diabetic Subjects and from Pancreatic Islet Cell Tumors, as Extracted on Cationic Exchange Resin and with Acid Ethanol. JURGEN STEINKE, ALBERT E. RENOLD,* JURGEN KNAACK AND HARRY N. ANTONIADES, Boston, Mass.

We have previously reported that a portion of the insulin contained in homogenates of pancreas can be adsorbed on cationic exchange resin at physiologic pH, while crystalline insulin remains unadsorbed. This suggests association of insulin in pancreas with cationic protein(s), probably representing the storage form(s) of insulin. Anomaly of this association is one of several possible pancreatic defects in diabetes. Accordingly, pancreatic insulin content has been measured subsequent to *a*) acid ethanol extraction (Scott and Fisher); and *b*) the cationic resin extraction procedure. Insulin was assayed by the $C^{14}O_2$ rat epididymal adipose tissue technique. Acid ethanol extraction of pancreas from 12 nondiabetic patients (mean age, 61 years), 6 growth-onset diabetics (onset between 7 and 17 years), and 7 patients with maturity-onset diabetes (mean age, 73 years) revealed insulin contents of 1.78 ± 0.25; less than 0.1; and 1.12 ± 0.16 U per g. In the same tissue, resin-adsorbable insulin content was 1.82 ± 0.29; less than 0.07; and 1.04 ± 0.21 U per g, respectively. In addition, the pancreatic homogenates contained 14, 13, and 16 per cent resin nonadsorbable insulin. These values agree well with those obtained by Scott and Fisher, and by Wrenshall, using a mouse convulsion assay procedure. The data suggest that in diabetic, as in normal pancreas, most if not all insulin is present in a complex form, associated with basic protein(s). Five islet cell tumors were extracted with resin and shown to contain between 2.1 and 24.1 U insulin per g, with a nonadsorbable component averaging 16 per cent. In one patient the "normal" pancreas adjacent to the tumor contained 0.09 U per g of tissue, a value in the juvenile diabetes range. This patient exhibited severe and prolonged postoperative hyperglycemia.

Estimation of Free Thyroxine Concentration in Serum. KENNETH STERLING,* New York, N. Y.

The demonstration of "free" or "unbound" thyroxine in serum by dialysis through cellophane has recently been reported, with identification of the hormone on chromatographic analysis of the dialysate (Sterling and Tabachnick). Further efforts to measure this minute moiety have led to the development of a technique employing the cation exchange resin Dowex 50W-X8 for the separation of thyroxine and iodide in the dialysate (Sterling, Tabachnick and Hegedus). The validity of the procedure was confirmed by chromatography. In the present work the sera of thyrotoxic subjects were studied by two methods: 1) dialysis of endogenously labeled sera 1 to 7 days after I^{131} therapy, and 2) addition of I^{131}-labeled thyroxine to sera in tracer amounts, such that the increment in thyroxine iodine concentration was 0.2 μg per 100 ml or less, a physiologically insignificant change. The two methods gave similar results; consequently, various other sera were investigated by the second method. The results obtained for free thyroxine concentration were of the order of 10^{-10} M. These values were in reasonable agreement with previous computations by Robbins and Rall and with recent data from this laboratory indicating four thyroxine binding sites on the albumin molecule (lysyl epsilon-amino groups) with an apparent association constant of approximately 10^5. In the various categories studied, the free thyroxine values, expressed as per cent of the total thyroxine content of the sera, were as follows (mean $\pm$ SD): thyrotoxicosis, 0.37 ± 0.09 per cent; euthyroidism, 0.26 ± 0.02 per cent; myxedema, 0.17 ± 0.03 per cent, pregnancy or estrogen administration, 0.16 ± 0.07 per cent. The findings are compatible with the assumption that physiological activity depends upon the unbound hormone.

Metabolic Changes in Normal and Glucose-6-Phosphate Dehydrogenase-Deficient Erythrocytes Induced by Acethylphenylhydrazine. NECHAMA STERNSCHUSS, GRACE A. VANDERHOFF, ERNST R. JAFFÉ AND IRVING M. LONDON,* New York, N. Y.

These studies are concerned with the mechanism by which glucose-6-phosphate dehydrogenase (G6PD) deficiency may result in accelerated destruction of erythrocytes. Washed erythrocytes of normal individuals and of Negro males with G6PD deficiency were incubated (37° C) in phosphate buffer with glucose, 200 mg per 100 ml, and with or without acetylphenylhydrazine (APH), 250 mg per 100 ml. In the presence of APH, the reduced glutathione (GSH) of normal erythrocytes declined from 67 (51–86) to 36 (23–51) mg per 100 ml after 7.5 hours, while the GSH of G6PD-deficient erythrocytes decreased from 46 (33–64) to 3 (0–6) mg per 100 ml after 2.5 to 5 hours. In the presence of APH, a significant difference in glycolysis was demonstrable between normal and G6PD-deficient cells: lactate production was diminished by only 10 per cent in normal cells as compared to 30 to 50 per cent in G6PD-deficient cells after 7.5 hours' incubation. Pyruvate accumulation did not occur in the absence of APH, but occurred in both types of cells in the presence of APH. The accumulation of pyruvate could account for the diminished lactate production in normal but not in G6PD-deficient cells. No diminution in the potential activity of glyceraldehyde-3-phosphate dehydrogenase or of lactic dehydrogenase was

demonstrable by assay of these enzymes. Furthermore, on addition of reduced diphosphopyridine nucleotide (DPNH) to lysates of both types of cells, pyruvate was rapidly converted to lactate. Although methemoglobin and sulfhemoglobin accumulated in the presence of APH, the two types of erythrocytes did not differ in the extent to which their hemoglobin was altered. These findings indicate that 1) APH treatment results not only in a diminution of GSH but also in a deficiency in the amount or availability of DPNH, and 2) that glycolysis as measured by lactate and pyruvate production is diminished in G6PD-deficient cells treated with APH.

Regenerative Macrocytosis in Experimental Phenylhydrazine Anemia. FREDERICK STOHLMAN, JR.* AND GEORGE BRECHER, Bethesda, Md.

The production of severe anemia in the rat by phenylhydrazine is followed by an increase in reticulocytes within 48 hours, reaching a maximum in 4 to 5 days. Five days after phenylhydrazine, reticulocyte values of 90 to 100 per cent are present. These cells are abnormal; the mean MCV is 100 to 110 (normal 55), MCHC 27 to 31; size distribution curves indicate that a significant number of cells have an MCV of as much as 150. The survival of these reticulocytes, measured in irradiated and hypertransfused animals, may be as long as 5 days compared with the normal of 2 to 3 days; the survival of the red cells derived from these reticulocytes, however, is shortened, 40 per cent of them being destroyed by Day 20. Following the peak reticulocytosis, size distribution curves suggest the elimination of the larger cells as a possible explanation for the shortening of the total red cell life span. Continued elevation of MCV (80 to 90) with return of the MCHC to normal after Day 8 argues against shrinkage as the cause for the loss of these larger cells. The early release of large numbers of reticulocytes in response to severe anemia might be explained by a shortened generation time or, more likely, skipped divisions within the erythroid compartment. The continued production of reticulocytes in rats with phenylhydrazine anemia, when given lethal doses of radiation (650 r, which produces complete aplasia in the normal rat), favors but does not prove the differentiation of substantial numbers of stem cells into erythroid elements which mature without dividing.

The Effect of Changes in Diet on Fat Mobilization and Transport in Man. JAMES M. STORMONT AND CHRISTINE WATERHOUSE,* Rochester, N. Y.

Variations in fasting plasma lipid components were induced by changes of diet composition in 9 subjects (3 control, 3 obese, 2 terminal carcinoma, 1 subacute cirrhosis). Each subject was placed on a metabolic ward and three diets were given: *a*) low calorie (700 calories); *b*) high carbohydrate (75 per cent carbohydrate, 1,700 calories); and *c*) high fat (75 per cent fat isocaloric with *b*). After 1 week on a constant diet, subjects were fasted 15 hours and plasma was obtained for measurement of free fatty acid (FFA), triglycerides, cholesterol and cholesterol esters. While fasting FFA concentrations were elevated following low calorie diet (range 0.81 to 2.29 mEq per L), fasting FFA levels fell in all subjects after 1 week of high carbohydrate diet (range 0.30 to 0.94), and rose when fat was substituted isocalorically for dietary carbohydrate (range 0.59 to 1.87 mEq per L). FFA concentrations in patients with cirrhosis and obesity were higher than others. Fasting plasma triglycerides rose in control and obese patients following high carbohydrate diet and fell following high fat diets. In patients with carcinoma and cirrhosis, triglycerides fell following high carbohydrate diets. Cholesterol esters rose slightly in obese patients after high carbohydrate diets but fell in other subjects. A rise in cholesterol esters occurred in all subjects following high fat diets. Oral administration of trace amounts of palmitic acid-1-C^{14} after each diet in a patient with carcinoma revealed no evidence of delayed incorporation of label into triglycerides; however, incorporation into cholesterol and cholesterol esters was delayed following high fat diets. The time course of specific activity for triglyceride and FFA was similar under all dietary conditions. These results further delineate dietary-induced adaptations in fat metabolism, and suggest specific changes in fat mobilization, transport and synthesis with alteration in diet, and with certain disease states.

Autoregulation of Intestinal Blood Flow. E. CLINTON TEXTER, JR., STEVEN MERRILL, MELVIN SCHWARTZ, GUIDO VAN DERSTAPPEN AND FRANCIS J. HADDY,* Chicago, Ill.

It has long been thought that the kidney was unique in its ability to hold its blood flow constant despite fluctuations in arterial pressure over the approximate range 70 to 200 mm Hg. However, Johnson recently reported that intestinal blood flow did not fall as much as expected when arterial pressure was lowered from normal to 40 mm Hg. Since this suggested that the intestinal circulation might also have an efficient local mechanism for regulating its flow, we have examined the relationship of pressure to flow in the intestinal bed of the dog over more extensive ranges and have compared this relationship with those obtained by the same method in the kidney and forelimb. A pump was interposed in the superior mesenteric artery, and pressures were measured in the superior mesenteric artery, a small artery (0.5 mm), a small vein (0.5 mm) and the portal vein as flow was increased over the range 20 to 360 ml per minute. In each of 10 experiments, pressures in the superior mesenteric and small arteries increased more rapidly than flow over the approximate range 90 to 270 ml per minute. Total resistance rose from 0.57 mm Hg per ml per minute at a pressure of 64 mm Hg to 0.76 at a pressure of 205 mm Hg. Corresponding values for resistance to flow through small vessels (less than 0.5 mm) were 0.26 at 39 and 0.53 at 150. These changes are similar to those obtained

in the kidney but differ from those obtained in the forelimb. These findings indicate that autoregulation is equally efficient in the intestinal and renal beds and that the regulation is accomplished by active vasomotion in vessels less than 0.5 mm diameter.

The Distribution of Iron Injected Intraperitoneally: Evidence of Serosal Absorption by the Small Intestine. PHAIROJANA THIRAYOTHIN, Washington, D.C. (introduced by William H. Crosby).

Three days after the intraperitoneal injection of 50 mg of iron as iron-dextran, much iron had accumulated as ferritin in the liver and spleen. (The animals were 200 g albino rats of the WRCF strain. The method of estimating ferritin was a micro-modification of Granick's $CdSO_4$ technique for crystallization of ferritin from tissue homogenates.) Ferritin could also be recovered from the intestine, especially the duodenum and upper jejunum. There was none in the stomach and colon. Ten days after injection ferritin could not be recovered from the gut, though it was present in heavy concentration in liver and spleen. When the iron was injected intravenously or subcutaneously in heavier doses (150 mg) it went to the liver and spleen without preliminary accumulation in the small intestine. In the significant experiment a loop of small intestine at the duodenojejunal junction was brought out of the peritoneum and tacked under the skin. Two weeks were allowed for healing of the wound. Iron-dextran was then injected intraperitoneally and after 3 days the animals were sacrificed. Ferritin was found in the small intestine adjacent to the extraperitoneal loop, but no ferritin was found in that loop. It is concluded that the ferritin which is present in the small intestine following intraperitoneal injection of iron-dextran is derived from iron or iron-dextran which migrates through the serosal surface of the intestine.

The Influence of Arterial Pressure on the Antihypertensive Action of a Normal Kidney, a Biological Servomechanism. LOUIS TOBIAN,* BEVERLY WINN AND JEANETTE JANECEK, Minneapolis, Minn.

An isolated normal kidney was connected to the carotid artery and jugular vein of rats with "Goldblatt" hypertension and was perfused for 3 hours. In one group of 22 hypertensive rats. the arterial connection was made with a short length of polyethylene tubing so that the isolated normal kidney was perfused with elevated pressures (around 203 mm Hg). In this group the blood pressure of the hypertensive rats fell 67 per cent of the way toward normal in 1.75 hours and 90 per cent of the way toward normal in 2.5 hours. In another group of 20 equally hypertensive rats, the arterial connection was made with a long length of tubing so that the isolated normal kidney was perfused with normal pressures (around 90 mm Hg). In this group the blood pressure of the hypertensive rats fell only 15 per cent of the way toward normal in 1.75 hours and only 35 per cent of the way toward normal in 2.5 hours. These differences were highly significant ($p < 0.00005$). In 8 other hypertensive rats, "nephrosclerotic" kidneys were used as the isolated kidney and were perfused with elevated pressures. They were able to bring the blood pressure down toward normal almost as effectively as normal kidneys. Six sham perfusions using a polyethylene arteriovenous fistula without any isolated kidney in the circuit induced only a 10 per cent reduction of arterial pressure toward normal in 1.75 hours and only a 14 per cent reduction in 2.5 hours. In summary, the antihypertensive mechanism of a normal kidney is brought into action when the kidney is perfused with elevated blood pressures. When it is perfused at normal pressures, the antihypertensive action receives little or no stimulation. This appears to be a feed-back arrangement designed to maintain normal arterial pressures.

Patterns of Estriol Conjugates in Body Fluids of the Human Fetus and Newborn. PHILIP TROEN AND EGON DICZFALUSY, Stockholm, Sweden (introduced by Herrman L. Blumgart).

It has been demonstrated previously that the estrogen pattern of cord blood, amniotic fluid and urine of the newborn is dominated by large amounts of conjugated estriol. As reported from this laboratory, there are at least three different conjugated forms of estriol in human body fluids: estriol-3-sulfate; estriol-16 (17?)-glucosiduronate; and another glucosiduronate-like conjugate, perhaps a 3,16-diglucosiduronate (I). A method has now been developed for a separation and estimation of these compounds in cord blood, amniotic fluid and urine of the newborn. In addition, estriol-3-sulfate and estriol-16 (17?)-glucosiduronate have now been isolated and identified in extracts of these body fluids. The pattern of estriol in cord blood is characterized by the preponderance of estriol sulfate; 79 per cent of the total estriol was found in this form. Free estriol was 4 per cent and estriol glucosiduronates were 17 per cent. In contrast, the glucosiduronate fraction constitutes the bulk of estriol both in the amniotic fluid and urine of the newborn. The pattern of estriol in the amniotic fluid was: free, 4 per cent; sulfate, 11 per cent; and glucosiduronate, 85 per cent. The distribution of estriol in the urine of newborn infants was: free, 2 per cent; sulfate, 8 per cent; and glucosiduronate(s), 90 per cent. The principal glucosiduronate obtained from cord blood appears to be the new compound referred to above (I); only a small part of this glucosiduronate fraction was estriol-16 (17?)-glucosiduronate. However, the glucosiduronate fraction obtained from amniotic fluid contained only estriol-16 (17?)-glucosiduronate. The finding that estriol sulfate is the major estrogen in cord blood, whereas estriol glucosiduronate is the principal estrogen of amniotic fluid and urine of the newborn, suggests that transconjugation of estriol takes place in the human fetus and newborn.

The Effect of Experimental Bronchomalacia on Pulmonary Mechanics. G. M. TURINO, R. M. GOLDRING AND L. A. KATZ, New York, N. Y. (introduced by A. P. Fishman).

The role of specific anatomic constituents of lung tissue in the maintenance of normal mechanics of breathing is unknown. The present study concerned the effects of alterations in the rigidity of tracheobronchial cartilage on both the distensibility of the lung and the resistance of the tracheobronchial tree to air flow. For this purpose, cartilage was softened in baby rabbits by the intravenous administration of 2 to 5 ml of a saline extract of crude papain (2 per cent). Pulmonary pressure-volume and pressure-flow relationships were compared before and after papain in unanesthetized animals breathing spontaneously. The techniques involved the simultaneous measurement of intrapleural pressure by direct needle puncture, and tidal volume and rate of air flow by a specially constructed body plethysmograph. An effect of papain on the rigidity of cartilage was manifested clinically by ear drop. An effect on tracheobronchial cartilage was confirmed by 1) abnormal pressure-volume characteristics of isolated tracheal segments and 2) alterations in staining characteristics upon histologic section. Following the administration of papain in 10 test animals, there was no significant difference from the control values either in pulmonary compliance (average 1 ml per cm H_2O, range 0.85 to 1.3) or in airway resistance (average 0.008 cm H_2O per ml per second, range 0.003 to 0.015) during normal breathing. However, the papain-treated animals showed an exaggerated susceptibility to obstruction of the cervical trachea by external compression; this was manifested by abnormally low compliance and high airway resistance. The results indicate that normal rigidity of tracheobronchial cartilage is not essential for patency of large or small pulmonary airways during normal breathing. However, the rigidity of cartilage may prevent airway obstruction during flexion of the neck or extreme variations in intrathoracic pressure.

Human Serum Growth Hormone: Measurements of Concentration and Turnover with a Radioimmunoassay. ROBERT D. UTIGER, MARY L. PARKER AND WILLIAM H. DAUGHADAY,* St. Louis, Mo.

A specific radioimmunoassay of human growth hormone (HGH) has been devised in which HGH competes with HGH-I^{131} for binding by rabbit anti-HGH γ-globulin, similar to the Berson and Yalow insulin assay. Unbound HGH-I^{131} was separated from bound HGH-I^{131} rabbit-antibody complex by goat antirabbit-γ-globulin serum. At present this assay can detect 3 mμg of HGH. Human serum, as well as porcine, ovine and bovine sera, contains a nonspecific inhibitor of HGH-I^{131} precipitation. This can be removed by extracting HGH from serum (Gemzell method). Recovery of millimicrogram amounts of HGH added to serum with each group of unknown sera has varied from 45 to 94 per cent. Correction for this loss has been made. Sera from 21 normal individuals contained less than 60 mμg HGH per ml, while sera from 14 acromegalic patients of varied clinical activity contained 20 to 260 mμg HGH per ml. HGH was injected intravenously in doses of 5 or 10 mg into one hypopituitary and three eupituitary subjects. HGH rapidly disappeared from serum (half-life 22 to 36 minutes) and became undetectable after 5 hours. No HGH was found in the urine. HGH-I^{131} was injected intravenously into two subjects. TCA and immunologically-precipitable I^{131} appeared promptly in the serum and was excreted in the urine. When HGH was given intramuscularly to four subjects, peak serum HGH levels were delayed 3 to 5 hours and were much lower than those obtained following intravenous administration. This radioimmunoassay of extreme sensitivity has demonstrated that HGH circulates in minute concentrations and has a rapid physiologic turnover.

Effect of Feeding Fat to Fasting Subjects. W. P. VANDERLAAN* AND R. R. HALL, La Jolla, Calif.

Although many factors affect the level of circulating fatty acids (FFA), the rise which occurs with fasting is dependent upon the pituitary gland and appears to be a reasonable test for presence of growth hormone. After fasting, the administration of large or small doses of glucose is followed by a prompt decline in FFA and a subsequent return to the previous high level. Albrink and Neuwirth have shown that a fat meal containing only 12 g of protein leads to a similar fall in FFA after 2 to 7 days of fasting but not after overnight fasting. They have interpreted this to indicate that an adaptation to fasting occurs such that a fat meal will lower FFA. We have studied five slightly to grossly obese subjects. After 60 or more hours of fasting, FFA averaged 1,165 μEq per L; 50 g of olive oil was then taken p.o. Two hours later FFA averaged 1,250 μEq per L; 4 hours later, 1,120 μEq per L. Blood sugar levels were unaffected, and ketonemia increased in three subjects. Our previous study suggested that the rise in FFA in the adaptation to fasting was reversed by glucose feeding only so long as blood sugar levels were elevated. This study suggests the specificity of carbohydrate or its precursors in this role.

The Effect of Hydrochlorothiazide on Magnesium Excretion. WARREN E. C. WACKER, Boston, Mass. (introduced by George P. Berry).

The disturbances of neuromuscular function which often accompany the administration of the chlorothiazide drugs have been attributed to the excessive renal loss of potassium. However, in some of these cases, potassium replacement fails to alleviate muscular spasms or cardiac arrhythmias, suggesting additional etiological factors. The intracellular distribution of magnesium, its role in neuromuscular excitation and its capacity to abolish cardiac arrhythmias due to digitalis intoxication, all suggest that this group of diuretic agents may

additionally effect magnesium metabolism. Eight normal persons were maintained on a constant diet throughout the experiment. After a 2-day control period, 200 mg of hydrochlorothiazide was administered on Day 3. The urinary excretion of magnesium during the control period was 180 ± 45 mg per 24 hours; it rose to 280 ± 43 mg during the 24 hours of hydrochlorothiazide administration. The increase in urinary magnesium concentration ranged from 50 to 157 mg per 24 hours and persisted on continued drug administration. Simultaneously, a transient decrease in serum magnesium concentration was observed. Symptomatic magnesium deficiency in man generally occurs as a conditioned deficiency in debilitated patients. These findings now add a drug, hydrochlorothiazide, to the nutritional, hormonal and anatomic conditioning factors already known to bring about the clinical manifestation of magnesium deficiency by an effect on absorption, utilization or excretion of this important intracellular electrolyte. Thus, it seems likely that signs and symptoms arising during the administration of hydrochlorothiazide and which do not respond to therapy with potassium may be alleviated by magnesium replacement therapy. An awareness of this circumstance may further assist to avoid the conditioning of severe magnesium deficiency in patients exhibiting the appropriate metabolic setting.

An Inherited Connective Tissue Syndrome Associated with a Specific Coagulation Defect. ROBERT L. WALL, DANTE SCARPELLI AND WILLIAM MOLNAR, Columbus, Ohio (introduced by Charles A. Doan).

Four generations of an Ohio family have been observed with what appears to be a unique inherited connective tissue disorder. Affected members of this family are of short stature (less than 160 cm). Their long bones are shortened and show multiple gross exostoses. Hemorrhage following surgery, and rarely spontaneously, has occurred from a vascular defect plus a decrease in a specific coagulation component. The vascular defect is a defective elastic fiber showing a total decrease, fragmentation, calcification, and rarely a redundant folding. The coagulation defect is thromboplastin antecedent component. Joint, skin and ocular manifestations have not been observed. The propositus, a 35 year old white female, died of hemorrhage from her pancreatic artery in the absence of pancreatitis 47 days post partum following surgical intervention on four occasions for profuse hemorrhage of pelvic origin. Her surgery was complicated by fragility of tissues to gentle surgical manipulation. Her sister, also in her third decade, bled fatally from her pancreatic artery 9 days post splenectomy for nontraumatic spontaneous rupture of her spleen. The pancreatic arteries of each patient showed decreased, fragmented and calcified elastic fiber. Multiple exostoses have been observed in 5 of 19 adults. Plasma thromboplastin antecedent assays show this deficiency in 18 relatives of the four generations. Genetic transmission is an autosomal dominant. Of the recognized inherited connective tissue syndromes, the present family most closely resembles the Ehler-Danlos syndrome where friability of tissues and unexplained hemorrhages are common, and short stature, while rare, has been described. They differ in the absence of hyperextensibility of joints and hyperelasticity of skin and by the presence of multiple exostoses, plasma thromboplastin antecedent deficiency and deficient elastic fibers of the pancreatic artery, none of which has previously been related to Ehler-Danlos syndrome.

Renal Discrimination between Alkaline Earth Cations. MACKENZIE WALSER,* Baltimore, Md.

The renal clearances of magnesium, calcium, and radiostrontium are highly correlated under a variety of circumstances, suggesting that alkaline earths may be reabsorbed via a common mechanism. This correlation has been examined in detail in dogs, both in daily urine collections while on varying diets and in clearance experiments in which mannitol or saline was infused. Protein binding differed significantly, averaging 27 per cent for radiostrontium, 33 per cent for magnesium, and 42 per cent for calcium. In dogs on normal or low sodium diets, the major determinant of the clearances of all three cations is the simultaneous clearance of sodium. Salt depletion results in markedly reduced clearances despite mannitol diuresis. Saline infusion or chlormerodrin (Neohydrin) injection increases clearances manyfold. The order of magnitude of the renal clearances, corrected for protein binding, is almost always $Sr > Mg > Ca$. The greatest proportional difference is seen at the lowest excretion rates, and is approximately 6 : 3 : 1. At higher rates, the ratios approach 2 : 1.5 : 1. The difference between percentage excretion of filtered radiostrontium and filtered calcium increases rapidly at first with increasing calcium clearance, but reaches a maximum of 20 per cent. Although these relationships were not altered by sodium administration, infusion of either calcium or magnesium salts augmented the clearance of the cation infused more than that of the other, or of sodium. On a magnesium-free diet, considerable calcium retention occurred; magnesium conservation was nearly complete, even after calcium infusion. On a calcium-free diet, however, calcium loss persisted unless magnesium was also removed. Under all conditions, radiostrontium clearance followed the clearance of calcium rather than of magnesium.

The Pathogenesis of Experimental Hepatosplenic Schistosomiasis mansoni. KENNETH S. WARREN, Bethesda, Md. (introduced by Robert S. Gordon).

The pathogenesis of the liver disease of schistosomiasis has not been elucidated. Conflicting theories hold that hepatosplenic schistosomiasis is due either to the presence in the liver of large numbers of eggs produced by the worms or of dead worms causing large areas of necrosis. Concepts of treatment vary widely depending on which

theory is believed. Those accepting the egg theory believe in complete and rapid destruction of the worms, whereas those supporting the dead worm theory feel that schistosomicidal therapy may be detrimental to the patient. Experimental investigations in the past have relied mainly on histopathological studies, often using inappropriate hosts. Recently the development of hepatosplenic schistosomiasis mansoni (HSSM) in mice has been reported by this laboratory. These animals have hepatosplenomegaly, esophageal varices, ascites, anemia, portal hypertension, sulfobromophthalein retention, hyperammonemia and hyperglobulinemia. Using the development of HSSM as a criterion of significant liver disease the influence of eggs and dead worms was studied both individually and in conjunction. Mice (862) were infected with a number of cercariae sufficient to produce HSSM. Treatment (stibophen) killing almost all of the schistosomes was instituted at 5 weeks when the worms were mature in size and oviposition was just beginning, at 7.5 weeks when many eggs were in the liver, and at 10 weeks when HSSM was completely developed in most of the mice. Untreated mice developed HSSM within 10 weeks. The animals treated at 5 weeks had only a mild transient splenomegaly. Moderate splenomegaly, hepatomegaly and portal hypertension in the group treated at 7.5 weeks rapidly returned to normal, and the mice with HSSM treated at 10 weeks recovered completely within 10 weeks following treatment. In conclusion, dead worms in a number sufficient to cause HSSM, if alive and producing eggs, neither produce nor exacerbate the syndrome.

Abnormalities of Erythrocyte and Plasma Lipids in Acanthocytosis. PETER WAYS, CLAUDE F. REED AND DONALD J. HANAHAN, Seattle, Wash. and Rochester, N. Y. (introduced by Cyrus E. Rubin).

Acanthocytosis is a hereditary syndrome manifested clinically by steatorrhea, progressive neurologic disability, and retinitis pigmentosa. Additional findings include vacuolization of intestinal absorptive cells, low levels of plasma lipids, and absence of β-lipoproteins and chylomicrons. The distinguishing feature is the presence of abnormal "spiny" erythrocytes. In this study such "acanthocytes" from 3 afflicted patients were analyzed; abnormalities in erythrocyte membrane lipids were found. First, despite normal quantities of total red cell phospholipids, their distribution was altered: the lecithin fraction was decreased in all three cases to 21.6, 18.6 and 15 per cent of total prospholipid, respectively (normal, 27 to 32 per cent), with concomitant increases in the sphingomyelin fraction to 28.6, 36.2 and 28.5 per cent (normal, 19 to 24 per cent). In addition, gas chromatography revealed a major abnormality in the profile of fatty acids esterified to pospholipid. Linoleic acid, normally comprising 10 to 12 per cent of the fatty acids present in total red cell phospholipid, averaged only 2.2 per cent in the acanthocyte. Percentages of linoleic acid in individual phospholipid fractions from the acanthocyte showed similar relative decreases when compared with corresponding phospholipids from normal cells. Because the lecithin fraction normally contains 75 per cent of all the linoleic acid found in erythrocyte phospholipid, it appears likely that the alterations in its distribution may be directly related to the reduction in linoleic acid. In plasma, lecithin was decreased both relative to sphingomyelin and in absolute terms. Linoleic acid was consistently decreased in all fractions in which it is normally found. In conjunction with the clinical picture, these abnormalities in erythrocyte and plasma lipid suggest that the composition of intestinal and neural structures may be similarly altered in acanthocytosis, and that the primary defect may reside in the absorption and/or metabolism of linoleic acid.

Influence of Flow Properties of Blood Upon Viscosity-hematocrit Relationships. R. E. WELLS, JR. AND E. W. MERRILL, Boston and Cambridge, Mass. (introduced by E. C. Eppinger).

Numerous investigators have shown that blood viscosity, plotted against hematocrit, rises steeply in an upwardly concave curve. It has also been clearly demonstrated (by relatively few workers) that the viscosity of whole blood is dependent on shear rate, decreasing as shear rate rises. Heretofore, connection between viscosity dependence on hematocrit and viscosity dependence on shear rate has been overlooked. Studies of blood viscosity, by instruments capable of measuring point values of shear stress and of shear rate, have been conducted in which the viscosity of whole blood in absolute values at specific shear rates was plotted against values of hematocrit. At low rates of shear (such as may occur in the arterioles) the viscosity rises very rapidly with increase in hematocrit, while at high rates of shear (such as may occur in the aorta) the relationship of viscosity to hematocrit is considerably more linear. The interpretation of data from studies of dynamics of blood circulation cannot, therefore, assume viscosity to be a single value, nor can changes in hematocrit during a series of observations lead to valid extrapolation of viscosity values, unless the values of viscosity are determined at equal shear rates.

Mechanism of the Anabolic Effect of Testosterone. J. D. WILSON, Dallas, Tex. (introduced by Marvin D. Siperstein).

Despite repeated demonstrations of the marked anabolic effect of testosterone, the mechanism by which this action is mediated is poorly understood. We have therefore studied the effect of testosterone on protein synthesis from lysine-C^{14}, tyrosine-C^{14}, and valine-C^{14} in slices of seminal vesicle and prostate. Testosterone administration markedly enhanced protein synthesis in slices from both normal and castrated rats, reaching a maximum of 10 to 20-fold in 48 to 72 hours. The demonstration of this effect *in vitro* makes possible an

evaluation of the biochemical site of testosterone action. Current concepts of protein biosynthesis are as follows: amino acids (AA) $\xrightarrow{1}$ intracellular AA $\xrightarrow{2}$ adenylates $\xrightarrow{3}$ RNA-AA $\xrightarrow{4}$ miocrosomal-ribonucleoprotein (RNP) $\xrightarrow{5}$ protein. The effect of testosterone on this pathway has been examined at several critical sites. Since intracellular transport of amino acids, and synthesis of new amino acids from acetate-1-C^{14} were both normal in these tissues, testosterone cannot act by regulating the level of intracellular amino acids. Indeed, since the rate of formation of RNA-AA was found to be normal in tissues from testosterone-treated animals, the first three steps of protein synthesis can be excluded as significant sites of testosterone action. These findings suggested that one of the final steps in protein synthesis is accelerated by testosterone. Two distinct types of experiment suggest that the site of this testosterone action is located specifically at step 4. Since step 4 is critically inhibted by reduced incubation temperature, the prevention of this effect by testosterone suggested that its site of action is microsomal-RNP synthesis. Moreover, the ability of testosterone to overcome the specific inhibition of microsomal-RNP synthesis by puromycin is added evidence for this locus of action. These studies constitute strong evidence that testosterone exerts its anabolic action by stimulating a specific site in protein synthesis, the conversion of RNA-AA to microsomal-RNP.

Hypercalcemia Simulating Hyperparathyroidism Induced by XV-2 Carcinoma in Rabbits. J. R. WILSON, H. MERRICK AND E. R. WOODWARD, Gainesville, Fla. (introduced by S. P. Martin).

Hypercalcemia has been observed in a variety of human cancers. This investigation was initiated on the possibility that hypercalcemia would be found in the presence of anaplastic carcinoma in the rabbit. Thirteen New Zealand white rabbits received a transplant of XV-2 carcinoma into the anterior thigh muscles. Seven control rabbits were maintained. Blood samples were obtained from 1 to 3 times weekly thereafter and plasma calcium, phosphorus, and blood urea nitrogen were determined. In 11 of the tumor-bearing rabbits there was an increasing degree of hypercalcemia as high as 38 mg% which usually occurred after the third week post implantation. Three animals demonstrated a sustained hypercalcemia with sudden return to control levels within 1 to 2 days prior to death. Plasma phosphorus usually decreased after the third week. Within 1 to 2 days prior to death plasma phosphorus was found at or above control levels in the same animals that demonstrated a rapid decrease in calcium. These same animals showed a progressive increase in BUN as high as 185 mg%. X-ray and histologic study revealed extensive bone resorption, associated with numerous giant cells. Microscopic study of the kidneys revealed marked hyperemia, infiltration with inflammatory cells, and degenerative changes of the proximal tubules with massive proteinaceous exudate. It is concluded that XV-2 carcinoma produces a hyperparathyroid-like state in rabbits, with hypercalcemia, hypophosphatemia, and bony resorption. Three animals apparently developed hypercalcemic nephropathy.

The Acute Hemodynamic Effects of α-Methyl-dopa in Man. WILLIAM R. WILSON, F. DAVID FISHER AND WALTER M. KIRKENDALL, Iowa City, Iowa (introduced by Horace M. Korns).

α-Methyl-3,4-dihydroxy-DL-phenylalanine (α-methyl-dopa) is an inhibitor of several decarboxylases. It has been suggested that its depressor effect in man is related to inhibition of decarboxylation necessary in the reaction, dopa to dopamine. α-Methyl-dopa (0.7 g per m^2) was given intravenously to 5 hypertensives. Observations were made before and at hourly intervals for 3 hours after the drug; identical control observations were made in 3 other hypertensive and 2 normotensive subjects given an equal volume of normal saline. When we compared data from the treated group with those from the controls, the following significant averaged findings were noted: supine intra-arterial mean blood pressure (B.P.) fell 26 mm Hg ($p < 0.02$) in treated hypertensives at 3 hours after the drug, compared with an average drop in the controls of 4 mm Hg ($p < 0.5$); upright B.P. decreased 37 mm Hg ($p < 0.01$) versus 2 mm Hg ($p = 0.9$); pulse rate fell 14 beats per minute ($p < 0.01$) compared with an increase of + 3 ($p < 0.4$); cardiac index declined 0.64 L per minute per m^2 ($p < 0.05$) in the treated group, while it increased 0.06 ($p = 0.9$) in the controls; calculated systemic peripheral resistance decreased in 3 of each group. Valsalva overshoot, tiltback overshoot, arterial hematocrit and oxygen saturation altered in the two groups. Mean pulmonary artery pressure was lowered and oxygen consumption was reduced after the drug. Somnolence was usually observed. The pressor effect seen soon after the intravenous administration of guanethidine and bretylium was not noted. These data support the view that acute blood pressure reduction after α-methyl-dopa is caused chiefly by a decrease in cardiac output. The depressor effect, the long latent period before its appearance, and the bradycardia are consistent with the hypothesis that the drug's hemodynamic effect is caused by partial blockade of peripheral synthesis of catecholamines.

The Relation of Insulin to Metabolic Defects in Adipose Tissue Metabolism in Diabetes. ALBERT I. WINEGRAD AND WALTER N. SHAW, Philadelphia, Pa. (introduced by F. D. W. Lukens).

Alterations in the metabolism of tissues from animals with experimental diabetes are usually attributed to a relative or absolute deficiency of insulin, particularly if insulin corrects the impaired glucose metabolism of these tissues. Alloxan diabetes produces several defects

in the metabolism of adipose tissues which can be demonstrated by *in vitro* experiments using rat epididymal fat pads. The response to insulin varies with the specific abnormality. 1) The impaired glucose oxidation to CO_2 and incorporation into long chain fatty acid are promptly stimulated by insulin *in vitro,* and insulin *in vivo* restores them to normal range within 3 hours. 2) Studies of Krebs' cycle activity demonstrate reduced $C^{14}O_2$ production from acetate-1-C^{14}, pyruvate-2-C^{14}, citrate-1,5-C^{14}, α-ketoglutarate-1,5-C^{14}, and fumarate-2,3-C^{14}, and decreased oxygen uptake by adipose tissue incubated with these substrates. Insulin *in vitro,* in the presence of glucose, has no effect on $C^{14}O_2$ production from these substrates or on oxygen uptake. When insulin is injected *in vivo* and adipose tissue removed at intervals and incubated with glucose, 24 hours is required before one observes a significant increase in oxygen uptake. 3) The increased rate of release of free fatty acid (FFA) by adipose tissue from alloxan diabetic rats incubated with glucose and bovine serum albumin is not affected by insulin *in vitro.* Twelve hours is required before injected insulin reduces the rate of FFA release to normal. Thus, in an "insulin sensitive" tissue some of the metabolic defects resulting from alloxan diabetes are not corrected by insulin *in vitro* and respond very slowly to insulin *in vivo,* the time course resembling that described by Renold for liver slices from these animals. These studies suggest that some defects may not result primarily from insulin deficiency but may reflect other hormonal adjustments.

The Metabolism and Distribtion of Angiotensin II-I^{131}. Robert L. Wolf, Julia Pick, Stanley E. Gitlow and Nosrat E. Naftchi, New York, N. Y. (introduced by Milton Mendlowitz).

I^{131}-labeled angiotensin II (ileu-5-angiotensin II and val-5-angiotensin II) has been employed in a quantitative study of the metabolism of this peptide in humans. Angiotensin II-I^{131} was administered intravenously to 11 normotensive subjects; 7 patients with primary benign hypertension (untreated); and to 1 patient with each of the following conditions: primary benign hypertension (treated), primary accelerated hypertension, secondary renal hypertension, pheochromocytoma before and after surgical removal of the tumor; and 1 patient who had previously had acute glomerulonephritis associated with hypertension. The results indicate a mean space of distribution of angiotensin II in the normotensive subjects of 23 L and in the primary untreated hypertensive patients of 26 L. The mean degradation half-time of angiotensin II in the normotensive controls was 10.3 hours compared with 15.8 hours in the primary untreated hypertensive subjects. The mean total exchangeable angiotensin II was calculated to approximate 0.41 μg in the normotensive controls, 0.99 μg in the subjects with primary untreated hypertension and 9.3 μg in one patient with accelerated hypertension. Unlabeled angiotensin II in 50 μg intravenous doses did not alter the degradation rate of angiotensin II-I^{131} in normotensive and hypertensive human subjects. The degradation of angiotensin II-I^{131} in these experiments, therefore, followed first order kinetics. The angiotensin II degradation rate of the patient who previously had hypertension associated with acute glomerulonephritis was normal. The primary hypertensive subject treated with hydrochlorothiazide with a normal blood pressure, the patient with secondary renal hypertension, and the subject with the pheochromocytoma both before and after surgical removal of the tumor had an angiotensin II degradation rate within the range of untreated patients with primary hypertension. The slow rate of degradation of angiotensin II-I^{131} in the circulation of subjects with untreated primary hypertension is partially if not completely responsible for the increased concentration of angiotensin II in the body fluids of these patients.

Genetic Control of the Ability of Human Blood to Neutralize Angiotensin and its Relationship to Essential Hypertension. J. Edwin Wood,* Augusta, Ga.

Variation in capacity of whole blood to neutralize angiotensin was investigated regarding its role in essential hypertension. Blood (30 ml), 45.0 μg of synthetic angiotensin (Ciba) and 3 mg of heparin were incubated (37° C) for 10 minutes, then infused into the donor at the rate of 1.91 ml per minute. The subject could not observe the onset of infusion. Arterial pressure was measured (auscultatory) by an observer unapprised of the blood pressure of the subject's parents. An increase of diastolic pressure of 10 mm Hg or more during infusion constituted a positive test. The result was not revealed to another observer who measured resting blood pressure of the subject's parents at their home. Parents (ages 41 to 69) with diastolic pressures of 90 mm Hg or more were considered hypertensive (35 per cent). Seventy-one freshman medical students were tested. Twenty-eight families were composed of 2 normotensive parents with 27 offspring having a negative test. Thirty-six families were composed of 1 hypertensive and 1 normotensive parent with 18 offspring having a positive test (predicted 20, $q = 0.80$) and 18 a negative test (predicted 16). Seven families were composed of 2 hypertensive parents with 4 offspring having a positive test and 3 a negative test. All parents in two of these families were tested and were positive. Five offspring demonstrated both positive and negative tests in each family. Ten of 12 patients thought to have essential hypertension had positive tests. Infusion of angiotensin with saline did not separate student subjects with positive and negative tests. Plasma hemoglobin concentration of infused bloods was negligible. These findings imply that a simple dominant gene may lead to essential hypertension through the characteristic of relative inability to destroy naturally occurring angiotensin.

Failure of Formiminoglutamic Acid (FiGlu) Excretion to Distinguish Vitamin B_{12} Deficiency from Nutritional Folic Acid Deficiency. RALPH ZALUSKY AND VICTOR HERBERT, Boston, Mass. (introduced by William B. Castle).

It has been suggested that measurement of FiGlu excretion in the urine following an oral histidine load distinguishes patients with folic acid deficiency from those with vitamin B_{12} deficiency. Studies in rats and chicks have shown that formiminoglutamicaciduria occurred with deficiency of either vitamin. To evaluate this subject in man, 12 patients with megaloblastic anemia were studied. FiGlu was measured qualitatively by paper electrophoresis at 400 v, and quantitatively by the enzymatic-spectrophotometric method and by the colorimetric alkaline ferricyanide nitroprusside reaction. Six patients had low serum vitamin B_{12} levels measured by *Euglena gracilis* (12 to 42 $\mu\mu$g per ml) and normal serum folic acid activity measured by *Lactobacillus casei* (7 to 21.5 mμg per ml). Six patients had low normal to high serum vitamin B_{12} levels (107 to 1,288 $\mu\mu$g per ml) and low serum folic acid activity (<1 to 1.5 mμg per ml). Two of the vitamin B_{12}-deficient patients and all of the folic acid-deficient patients had abnormal FiGlu excretion following 5 to 20 g L-histidine orally. Of the two vitamin B_{12}-deficient patients with abnormal FiGlu excretion, 0.82 mg folic acid intravenously in one, and 15 mg folic acid intramuscularly daily for 2 days in the other, resulted in an increase rather than decrease of FiGlu excretion. Vitamin B_{12} therapy, 5 μg daily for 7 days in one case, and 30 μg daily for 15 days in the other, led to a marked diminution of FiGlu excretion within 10 days. These results indicate that while formiminoglutamicaciduria after histidine-loading occurs in nutritional folic acid deficiency, it may also occur in vitamin B_{12} deficiency. It is suggested that vitamin B_{12} may be required for adequate utilization of folic acid.

The Influence of Thyroid Hormone on Cortisol Production and Metabolism. BARNETT ZUMOFF, LEON HELLMAN,* H. L. BRADLOW, D. K. FUKUSHIMA AND T. F. GALLAGHER, New York, N. Y.

Steroid isolation studies in myxedematous, euthyroid and hyperthyroid subjects have demonstrated that profound alterations in the metabolism and production of cortisol (F) occur in direct relation to the level of thyroid function. 1) Following administration of thyroid hormone or in spontaneous hyperthyroidism there is increased conversion of endogenous F to its 11-ketonic metabolites (tetrahydrocortisone, cortolone, 11-ketoetiocholanolone). In contrast, hypothyroidism or treatment of hyperthyroidism favors the transformation of F to the 11-hydroxy metabolite, tetrahydrocortisol. These findings are the result of changes in peripheral metabolism as demonstrated by parallel tracer studies with cortisol-4-C^{14}. 2) In hyperthyroidism, the total production of F is markedly increased, frequently to levels observed in Cushing's syndrome. Reduction in F secretion is found in hypothyroidism. 3) This modification of the route of metabolism of the carbon-11 position in the corticoid series is a new effect of thyroid hormone and is to be compared with the previous demonstration that thyroid favors the formation of 5-α reduction products (androsterone) in the androgen series. 4) The increased F production in hyperthyroidism appears to be dependent on the augmented formation of the 11-ketonic metabolites; i.e., these represent increased metabolic inactivation of F. The subsequently diminished availability of F results in operation of a negative feedback mechanism for the stimulation of pituitary ACTH production until an additional quantity of F is produced, sufficient to operate the pituitary suppressive mechanism. This is interpreted as evidence for the feedback control of a tropic hormone by alterations in the metabolism of the secretory product of the target organ. 5) This thyroid-induced metabolic acceleration of F inactivation accounts for the failure to develop a typical Cushing's syndrome in hyperthyroidism in spite of the suitably elevated F production and, probably, for the finding of signs and symptoms of adrenal insufficiency in thyroid storm.