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PROCEEDINGS OF THE FIFTY-SIXTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, INC., HELD IN ATLANTIC CITY, N. J., MAY 4, 1964

Presidential Address

The Impact of the Revolution in Biology on Clinical Investigation

IRVING M. LONDON

Since custom demands that I address you this morning, I have considered a number of subjects that might be of interest to you and to which we might direct our attention. As a biologist engaged in the study of man, the clinical investigator is keenly sensitive to the social, political, and intellectual climate of our human society. I was tempted, therefore, to speak of the turbulence of the atmosphere in which we live; of the clash of the world's rival political philosophies; of the revolts of the colored peoples of the world against deprivation and indignity; of the grave medical problems generated by the growth of our population; or of the influences of governmental support for biological and medical research on American medical education. I was tempted too to speculate on the potential implications for medical science of spatial exploration, which has made a reality of what was fantasy only a short time ago. But though these questions are tempting and invite discusion, you may be relieved to know that I finally decided that I might better discuss the impact on clinical investigation of the revolution in biology, a revolution in which clinical medicine is a very active participant.

Biology and the natural sciences related to biology are advancing at an explosive pace. Let me indicate just a few of these advances. The mechanisms of biosynthesis of nucleic acids and of proteins are rapidly being elucidated. The transmission of genetic information from DNA via RNA to proteins is being delineated. The nucleotide code for each of the amino acids is largely established. The determination of the fine structure of the gene and of the colinearity of its structure and of the structure of the proteins whose synthesis it controls is well under way. The genetic control of regulatory processes in cellular metabolism is increasingly understood. The mechanisms of viral invasion and replication and the role of viruses in the etiology of animal tumors are being defined. The physical and chemical properties of macromolecules are being correlated with their biologic function, as, for example, in the determination of the detailed structure of the active sites of enzymes. The functions of structural constituents of cells, such as the endoplasmic reticulum, or the ribosome, or the lysosome, are being revealed. These are but examples of the extraordinary advances that have occurred in recent years. But clinical medicine too has shared in this crescendo of discovery. In the past fifteen years we have savored the recognition of numerous new diseases; we have witnessed the extraordinary growth of human genetics; our understanding in all branches of internal medicine has been greatly enhanced, and there have been monumental achievements in pharmacotherapy and in surgical treatment.

In the advancement of knowledge in the biological sciences, the clinical investigator has an important role to play. For the most part, he plays this role as a member of a Department of Medicine, or of another clinical department, in a university medical school. I hope that you will permit me to say that the Department of Medicine occupies the cardinal position in the medical school and serves as the principal bridge between the basic medical sciences and clinical medicine. With growing recognition of the necessity for maintaining close integration in the teaching of the basic medical sciences and clinical medicine, various curricula have been designed to meet this objective. In the final analysis, however, the teaching of clinical medicine in terms of a deep understanding of the basic medical sciences is best done by physicians who themselves are well trained, both in clinical medicine and in one or more of the basic medical sciences, in other words, by well integrated physician-scientists who are engaged in creative scholarship.

This objective is so easy to state but so difficult to achieve. For of all the problems faced by the clinical investigator, the most critical is the intellectual and emotional challenge of achieving and maintaining both clinical excellence and excellence in medical scientific investigation. It is true now, and it will be increasingly true in the future, that the clinical investigator must be prepared to utilize and to develop the most advanced and sophisticated concepts, methods, and techniques of the basic medical sciences. But he also feels deeply the need to be an expert and knowledgeable physician and teacher of medical students and house officers.

It is obvious that if he is to be successful in the achievement of medical scientific and clinical excellence, the clinical investigator must be intelligent, exceptionally hard-working, and, of equal importance, he must have a deep and genuine interest in clinical medicine. And he also needs just a little bit of luck. With these prerequisites, a program of clinical and scientific training will help to prepare him for the long road ahead. Let me emphasize that there is no unique formula for the edu-

cation and training of a clinical investigator. There are numerous examples of brilliant men who, largely self-taught, have made great contributions in clinical investigation.

The essence of fundamental investigation lies not in whether it is done in a preclinical or in a clinical department, in a laboratory, or on a ward. It is rather the quality of the question which is asked and the quality of the experiment which is designed to answer the question that determine whether research is fundamental in character. But as the medical sciences become increasingly complex and difficult, it is clear that the clinical investigator who hopes to ask and to answer questions of fundamental importance will require advanced scientific training as well as the excellent clinical training offered by our best internship and residency programs.

In seeking to achieve excellence as a clinician and as a scientist, many a clinical investigator feels himself riding two horses, often with a sense of their incoordinate pacing or of his inadequate mastery of them. He feels an enormous challenge to his intellect and to his capacity for hard sustained work. He may find himself frustrated at times, unable to devote himself as fully as he would like to his clinical and teaching responsibilities or to his investigative work.

It is worth noting, therefore, that in meeting the challenges of modern science, the clinical investigator is afforded major opportunities. Careful study of the human organism in health and in disease can provide stimulating leads to an understanding of fundamental physiologic or chemical or physical processes. One need only reflect on such classic examples as the studies on pernicious anemia that led to the discovery of vitamin B_{12} and the mechanism of its absorption; or the observations in a variety of clinical disorders associated with hyperglobulinemia that are leading to important advances in our knowledge of protein structure and synthesis; or the studies in the human endocrine diseases that have provided essential data for an understanding of the physiologic roles of endocrine secretions. A striking example is afforded by the history of studies in sickle cell anemia, which ushered in a whole new era of molecular diseases. Sickle cell anemia was first described by J. B. Herrick in 1910 who noted the peculiar elongated and sickle-shaped red cells in an East Indian medical student in Chicago. In 1927, Hahn and Gillespie, studying a child with sickle cell anemia, indicated that the basis of the sickling phenomenon is an abnormality of the hemoglobin and that with deoxygenation, sickling occurs, whereas the oxygenated cell does not sickle. In 1940, Ham and Castle noted that the viscosity of sickle cell blood was greatly increased on deoxygenation, and Sherman observed that the deoxygenated sickle cell displayed birefringence in polarized light. In a conversation with Pauling, Castle mentioned the likelihood of molecular orientation as a basis for the sickling phenomenon. With these clues, Pauling and Itano in 1949 carried out their brilliant experiment that demonstrated a difference in the electrophoretic behavior of normal human hemoglobin and of sickle hemoglobin. Shortly thereafter Harris demonstrated the formation of sickle-shaped tactoids in stroma-free solutions of deoxygenated sickle hemoglobin. This was clear proof that the abnormal shape of the sickle cell is a function of its hemoglobin and not of the cell membrane. Electrophoretic techniques were quickly employed to study the genetics of this disorder as well as of the other hemoglobinopathies that were soon discovered. And then in 1956 Ingram demonstrated that normal human hemoglobin and sickle hemoglobin differ by only one amino acid residue, the substitution in sickle hemoglobin of valine for glutaminic acid. A single gene defect was shown to be responsible for a single amino acid substitution. These studies, which began with careful and astute clinical observation, have led to major advances in human genetics and in our understanding of the structure and mechanism of synthesis of proteins. In several respects, modern molecular biology has been fathered by clinical medicine.

As clinical investigators, we want to do more than to discover leads, important and crucial as these are. We want to be able to follow these leads in depth and to explore their significance. It is for this further exploration that advanced scientific training is usually essential. Armed with such training, the clinical investigator who is alert to nature's experiments in man can make contributions to medical science that are of major importance. He is doing so now and I am confident that he will do so in the future.

I have spoken until now of the intellectual and emotional impact of the revolution in biology on the clinical investigator. But more important, I believe, is the much broader question of the impact of this scientific revolution on our society and on the contributions of clinical medicine and clinical investigation to our society.

The achievements of modern science and technology and of modern medicine have resulted in the prolongation of the average human life span and in diminished infant and child mortality. The consequent rapid rise in the world's population, though an impressive testimonial to the advancement of science, is nonetheless a cause for much concern because of the economic and political problems that have been created. But for us as clinical investigators there is yet another series of questions with which we must wrestle.

The science of human genetics, which has grown so rapidly in the past two decades, has already revealed an enormous number of human hereditary disorders, and the number appears to increase with every passing day. There are at least 60 disorders linked to the X-chromosome alone and another 20 that are probably linked to it. There are more than 400 autosomal dominant and at least 175 autosomal recessive genetic disorders. If the estimate is correct that man has approximately 40,000 to 50,000 genetic loci, then we have so far recognized genetic substitution or mutation in only about 1% of the total. If we further assume for man a spontaneous mutation rate similar to that which obtains in other higher

forms, then we may expect an appreciable and steady increase in the number of newly created as well as newly recognized genetic disorders.

But the rate of mutation may be further accelerated by environmental factors or mutagens such as chemical agents that are poured into the air we breathe, added to the food we eat, or dumped into the water we drink. And I need not speak of the obvious mutagenicity of ionizing radiation. Clearly there is reason to expect that the rate of genetic mutation in man will increase.

As physicians we have the responsibility of healing the sick and of prolonging life. But we must realize that in genetic and evolutionary terms, such successful prolongation of life may often aid in the propagation of deleterious genes. The very successes of clinical medicine, which may permit the preservation and dissemination of harmful genetic traits, may create grave problems from the standpoint of human evolution. It is obvious that as physicians we shall continue to do our utmost to heal the sick and to prolong life. But I believe that we have an equal responsibility to do all that is possible to render the deleterious gene harmless and to foster favorable progress in man's evolution.

The challenge that is offered to us as clinical investigators is formidable. It involves the recognition of known hereditary disorders and the discovery of new ones; the discovery of the latent asymptomatic carriers of mutant genes; the determination of the nature of the genetic defect, ultimately in terms of the modification in the gene and the modification in the gene product; the determination of the factors in our environment that are beneficial and of those that are harmful to individuals genetically predisposed to human diseases; and the institution of measures designed to prevent the symptomatic expression of the genetic disorder. We may find that the elimination or attenuation of harmful factors in our environment will require major changes in our social and cultural patterns; if we are convinced that such changes are wise and necessary, I hope that we shall accept our social responsibility and shall help bring them to pass.

I should not close without saying a word about our good fortune in being clinical investigators at this point in history. I realize that life in academic medicine is not easy; we seem chronically harried and harassed with too little time to think; the calm contemplative existence we yearn for is often supplanted by committee meetings, project site visits, time sheets, and querulous comments of seemingly unsympathetic editors or obtuse referees; and the wives and children of young investigators are not convinced that academic medicine has been admitted to the affluent society. Granted these irritations, which I hope will be remedied, I feel that we are extraordinarily lucky to be taking part in the greatest leap forward in man's knowledge of living matter that has yet occurred. We live during a glorious era in science, and we are very fortunate indeed that our work is so much fun and is such high adventure. The meetings of these societies testify to the vigorous healthy state of clinical investigation today. I hope that you will all continue to prosper in your work and that you will enjoy your adventure to the full.

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PAPERS PRESENTED AT THE FIFTY-SIXTH ANNUAL MEETING 1964

1. Repopulating Potential of Blood and Marrow. F. E. TROBAUGH, JR., and J. P. LEWIS, Chicago, Ill. (introduced by T. B. Schwartz). (1306)
2. Erythrocyte Membrane Alteration Associated with Marrow Stress. R. S. HILLMAN and E. R. GIBLETT, Seattle, Wash. (introduced by C. A. Finch). (1298)
3. The Mechanism of Action of Erythropoietin. S. B. KRANTZ and E. GOLDWASSER, Chicago, Ill. (introduced by L. O. Jacobson). (1234)
4. Studies on the Mechanism of Action of Aldosterone. G. A. PORTER, R. BOGOROCH, and I. S. EDELMAN,* San Francisco, Calif. (1246)
5. An Aldosterone Biosynthetic Defect in a Salt-wasting Disorder of Infancy. S. ULICK,* K. K. VETTER, E. GAUTIER, G. L. NICOLIS, J. R. MARKELLO, and C. U. LOWE,* New York and Buffalo, N. Y. (1261)
6. Steroids Secreted by the Fetal Adrenal Cortex. W. R. EBERLEIN,* Philadelphia, Pa. (1255)
7. The Source of Energy for Active Sodium Transport in the Toad Urinary Bladder. R. P. DAVIS, M. CANESSA-FISCHER, C. M. EDELMANN, JR., and L. HOFFMAN, New York, N. Y. (introduced by H. A. Eder). (1292)
8. Specificity of Sodium Transport and the Biologically Active Form of Sodium Ion. H. S. FRAZIER, Boston, Mass. (introduced by P. C. Zamecnik). (1265)
9. Evidence for a Common Carrier in the Renal Reabsorption of All Alkali Cations. M. WALSER* and W. J. RAHILL, Baltimore, Md. (1295)
10. Isolation and Characterization of the Long-acting Thyroid Stimulator of Graves' Disease. J. C. MEEK, A. E. Jones, U. J. LEWIS, and W. P. VANDERLAAN,† La Jolla, Calif. (1258)
11. The Carnitine-dependent Distribution of Fatty Acyl CoA's into Cellular Compartments. R. BRESSLER and R. I. KATZ, Durham, N. C. (introduced by W. Nicholson). (1263)
12. The Impairment of Carbohydrate Tolerance by Elevated Plasma Free Fatty Acids. D. S. SCHALCH and D. M. KIPNIS,* St. Louis, Mo. (1283)
13. Black Liver Disease in Corriedale Sheep: A New Mutation Affecting Hepatic Excretory Function. I. M. ARIAS,* L. BERNSTEIN, R. TOFFLER, C. CORNELIUS, A. B. NOVIKOFF, and E. ESSNER, New York, N. Y. (1249)
14. Inhibition of Protein Synthesis: A Mechanism for the Production of Impaired Fat Absorption. S. M. SABESIN, G. D. DRUMMEY, D. M. BUDZ, and K. J. ISSELBACHER,* Boston, Mass. (1281)
15. The Role of Serum Factors in Reticuloendothelial Blockade. M. G. KOENIG, R. M. HEYSSEL, M. A. MELLY, and D. E. ROGERS,* Nashville, Tenn. (1261)
16. Glomerular Lesions Induced by Intravenous Injection of Streptococcal M Protein. F. S. KANTOR, New Haven, Conn. (introduced by E. Atkins). (1251)
17. Immunoglobulins: Clarification of Their Significance in Renal Disease and Demonstration of Response to Immunosuppressive Therapy. A. F. MICHAEL, K. N. DRUMMOND, R. A. GOOD,* and R. L. VERNIER,* Minneapolis, Minn. (1291)
18. Human Antibody to Bence Jones Proteins. W. V. EPSTEIN and D. GROSS, San Francisco, Calif. (introduced by E. Jawetz). (1258)
19. Single Peptide Differences between γ-Globulins of Different Genetic (Gm) Types. E. C. FRANKLIN,* M. Meltzer, H. H. FUDENBERG,* and B. FRANGIONE, New York, N. Y., and San Francisco, Calif. (1268)
20. Genetic Abnormalities in Hereditary Angioneurotic Edema. P. CHARACHE, V. DONALDSON, J. PENSKY, P. FIREMAN, F. S. ROSEN, and C. A. JANEWAY,† Boston, Mass., and Cleveland, Ohio. (1250)
21. Force-Velocity Relations in the Human Heart. E. H. SONNENBLICK, G. GLICK, A. G. MORROW, and E. BRAUNWALD,* Bethesda, Md. (1245)
22. Pacemaker Periodicity in Atrial Fibrillation. E. J. BATTERSBY, Nashville, Tenn. (introduced by E. V. Newman). (1264)
23. Determination of Digitoxin in Plasma by Double Isotope Dilution Derivative Assay. D. S. LUKAS and R. E. PETERSON,* New York, N. Y. (1242)
24. Implication of the Kallikrein System in Production of the Carcinoid Flush. K. MELMON, W. LOVENBERG, J. A. OATES, L. GILLESPIE, JR., and A. SJOERDSMA,* Bethesda, Md., and Nashville, Tenn. (1308)
25. The Role of Connective Tissue in Pulmonary Mechanics. G. M. TURINO,* R. V. LOURENÇO, and G. H. MCCRACKEN, New York, N. Y. (1297)
26. The Response of the Retinal Circulation to Hyperbaric Oxygenation. H. A. SALTZMAN, L. HART, B. ANDERSON, JR., E. DUFFY, and H. O. SIEKER,* Durham, N. C. (1283)

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* ASCI, active member.

† ASCI, emeritus member.

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SECTIONAL MEETINGS

CARDIOVASCULAR

1. Phospholipid Synthesis in Human Blood Vessels. A. V. CHOBANIAN and W. HOLLANDER,* Boston, Mass. AFCR
2. Uptake and Metabolism of Glucose by the Ischemic Myocardium. N. BRACHFELD and J. SCHEUER, New York, N. Y. (introduced by R. F. Watson). ASCI (1301)
3. Interrelationships between Heart Rate and Cardiac Output in Man Studied by Electrical Pacing of the Right Atrium. J. W. LINHART, J. ROSS, JR., and E. BRAUNWALD,* Bethesda, Md. AFCR
4. Reduction of Estimated Hepatic Blood Flow during Mild to Maximal Exercise in Upright Man. L. B. ROWELL, J. R. BLACKMON, and R. A. BRUCE,† Seattle, Wash. ASCI (1307)
5. Left Ventricular Function during Angina Pectoris. L. S. COHEN, W. C. ELLIOTT, and R. GORLIN,* Boston, Mass. AFCR
6. Release of Metaraminol (Aramine) from the Heart by Sympathetic Nerve Stimulation. J. R. CROUT and P. A. SHORE, Dallas, Texas. AFCR
7. Analysis of Sympathetic Activity and Cardiac Norepinephrine Stores in Congestive Heart Failure. C. A. CHIDSEY and A. G. MORROW, Bethesda, Md. AFCR
8. Relationship of Myocardial Metabolism and Hemodynamics to the Etiology of Zonal Lesions in Hemorrhagic Shock. A. M. MARTIN, D. B. HACKEL, H. O. SIEKER,* and M. S. SPACH, Durham, N. C. AFCR
9. The Transmyocardial Temperature Gradient in the Intact Dog. P. L. BLEAKLEY, JR., E. W. REYNOLDS, and P. N. YU, Rochester, N. Y., and Ann Arbor, Mich. AFCR
10. Coronary Vascular Reactivity Following Arterial Versus Arteriolar Obstruction. C. B. MOSCHOS, P. H. LEHAN, H. A. OLDEWURTEL, P. CASANEGRA, and G. KOROXENIDIS, Jersey City, N. J. AFCR

ELECTROLYTES

1. Anaerobic Sodium Transport by the Isolated Turtle Bladder: Dissociation of Glycolysis from Transport. S. KLAHR and N. S. BRICKER,* St. Louis, Mo. ASCI (1277)
2. The Effect of Vasopressin and of Theophylline on the Concentration of Adenosine 3′,5′-Monophosphate in the Intact Urinary Bladder of the Toad. J. S. HANDLER, R. W. BUTCHER, E. W. SUTHERLAND, and J. ORLOFF,* Bethesda, Md., and Nashville, Tenn. ASCI (1297)
3. The Surface Charge of Isolated Toad Bladder Epithelial Cells. R. M. HAYS and K. M. LIPMAN, New York, N. Y. (introduced by Q. B. Deming). ASCI (1272)
4. The Effect of Changes in Boundary Layer Fluids on Osmotic Flow across the Isolated Turtle Bladder. W. A. BRODSKY,* T. P. SCHILB, H. WYSSBROD, and C. GONZALEZ, Louisville, Ky. ASCI (1264)
5. Micropuncture Study of the Effect of Reduced GFR on K and Water Reabsorption in Proximal Tubule of the Dog Kidney. J. F. WATSON, Burlington, Vt. AFCR
6. On the Mechanism of Hyposthenuria in Hypercalcemia. N. BANK, D. J. MARSH, and H. S. AYNEDJIAN, New York, N. Y. AFCR
7. Augmented Intestinal Transport of Calcium in Magnesium Deficiency. D. M. KESSNER, New Haven, Conn. AFCR
8. Metabolic Control of Cell pH. S. ADLER, A. M. ROY, and A. S. RELMAN,* Boston, Mass. ASCI (1251)
9. The Effect of Selective Ionic Restriction on the Development of Gastric Alkalosis. G. LEMIEUX, M. GERVAIS, J. MEYERS, and J. PARENTEAU, Montreal, Quebec, Canada. AFCR
10. Natriuretic Effect of Epinephrine and Norepinephrine: A Study of Its Mechanism. P. J. SPELLER and D. H. P. STREETEN, Syracuse, N. Y. AFCR

ENDOCRINOLOGY

1. Hormonal Control of Free Tyrosine in Thyroid Gland. K. MELMON, J. V. HODGE, and A. SJOERDSMA,* Bethesda, Md. AFCR
2. Decreased Plasma Clearance and Hepatic Extraction of Aldosterone in Patients with Heart Failure. C. A. CAMARGO, E. W. HANCOCK, A. J. DOWDY, and J. A. LUETSCHER,† Palo Alto, Calif. ASCI (1299)
3. Competition between Several Antithyroid Compounds and Iodide for a Common Oxidizing Enzyme System in Thyroid Tissue. F. MALOOF* and M. SOODAK, Waltham and Boston, Mass. ASCI (1292)
4. Effect of Epinephrine on Radiothyroxine Turnover Rate in Euthyroid Men. M. T. HAYS and D. H. SOLOMON, Los Angeles, Calif. AFCR
5. Hypothalamic Regulation of Growth Hormone Secretion. R. L. ABRAMS, M. PARKER, S. BLANCO, S. REICHLIN,* and W. H. DAUGHADAY,* Rochester, N. Y., and St. Louis, Mo. ASCI (1242)
6. Effects of Structural Deletions on the Activity of Lysine Vasopressin. I. L. SCHWARTZ* and L. LIVINGSTON, Cincinnati, Ohio, and Upton, N. Y. ASCI (1267)
7. The Effects of Angiotensin, ACTH, and Potassium upon Steroid Biosynthesis *in Vitro*. N. M. KAPLAN, Dallas, Texas (introduced by G. J. Fashena). ASCI (1288)
8. An Insulinotropic Effect of Corticotropin. S. GENUTH and H. E. LEBOVITZ, Durham, N. C. AFCR

9. The Character of the Aldosterone Response to Changes in Potassium Balance and to Angiotensin: Dependence of Both Effects upon Sodium Balance. P. J. CANNON, R. P. AMES, and J. H. LARAGH,* New York, N. Y. ASCI (1294)
10. Secretion of Cortisol in Acromegaly. M. S. ROGINSKY and N. P. CHRISTY,* New York, N. Y. AFCR

GASTROENTEROLOGY

1. A Disease of Steriod Sterioisomerism: Gallstone Formation in the Cholestanol-fed Rabbit. A. F. HOFMANN and E. H. MOSBACH, New York, N. Y. (introduced by J. Hirsch). ASCI (1257)
2. Pathways of Iron Metabolism in the Intestinal Mucosa and the Regulation of Iron Absorption. J. MANIS and D. SCHACHTER,* New York, N. Y. ASCI (1240)
3. Iron Absorption Kinetic Studies in the Normal Dog. E. W. MOORE, W. G. LINSCHEER, and W. R. KEENE, Boston, Mass. (introduced by J. M. Hayman, Jr.). ASCI (1282)
4. Glucose and Electrolyte Absorption in the Normal Human Small Intestine: Inhibition by Xylose. H. P. SCHEDL, R. B. TALLEY, and J. A. CLIFTON, Iowa City, Iowa (introduced by W. B. Bean). ASCI (1232)
5. Sucrose Absorption in Man: Differential Absorption of Hydrolysis Products. G. M. GRAY and F. J. INGELFINGER,† Boston, Mass. ASCI (1305)
6. Chemical-histologic Demonstration of Nonicteric Hepatitis after Blood Transfusion in High Percentages of Recipients. C. L. HAMPERS, D. PRAGER, and J. R. SENIOR, Philadelphia, Pa. AFCR
7. A Double-blind Study of Gastric Freezing in Duodenal Ulcer. H. ROSE, J. FORDTRAN, and B. FRIEDMAN,† Dallas, Texas. AFCR
8. Isolation, Physicochemical and Physiological Characterization of Gastrin. S. D. TAUBER and L. L. MADISON,* Dallas, Texas. ASCI (1271)
9. The Gastric Secretion of Intrinsic Factor Following Histamine, Histalog, and Methacholine Stimulation. G. H. JEFFRIES, L. L. BENJAMIN, and M. H. SLEISENGER,* New York, N. Y. AFCR
10. The Effects of Antimetabolites on Cell Renewal in the Colon of Man. M. LIPKIN, B. BELL, and P. SHERLOCK, New York, N. Y. (introduced by T. P. Almy). ASCI (1239)

GENETICS

1. Study of a Kindred with a New Inherited Enzymatic Deficiency of Erythrocytes: 6-Phosphogluconic Dehydrogenase (6-PGD) Deficiency. G. J. BREWER and R. J. DERN, Ann Arbor, Mich., and Chicago, Ill. AFCR
2. Hereditary Methemoglobinemia Associated with Severe Mental Retardation. E. R. JAFFÉ,* G. NEUMANN, H. ROTHBERG, F. T. WILSON, and R. M. WEBSTER, New York, N. Y., and Princeton, N. J. AFCR
3. The Effects of Colchicine on RNA Synthesis in Cultured Blood Leukocytes. A. A. SANDBERG,* R. N. HOLDSWORTH, and H. WEINFELD, Buffalo, N. Y. ASCI (1252)
4. Possible Identification of the Chromosome Bearing the Haptoglobin Locus. P. S. GERALD,* S. WARNER, J. D. SINGER, P. A. CORCORAN, and I. UMANSKY, Boston, Mass. ASCI (1297)
5. Cytogenetic Studies in Pernicious Anemia, Megaloblastosis, and the DiGuglielmo Syndrome. K. A. KIOSSOGLOU and W. J. MITUS, Boston, Mass. AFCR
6. Hgb A_4, a Naturally Occurring Hemoglobin Possessing Only α Chains. A. I. CHERNOFF,* Knoxville, Tenn. ASCI (1266)
7. Studies on the Control of Hemoglobins A and F Synthesis in Man. E. R. BURKA and P. A. MARKS,* New York, N. Y. ASCI (1255)
8. Hereditary Resistance to Coumarin Anticoagulant Drugs: The First Reported Kindred. R. A. O'REILLY, P. M. AGGELER,† M. S. HOAG, L. S. LEONG, and M. KROPATKIN, San Francisco and San Jose, Calif. AFCR
9. Studies of Osteopetrosis. C. C. JOHNSTON, JR., T. J. LORD, N. W. LAVY, A. D. MERRITT, and W. P. DEISS, JR.,* Indianapolis, Ind. AFCR
10. Genetic Control of Lactate Dehydrogenase in Testis. A. BLANCO and W. H. ZINKHAM,* Baltimore, Md. ASCI (1270)

HEMATOLOGY

1. Prolongation of Remission in Acute Lymphocytic Leukemia by Alteration in Dose Schedule and Route of Administration of Methotrexate. O. S. SELAWRY and E. FREI III, Buffalo, N. Y., and Bethesda, Md. AFCR
2. The L-Chain Types of Erythrocyte Autoantibodies. J. P. LEDDY and R. F. BAKEMEIER, Rochester, N. Y. (introduced by J. H. Vaughan). ASCI (1300)
3. Effect of Anticoagulant, ABO Incompatibility, and Spleen Size on Recovery and Survival of Transfused Human Platelets. R. H. ASTER, Boston, Mass. AFCR
4. Altered Metabolism of Leukocytes in Down's Syndrome. D. Y. HSIA, T. INOUYE, and P. WONG, Chicago, Ill. AFCR
5. Intracellular Protein Denaturation: An Ultrastructural Analysis. R. A. RIFKIND, New York, N. Y. (introduced by D. F. Tapley). ASCI (1271)
6. Normalization of the Oxyhemoglobin Dissociation Curve in Sickle Cell Disease by Fetal Hemoglobin. P. A. BROMBERG and W. N. JENSEN,* Pittsburgh, Pa. ASCI (1269)
7. Human Erythrocyte Catalase Activity in Iron Deficiency. S. P. BALCERZAK, A. P. DOYLE, and J. W. VESTER, Pittsburgh, Pa. AFCR
8. The Determinants of Red Cell Size. B. LEVENTHAL, J. DONOVAN, and F. STOHLMAN, JR.,* Boston, Mass. AFCR
9. Haptoglobin Binding Capacity of Certain Abnormal Hemoglobins. R. NAGEL and H. M. RANNEY,* New York, N. Y. (introduced by E. E. Gordon). AFCR

10. Membrane Surface Sulfhydryl Groups, Red Cell Survival, and Glucose Transfer. R. I. WEED and J. VAN STEVENINCK, Rochester, N. Y. (introduced by S. N. Swisher). ASCI (1298)

IMMUNOLOGY AND CONNECTIVE TISSUE

1. The Identification of Antigen "E" as the Principal Allergen of Ragweed Pollen. P. S. NORMAN and T. P. KING, Baltimore, Md., and New York, N. Y. (introduced by D. P. Jackson). ASCI (1262)
2. Direct Demonstration of Reagin Activity in Purified $Gamma_{1A}$ Globulin. J. P. VAERMAN, K. ISHIZAKA, W. EPSTEIN, and H. H. FUDENBERG,* San Francisco, Calif., and Denver, Colo. AFCR
3. Characteristics of an Immune System Common to Certain External Secretions. T. B. TOMASI, JR., Burlington, Vt. (introduced by K. J. Thomson). ASCI (1290)
4. Cryoglobulins Behaving as "Cold Autoantibodies." M. GOKCEN, Minneapolis, Minn. AFCR
5. Mechanism of Neuromuscular Block in Myasthenia Gravis. D. GROB,* H. HIMEI, and T. NAMBA, Brooklyn, N. Y. ASCI (1273)
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7. Possible Protective Activity of Antilysosomal Autoantibodies in Patients with Hepatitis. P. A. MIESCHER,* G. WIEDERMANN, R. HIRSCHHORN, and G. WEISSMANN, New York, N. Y. ASCI (1266)
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6. A Cyclical Diuresis from Exposure to Cold. G. A. BRAY, Boston, Mass. AFCR
7. Importance of the Adrenergic Nervous System for Sodium Conservation during Sodium Deprivation in Normal Man. J. R. GILL, JR., and F. C. BARTTER,† Bethesda, Md. ASCI (1272)
8. Use of Radioisotopic Renal Function Studies to Select Patients for Surgery in Renal Arterial Stenosis. M. H. FARMELANT, C. E. SACHS, G. J. HINE, and B. A. BURROWS,* Boston, Mass. ASCI (1308)
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3. Direct Measurements of Human Bone Metabolism in Normal and Hyperparathyroid Subjects. B. Flanagan and G. Nichols, Jr.,* Boston, Mass. AFCR
4. Measurement of Intestinal Absorption of Calcium^{45} in Man by Double Isotope Dilution. C. Rich, J. A. DeGrazia, P. Ivanovich, and H. Fellows, Seattle, Wash. AFCR
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5. CSF—Blood Acid-Base Relationships in Respiratory Insufficiency. J. B. Posner and F. Plum,* New York, N. Y. ASCI (1302)
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ABSTRACTS ‡

Study of Δ^5,3β-Hydroxysteroid Dehydrogenase in Human Adrenal Cortical Tissue. ALLEN S. GOLDMAN, ALFRED M. BONGIOVANNI,* AND WILLIAM C. YAKOVAC, Philadelphia, Pa.

Normal, hyperplastic, and neoplastic adrenal cortical tissues have been assayed for activity of Δ^5,3β-hydroxysteroid dehydrogenase (3β-HSD) and 21- and 11-steroid hydroxylases (21-SH and 11-SH). The *in vitro* activity of each enzyme has been measured by determining amount of steroidal product formed. The activity of 3β-HSD has also been demonstrated histochemically by reduction of Nitro-blue tetrazolium. A selective deficiency of 3β-HSD has been demonstrated both histochemically and *in vitro* in adrenal cortical tissue of patients excreting abnormally high levels of Δ^5,3β-hydroxysteroids. These include the newly described rare type of "salt-losing" adrenogenital syndrome, a virilizing adrenal carcinoma, and several adenomas. The hyperplastic adrenal cortex of the usual form of adrenogenital syndrome with a selective deficiency of 21-SH *in vitro* exhibits diffuse staining of 3β-HSD. A virilizing carcinoma without excess Δ^5,3β-hydroxysteroid excretion shows 21-SH (104 units), 11-SH (8.6), and 3β-HSD (44), and comparable focal staining of 3β-HSD. A recurrence of this carcinoma shows increased activities of 21-SH (183), 11-SH (26.8), and 3β-HSD (470), and more intense 3β-HSD staining throughout the lesion. Staining of 3β-HSD occurs in the adrenal neocortex of the normal fetus of 6 months gestation and of normal neonates and is absent in the fetal zone. In older children staining of 3β-HSD is most intense in the outer half of the cortex and occurs occasionally in scattered areas of the zona reticularis. Each normal tissue studied shows activity of all three enzymes *in vitro*. The studies reveal a good correlation between the two methods and support the validity and specificity of the histochemical method. Postmortem tissues appear to be reliable for the demonstration of these enzymic activities, since 3β-HSD staining is not adversely affected by up to 18 hours delay in freezing the tissues after death of the patient, and *in vitro* demonstration of 3 enzymes by incubation with suitable steroid substrates is possible.

Glucose and Electrolyte Absorption in the Normal Human Small Intestine: Inhibition by Xylose. HAROLD P. SCHEDL, ROBERT B. TALLEY, AND JAMES A. CLIFTON, Iowa City, Iowa (introduced by William B. Bean).

Small intestinal transport of both D(+)glucose and D(+)xylose in the hamster *in vitro* is mediated at least in part by the same carrier systems, since glucose inhibits absorption of xylose and causes it to be countertransported. To examine these relationships in man, we studied the effect of xylose on glucose and electrolyte absorption by the proximal and distal small intestines. Absorption rates were compared between buffered isotonic glucose-Ringer's control solutions and xylose-containing glucose-Ringer's experimental solutions. Solutions were adjusted to equal osmolality and electrolyte content by adding mannitol to control solutions. Absorption was measured as disappearance rate from the bowel lumen using steady-state small intestinal perfusion with the transintestinal tube. In 28 studies xylose inhibited glucose, sodium, and chloride absorption in the proximal small intestine ($p < 0.001$). The trend was for higher proportions of xylose to glucose at a given carrier concentration (estimated from glucose absorption rate from the control solution) to cause greater inhibition. Xylose could not be shown to inhibit glucose or electrolyte transport in the distal small gut. Since glucose and xylose have high proximal and low distal absorption rates, the distal gut was studied at higher substrate to carrier ratios and should have demonstrated inhibition more readily than the proximal. These paradoxical findings must be related to kinetic differences between proximal and distal glucose transport systems. The proximal system has high capacity and operates optimally at high glucose concentrations; the distal mechanism has low capacity and operates optimally at low glucose concentrations. Functionally the differential inhibition by xylose is consistent with low glucose-carrier affinity proximally and high glucose-carrier affinity distally. Transport of glucose, sodium, and chloride is linked, explaining the inhibition of absorption of all three by xylose.

Absorption and Metabolism of Vitamin A in the Rat. DEWITT S. GOODMAN, HELEN S. HUANG, AND TATSUJI SHIRATORI, New York, N. Y. (introduced by Stanley E. Bradley).

Vitamin A alcohol-15-C^{14} was dissolved in 0.3 ml triolein, trilinolein, or a corn oil-olive oil mixture, and was fed by gastric tube to rats with cannulated thoracic ducts. Chyle was collected and the lipid extracted and chromatographed on columns and then on thin layer plates of alumina. Washed chylomicrons contained 80% of the recovered C^{14}. In all samples, 90% of the C^{14} resided in long-chain fatty acid esters of vitamin A. These were separated into saturated, mono- and di-unsaturated fatty acid esters of vitamin A by thin layer chromatography on silver nitrate impregnated alumina, together with carrier vitamin A palmitate, oleate, and linoleate. The saturated esters comprised 70% of the labeled esters, regardless of the composition of the dietary fat. Subsequent reversed phase paper chromatography demonstrated that vitamin A palmitate was the major saturated ester in every sam-

* ASCI, active member.
† ASCI, emeritus member.
‡ Listed in order of receipt.

ple. Washed chylomicrons containing newly absorbed C^{14}-vitamin A were injected intravenously into intact rats, and the tissue distribution of C^{14} was determined at varying time intervals. Recovery of injected lipid-soluble C^{14} in the entire animal varied from 92% after 17 minutes to 56% after 6 days. In every instance the liver contained approximately three-fourths of the recovered C^{14}, predominately (90%) present as vitamin A ester. Saturated fatty acid esters of vitamin A comprised 90% of the labeled liver esters after 3 hours, and vitamin A palmitate was the major ester in all samples. Relatively large amounts of C^{14} were also found in the kidneys, with lesser amounts found in the ten other tissues examined. Only plasma contained the labeled vitamin A almost entirely as the alcohol. From 24 hours on more than two-thirds of the radioactivity in all other tissues examined was present as vitamin A ester, and saturated fatty acid esters of vitamin A predominated in every tissue. Vitamin A palmitate was the major saturated ester in kidney, lung, and intestines, but vitamin A stearate predominated in the adrenals.

Effect of Amino Acid Intake upon Serum Cholesterol in Man. ROBERT E. OLSON,* MILTON Z. NICHAMAN, JUDITH NITTKA, AND LAKELES DORMAN, Pittsburgh, Pa.

It has been shown that low protein diets (25 g per day) containing 36% of calories from relatively saturated fat induce a fall in serum cholesterol of 20% in human subjects (Olson and associates, Amer. J. clin. Nutr. 1958, **6**, 310). Supplementation of this diet with single amino acids produced no consistent response. The effect of various pure 1-amino acid mixtures was, therefore, studied to afford better control of the amino acid intake. For 4 weeks four male subjects were fed a control diet containing 100 g of protein, 36% of calories from fat, and all of the essential nutrients. Linoleate supplied 4% of total calories. Each subject was then given a formula diet containing either all of the 1-amino acids in the same proportions as in the control protein mixture or a diet containing the eight essential amino acids in adequate amounts plus glutamate as a source of nonessential nitrogen for 3 to 4 weeks. The caloric value and fat content of these diets were kept constant. The control diet was refed at the end of the experimental period. We found that when all the amino acids were fed, there was no significant change in serum lipids or beta-lipoproteins from control values. When eight essential amino acids plus glutamate were fed, however, serum cholesterol fell 37 mg per 100 ml, phosphatides 19 mg per 100 ml, and beta-lipoproteins 73 mg per 100 ml. Triglycerides actually increased 49 mg per 100 ml. All subjects remained in nitrogen balance during all experimental periods, showing that the hypolipidemic effect is not due to nitrogen imbalance. These data suggest that the formation of beta-lipoproteins by the liver is a function of the nonessential as well as the essential amino acid nitrogen in the diet.

Pulmonary Venous Responses to Hypoxia. A. L. HYMAN, G. E. BURCH,† AND N. P. DEPASQUALE, New Orleans, La.

There is general agreement that airway hypoxia produces an increase in pulmonary arterial pressure. However, controversy remains concerning the mechanism of the increase in pulmonary arterial pressure with both pre- and postcapillary constriction being postulated as the cause. Using a modification of the transeptal technic, we were able to catheterize small pulmonary veins, 1.5 mm in diameter or less, in intact dogs. Simultaneous recordings of right atrial, pulmonary arterial, small pulmonary venous, left atrial, and peripheral venous pressures were obtained with measurements of cardiac output (CO) and pulmonary blood volume (PBV) during the inhalation of air containing 15%, 10%, and 5% oxygen. Fifteen of 24 dogs had an increase in pulmonary arterial pressure. The increase in pulmonary arterial pressure was associated with an increase in pulmonary venous pressure, a decrease in left atrial pressure, and an increase in total pulmonary resistance (TPR) and pulmonary venous resistance (PVR). There were no changes in CO or PBV. The increase in small pulmonary venous pressure associated with a decrease in left atrial pressure with no change in CO or PBV must have been due to active constriction of the small pulmonary veins. Fractionation of TPR into prevenous and venous resistances indicated that the increase in PVR accounted for the entire increase in TPR during hypoxia. We conclude that in these studies pulmonary hypertension during hypoxia was due primarily to active constriction of the pulmonary veins.

A General Method of Catheter Correction: Its Effect on Calculated Hepatic Sinusoidal Blood Volumes. CARL A. GORESKY AND MELVIN SILVERMAN, Montreal, Quebec, Canada (introduced by Francis P. Chinard).

The use of a collecting system to obtain samples in the multiple indicator dilution technique produces delay in and distortion of the dilution curves. If the response of the collecting system alone to a step concentration input function is determined experimentally, the transfer characteristic of the system may be ascertained. An analog computer may then be utilized to remove the delay and distortion introduced by the transfer characteristic of the collecting system. This method is applied to previously published hepatic venous dilution curves (Amer. J. Physiol. 1963, **204**, 626). In each case, the corrected curve rises more sharply, reaches a higher and earlier peak, and decays more rapidly than the observed curve. The correction increases with the rate of change of concentration and so is greatest for labeled red cell dilution curves and least for labeled water dilution curves. The effect of the distortion on the linear method for determining liver sinusoidal and extravascular volumes for these dilution curves has been determined. Extravascular volumes of distribution of rapidly diffusible completely recovered indicators (i.e., transit time volumes) are found to be unaffected, whereas catheter distortion is found,

with the collection system used, to produce an increase in estimated sinusoidal blood volume of an average magnitude of 15%. These results suggest that the value obtained after removal of distortion of the collecting system is still in error by approximately the same order of magnitude because of the effect of portal and hepatic veins. The distorting effect of large nonexchanging vessels on indicator dilution curves precludes obtaining an accurate estimate of capillary blood volume from the multiple indicator dilution technique in any organ.

Lipid Metabolism in the Diabetic Rat Heart. J. R. EVANS AND C. H. HOLLENBERG, Toronto, Ontario, and Montreal, Quebec, Canada (introduced by Douglas G. Cameron).

Hearts from untreated alloxan-diabetic rats were perfused in a recirculation system with a variety of nonesterified fatty acids bound to albumin in bicarbonate buffer. The relative myocardial extraction and utilization of different fatty acids presented either individually or in equimolar mixtures followed the pattern previously observed in hearts from normal rats. With saturated fatty acids of increasing chain length, extraction and oxidation decreased, and incorporation into tissue lipid increased. Among the 18-carbon fatty acids, extraction and oxidation of oleic and linoleic acid were greater and tissue lipid formation less than with stearic acid. However, the principal fates of each of the C^{14}-labeled fatty acids extracted by diabetic hearts differed markedly from normal. Incorporation of label into triglyceride increased twofold, whereas other fractions of tissue lipid showed only minor changes. Less label was recovered in CO_2, and respiration of endogenous lipid appeared to account for a larger fraction of total CO_2 production by the isolated heart. Insulin pretreatment of alloxan-diabetic rats for 3 days reverted the pattern of fatty acid metabolism in the isolated heart to normal, but addition of 0.1 U per ml insulin directly to the perfusate was without effect. Tissue triglyceride labeled during preliminary perfusion of the diabetic heart with palmitate-C^{14} was used to support respiration in a subsequent perfusion without exogenous substrate. We conclude that in the isolated heart, the diabetic state does not affect the relative utilization of fatty acids of differing chain length and saturation but does alter the fate of fatty acids which are extracted. The increased incorporation of exogenous fatty acid into triglyceride together with the greater use of preformed tissue lipid for respiration suggests that the turnover of tissue triglyceride is accelerated in hearts from diabetic animals.

The Detection of Hereditary Angioneurotic Edema and Certain Immunologic Reactions of Man by Specific Titration of the Second Component of Human Complement. K. FRANK AUSTEN, ALBERT L. SHEFFER, AND J. LYNDA SEYMOUR, Boston, Mass. (introduced by Morton N. Swartz).

The concentration of second component of complement should be a more sensitive index of antigen-antibody interaction than the whole complement titer (CH_{50}) and can be assayed in human serum by its specific interaction with a cellular intermediate prepared with guinea pig complement. In this interaction the conversion of $SAC'1a^{gp},4^{gp}$ sites (sensitized sites on sheep erythrocytes carrying first and fourth components of guinea pig complement) to $SAC'1a^{gp},4^{gp},2^{hu}$ sites is stoichometrically related to the relative concentration of second component ($C'2^{hu}$) in the human serum examined. The titration is linear even when the $C'2^{hu}$ titer is as low as 1% or as high as 200% of normal. The fact that the CH_{50} titer need not reflect the extent of complement utilization was documented in patients with a reduced $C'2^{hu}$ titer and a normal CH_{50} value. The $C'2^{hu}$ titer was measured in three general clinical situations: hereditary angioneurotic edema, Coombs positive hemolytic anemia or poststreptococcal nephritis, and renal homograft rejection. Sera of patients with hereditary angioneurotic edema lack the inhibitor of the first component of complement, C'1a esterase (Donaldson and Evans, Amer. J. Med. 1963, 35, 37), leaving $C'2^{hu}$, a natural substrate of C'1a esterase, unprotected. Three families with angioneurotic edema of the hereditary type were detected because sera from the proband was severely depleted of $C'2^{hu}$; the diagnosis was confirmed by Donaldson, who demonstrated the lack of C'1a esterase inhibitor. The relationship of the $C'2^{hu}$ titer to the attacks of angioedema was determined. In two patients with renal homografts, the $C'2^{hu}$ titers were significantly diminished in association with actual or threatened rejection. The specific titration of $C'2^{hu}$ not only offers a simple method of differentiating hereditary angioneurotic edema from other types but also can be more sensitive than the CH_{50} assay in detecting immunologic reactions in man.

The Mechanism of Action of Erythropoietin. SANFORD B. KRANTZ AND EUGENE GOLDWASSER, Chicago, Ill. (introduced by Leon O. Jacobson).

We have recently reported on an *in vitro* marrow culture system that responds to erythropoietin with a marked increase in hemoglobin production. This system can provide an *in vitro* assay method for erythropoietin and also serve for the study of the mechanism of action of the hormone. Since erythropoietin is believed to act on stem cells to initiate differentiation into normoblasts, and since the formation of a new protein such as hemoglobin requires messenger RNA synthesis, we examined the action of erythropoietin on RNA production. Pulse-labeling experiments with uridine-2-C^{14} demonstrated increased RNA synthesis in erythropoietin-treated cells by 4 hours after addition of the hormone. Trypsin treatment of the erythropoietin inactivated it as is the case *in vivo*. After the RNA was extracted by the phenol technique and centrifuged in a sucrose density gradient, the 12 to 24 S component showed increased radioactivity when erythropoietin was present. This effect could be seen as early as 15 minutes after addition of the hormone. The

labeling in this particular RNA was inhibited by the presence of actinomycin D, demonstrating its dependence on DNA for synthesis. The early and rapid synthesis, actinomycin sensitivity, and position in the sucrose gradient indicate that this is messenger RNA and that the primary mechanism of action of erythropoietin proceeds through the regulation of its formation.

Spurious Signs of Cardiorespiratory Disease in Obesity and Ascites: Obligatory Venous Hypertension and Respiratory Arterial Pressure Variation. RAMON L. LANGE, JAMES T. BOTTICELLI, AND THEOFILOS T. TSAGARIS, Milwaukee, Wis. (introduced by William W. Engstrom).

Wide variation in central venous and arterial pressure with quiet respiration or elevation of mean venous pressure is ordinarily evidence of myocardial, pericardial, or respiratory disease. Obesity and ascites may evoke spurious signs of disease because of obligatory central venous hypertension and altered venous return during respiration. Twelve patients with obesity or ascites exhibited unusual patterns of venous and arterial pressure despite normal cardiorespiratory function. Inspiratory (I), expiratory (E), and average (Av) values for right atrial (RA), extrathoracic superior caval (SVC) and inferior caval (IVC), pulmonary arterial (PA), pulmonary arterial wedge (PAW), and systemic arterial (SA) pressures were measured during quiet breathing. Although all RA tracings showed abnormally wide respiratory variation with sample means ($I = -1.3$, $E = 12.3$, and $Av = 7.33$ mm Hg), obesity caused the most striking variation: $I = 0.1$, $E = 18$, and $Av = 15$ mm Hg. Mean PAW pressures were these: $I = 0.2$, $E = 18$, and $Av = 15$ mm Hg. On inspiration the mean SA pressure decrease was 15 mm Hg systolic and 7 mm Hg diastolic with similar changes in the PA. The large and abrupt drop in inspiratory intrathoracic venous pressure disappeared during catheter transversal of short extrathoracic IVC segments with values of $I = 18.5$ and 11.6, $E = 16$ and 10.6, and $Av = 17.5$ and 10.7 mm Hg in obesity and ascites, respectively. SVC tributaries showed a damped RA pressure (ascites) or resembled IVC pressures (obesity). At times a 60° tilt abolished the inspiratory IVS to RA gradient and the SA pressure variation in ascites. Inspiration apparently depletes venous volume adjacent to the thoracic cavity with a local drop in transmural pressure and venous collapse (due to high extravascular pressure). Inspiratory increase in venous return is prevented despite high intravascular pressure gradients (waterfall phenomenon). Low resistance flow is possible only when RA, IVC, and SVC pressures exceed extrathoracic extravascular pressures. Normal increase in pulmonary vascular compliance on inspiration without augmented venous return causes reduced LV filling and decreased arterial pressure. Such functional restriction of venous return produces misleading signs of cardiorespiratory disease.

Abnormal Erythrocyte Cholesterol Accumulation in Spur Cell Anemia. KENNETH STERLING,* HERBERT G. ROSE, JEANNE ALLEN SMITH, AND EDMUND T. LONERGAN, New York, N. Y.

A unique morphological abnormality of erythrocytes, consisting of spur-like projections, has been observed in a young man with cirrhosis and severe hemolytic anemia. The spur cell abnormality could be induced by incubation of normal erythrocytes with the patient's plasma. Because of presumed disorder of the cell membrane, studies of the erythrocyte lipids were undertaken. The cholesterol content in spur cell anemia erythrocytes was above normal (118% to 230% of the normal mean). Normal values were obtained for lecithin, sphingomyelin, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, and cardiolipin. Incubation of normal red cells with the patient's plasma resulted in marked increase of cholesterol content but not of the other lipids. The influx of 4-C^{14}-cholesterol from plasma into normal erythrocytes was greater with the patient's plasma than with normal plasma. Increased erythrocyte cholesterol has been reported in jaundice of various types and in malignancies without marked hemolytic anemia; therefore, the increased erythrocyte cholesterol in the present case was not considered to play a causal role in the hematologic abnormalities. We believe that probably some factor in the patient's plasma produced an injury to red cells causing the spur formation, the susceptibility to hemolysis, and the abnormal accumulation of cholesterol.

The Activity of Glyceraldehyde-3-Phosphate Dehydrogenase in Hemolysates of Normal and Glucose-6-Phosphate Dehydrogenase-deficient Erythrocytes. ROBIN D. POWELL, GEORGE J. BREWER, RICHARD L. DEGOWIN, AND ALF S. ALVING,† Chicago, Ill.

The mechanism of drug-induced lysis of glucose-6-phosphate dehydrogenase(G6PD)-deficient erythrocytes is not completely understood, but considerable evidence implicates the oxidant properties of hemolytic agents. The cellular content of reduced glutathione (GSH) decreases abruptly just before the major hemolytic episode that results from administration of primaquine or certain other drugs. It has been suggested *1*) that GSH may protect key sulfhydryl-dependent enzymes, such as glyceraldehyde-3-phosphate dehydrogenase (GA3PD), against oxidative damage and *2*) that oxidative destruction of GA3PD, coincident with the decreased cellular content of GSH, may play a role in primaquine-type hemolysis. We administered 30 mg of primaquine daily to each of four G6PD-deficient Negro men. The content of GSH in erythrocytes decreased markedly, and acute hemolysis ensued; during this period there was no diminution in activity of GA3PD in hemolysates. Primaquine preferentially destroys older erythrocytes of G6PD-deficient Negro men. Activity of G6PD in circulating normal and G6PD-deficient erythrocytes apparently decreases considerably as the cells age *in vivo*. On the basis of unconfirmed studies of normal erythrocytes indicating that activity of GA3PD decreases even more

markedly during aging than does activity of G6PD, it has been suggested that diminished activity of GA3PD may be important in the destruction of aged erythrocytes. We studied activities of G6PD and GA3PD during reticulocytosis after primaquine-induced hemolysis and after using differential centrifugation and graded osmotic lysis to separate both normal and G6PD-deficient erythrocytes according to age. Results indicate that as normal and G6PD-deficient erythrocytes age *in vivo,* activity in G6PD decreases markedly, whereas activity of GA3PD decreases only slightly. We conclude that the data do not support the concept that oxidative destruction of GA3PD plays a role in primaquine-type hemolysis. The findings indicate that there is no marked decrease in activity of GA3PD during aging of either normal or G6PD-deficient erythrocytes.

A New and Simple Test of Calcium Absorption. LOUIS V. AVIOLI, RICHARD SINGER, JOSEPH E. MCDONALD, AND PHILIP H. HENNEMAN,* Jersey City, N. J.

In the past, evaluation of calcium absorption has been limited by the requirement of 18 to 24 days of balance study. A simple new test has been evaluated in 26 normal subjects, in 23 patients with known malabsorption, and in 24 patients with known hyperabsorption. After an overnight fast, 5 to 10 μc $Ca^{47}Cl_2$ in 20 mg carrier $CaCl_2$ was administered orally, and plasma levels of radioactivity were measured over the next 4 hours. In normal subjects plasma radioactivity rose to a peak of $1.69 \pm 0.05\%$ of dose per L plasma at 1 hour and declined thereafter. A similar pattern was seen in patients with known hyperabsorption, but peak plasma radioactivity (2.85 ± 0.05, $p < 0.001$) at 1 hour was increased. These values became normal with appropriate therapy. In the malabsorption patients 1-hour plasma radioactivity was reduced (0.74 ± 0.07, $p < 0.001$), and peak levels were not reached until 2 to 3 hours. Correction of malabsorption restored the Ca^{47} absorption pattern to normal. Vitamin D therapy produced the expected increase in Ca^{47} absorption. No constant relationship between serum calcium and Ca^{47} absorption was noted. Reproducibility of test results was excellent. Although Ca^{47} absorption was similar in Negro and white males, it was 2 to 3 times greater in Negro females than in white females. We conclude that calcium absorption may be measured in 4 hours with a simple, safe, reproducible oral Ca^{47} test and that this test distinguishes hyper- and hypoabsorption from normal and accurately reflects effects of specific therapy.

Behavior of Autotransfused in Vitro H^3-Cytidine (H^3-CDR)-labeled Lymphocytes in Chronic Lymphocytic Leukemia (CLL). JANET CUTTNER, EUGENE P. CRONKITE,† MATINA KESSE, AND THEODOR M. FLIEDNER, Upton and New York, N. Y.

The net increase in mass of leukemic cells in CLL may involve increased production or increased life span, or both. It was shown earlier, utilizing H^3-thymidine in acute leukemias, that a significant fraction of blast cells was "sterile," implying increased life span. H^3-cytidine was found to be an *in vitro* label for RNA and DNA of lymphocytes in normal and leukemic states. To obtain information about the behavior of autotransfused H^3-CDR, *in vitro* labeled lymphocytes, and perhaps RNA to DNA conversion, five patients with CLL were studied. Five hundred ml blood was removed and incubated in ACD for 1 hour with 0.5 mc H^3-CDR (SA, 1.4 c per mmole). After reinfusion serial blood samples were taken for up to 2 weeks and processed for radioautography. In the autotransfused blood, 70 to 98% of all lymphocytes showed cytoplasmic and nuclear label. Thirty minutes after completion of the autotransfusion, approximately 4% of all circulating lymphocytes was labeled. After 24 hours the fraction labeled and intensity of label had dimished by a factor of two. Thereafter the fraction of labeled lymphocytes and their intensity remained constant for the observation periods. The findings are consistent with the hypothesis that the leukemic cells studied either have a generative cycle greater than 5 to 14 days or a large fraction are long lived cells or both. This approach to study of leukemia shows promise for determination of many parameters of the life cycle of lymphocytes in CLL and may be applicable to the study of acute leukemias.

Thrombocytopenia Due to Digitoxin: Demonstration of Antibody and Mechanism of Action. HERBERT I. HOROWITZ, ROBERT C. YOUNG, AND RALPH L. NACHMAN, New York, N. Y. (introduced by Edward E. Fischel).

A 55-year-old male developed thrombocytopenia while taking digitoxin. Studies revealed the presence of a factor in his serum that reacted with digitoxin and normal platelets. Inhibition of clot retraction was demonstrated. The Russell's viper venom (Stypven) time shortened markedly in a mixture of normal intact platelet-rich plasma, patient's serum, and drug, indicating an increase in the platelet factor 3 activity. Incubation of normal washed platelets, patient's serum, and drug for an hour at 37° C resulted in generation of alpha-amino nitrogen in the mixture. Tests for platelet factor 3 and alpha-amino nitrogen changes have previously been reported to be sensitive parameters of immune platelet injury; they were significantly more sensitive than clot retraction inhibition. The antiplatelet activity was localized to the DEAE-separated gamma globulin fraction of the patient's serum. Requirement for drug was highly specific; negative results were obtained with digoxin, Cedilanid, ouabain, and digitoxinogen. Tritiated digitoxin was bound to the patient's gamma globulin in the absence of platelets. When normal platelets were added, the drug-antibody was loosely bound to the platelets; it could be removed with a single saline wash. These studies indicate that the mechanism of digitoxin-induced thrombocytopenia is similar to that of sedormid or quinidine purpura, and provide direct evidence in support of N. R. Shulman's concept that platelets are

damaged in these diseases by nonspecifically absorbing drug-antibody complex.

Asynchronous Synthesis of Hemoglobins A and A_2 by Human Erythrocyte Precursors. RONALD F. RIEDER, Baltimore, Md. (introduced by C. Lockard Conley).

Relative rates of synthesis of hemoglobins A and A_2 in erythrocyte precursors were determined by measuring rates of incorporation of Fe^{59} into hemoglobin components after intravenous injection of Fe^{59} citrate and after incubation of Fe^{59} citrate with marrow aspirates or with reticulocyte-rich blood *in vitro*. Hemoglobins A and A_2 were measured and their specific activities determined after separation by starch block electrophoresis of cleared, dialyzed hemolysates. On days 5 and 6 after intravenous administration of Fe^{59} to a patient with hereditary spherocytosis, specific activities of hemoglobins A and A_2 were equal, and relative rates of synthesis were 40 to 1. In 4 experiments in which marrow was incubated with Fe^{59} *in vitro,* the mean ratio of specific activities of hemoglobins A and A_2 was 1.3 $\pm$ 0.1; in all of the experiments with marrow, nonhemoglobin protein containing Fe^{59} was removed on Amberlite CG-50 columns before electrophoretic separation of the hemoglobin components. When blood from 12 patients with various types of anemia (reticulocytes, 5 to 40%) was incubated with Fe^{59}, the mean ratio of specific activities of hemoglobins A and A_2 was 2.0 $\pm$ 0.4. In 3 additional experiments with blood, nonhemoglobin protein was removed by chromatography before electrophoresis; the mean ratio of specific activities was 2.5 $\pm$ 0.6. These observations suggest that synthesis of hemoglobin A does not parallel that of hemoglobin A_2 throughout the maturation of the erythrocyte. Because of the more rapid decline in synthesis of hemoglobin A_2 in the maturing red cell, the relative rates of synthesis of hemoglobins A and A_2 in reticulocytes are about 80 to 1. Similar results were obtained with reticulocyte-rich blood of patients with sickle cell anemia; the ratio of specific activities of hemoglobins S and A_2 was 2.3 in one case and 1.8 in another.

Determinants of Cardiovascular Responsiveness to Ischemic Pain. ALVIN P. SHAPIRO* AND JOSEPH D. SAPIRA, Pittsburgh, Pa.

Although cardiovascular responses to noxious stimuli usually are mediated through the sympathetic nervous system, individuals with adrenergic impairment due to disease or drugs can often display pressor reactivity. To investigate this phenomenon and to examine "adaptation" to repetitive stimuli, we performed the following study. Eleven normotensive volunteers were subjected to four periods of ischemic pain of the arm. Blood pressure and pulse rate were measured by indirect continuous recorders. Plasma free fatty acids (FFA) and urinary vanilmandelic acid (VMA) were determined before and after the experiment. One to three weeks later, each subject returned for replication of the procedure. Mean blood pressure increases over base line were 26.5, 27.4, 26.7, and 25.9 mm Hg for the four ischemic periods respectively on day 1 and 30.5, 28.5, 29.3, and 28.6 on day 2. Pulse rate elevations were 16.5, 17.0, 14.8, and 15.4 per minute on day 1 but only 13.7, 10.7, 12.6, and 12.8 on day 2. Analysis of variance indicated that differences between periods on each day were not significant; when all periods were combined and analyzed by paired differences, the increased pressor and decreased rate responses on day 2 were significant ($p = 0.01$). FFA increased from 661 to 906 μEq per L originally but fell from 737 to 658 μEq per L during the repeat session; this diminished response occurred in all subjects ($p < .001$). VMA excretion rose 3.93 μg per minute on day 1 but only 2.90 μg per minute on repetition ($p = 0.10$). Thus pressor and pulse responses did not adapt to the stimulus of ischemic pain on any given day. With replication, the pressor response increased, whereas the pulse response diminished, biochemical indicators of sympathetic activity decreased, and subjects reported less anticapatory anxiety. These findings suggest that although pulse response with ischemic pain may correlate with adrenergic arousal, the pressor response is mediated to a significant extent by nonadrenergic mechanisms.

Demonstration of Functioning Lymphaticovenous Communications in the Living Animal. SAM A. THREEFOOT, MELVYN F. KOSSOVER, AND DAVID W. AIKEN, New Orleans, La. (introduced by J. A. Abildskov).

Having demonstrated lymphaticovenous communications by plastic corrosion models in rats and postmortem lymphangiography in man, we studied factors influencing function of lymphaticovenous communications in living dogs. Cisterna chyli or thoracic ducts, or both, were ligated or excised 0 to 140 days before injection of radioactive material into a leg lymphatic. Blood from the inferior vena cava and femoral artery or superior vena cava was circulated through coils surrounding scintillator crystals connected to dual recorders. None of the 25 animals developed edema. Three unobstructed animals served as controls. Lymphangiography was performed in both groups. In some animals 70% of I^{131}-albumin injected intralymphatically entered the circulating blood through lymphaticovenous communications. In others, intralymphatic I^{131}-Ethiodol entering the circulation did not recirculate but was filtered completely by the lungs; 60% was recovered from some postmortem lung homogenates. Functioning lymphaticolymphatic bypass of obstructed channels was demonstrated in 10 animals, lymphaticovenous communications below the diaphragm in 14, and above the diaphragm in 4. Results indicate that prolonged obstruction, increased intralymphatic volume and pressure, external abdominal pressure, and sometimes drugs such as hexamethonium contribute to functioning communications. These and supporting data suggest that lymphaticovenous communications serve as accessory channels for return of fluid and protein accumulated in tissue and organs during

disease; their failure to function may contribute to more rapid and pronounced pathologic manifestations of fluid accumulation, and methods for increasing flow through these channels may be of assistance in therapy.

Fluid and Electrolyte Metabolism in Spontaneously Occurring Canine Heart Failure. HADLEY L. CONN, JR.,* AND GORAN E. BOJS, Philadelphia, Pa.

Spontaneously occurring cardiac failure is found in approximately 15 of every 1,000 dogs examined in a veterinary cardiac clinic. Mitral insufficiency is the most common cause. Although the process is of undetermined etiology, the pathology and the ensuing pathophysiology in many respects mimic those of rheumatic heart disease. Fluid and electrolyte metabolism, renal hemodynamics, and aldosterone excretion were examined in 13 such animals with heart failure. Twenty-four hour exchangeable sodium and potassium, total body water (H^3), interstitial water (S^{35} space), Cr^{51}-labeled red cell mass, creatinine clearance, renal E_{PAH}, 24-hour urinary aldosterone excretion, and related standard measurements were used to evaluate the pathophysiologic state both in heart failure and in the compensated condition achieved by cage rest, digitalis, and occasionally added diuretic therapy. A standard low normal salt intake was maintained. In the compensated state the spectrum of results approximates the range of normal values for man and dog. In heart failure plasma and red cell mass were significantly (87% and 60%) increased. Interstitial water and sodium and potassium content were significantly increased (approximately 103%) but so proportionately that only a minor increase in potassium concentration was found consistently. Cell water was increased slightly but not significantly and cell sodium even less. A small cellular potassium loss was calculated. Renal blood flow averaged 0.68 of the compensated state, whereas glomerular filtration varied from near control to about 50% of control. Urinary aldosterone averaged one-tenth that of human urine, but as in man was increased twofold in the state of equivalent right-heart failure. However, in early heart failure normal values were found, and administration of aldosterone blocking agents failed to prevent the sodium-retaining effects of exercise. We conclude that spontaneously occurring right-heart failure in the dog is associated with the fluid, electrolyte, and renal-adrenal pattern qualitatively and quantitatively similar to that observed in human heart failure.

Intestinal Absorption and Mode of Transport in Portal Vein of Medium Chain Fatty Acids. SAMI A. HASHIM, STANLEY S. BERGEN, JR., KENNETH KRELL, AND THEODORE B. VAN ITALLIE,* New York, N. Y.

Although there is indirect evidence that medium chain fatty acids (C8 to C10) travel in the portal vein after absorption, the mode of transport has not been clarified. In a direct approach to the problem, C^{14}-labeled tricaprylin diluted with nonradioactive medium chain triglyceride (MCT) was instilled in the duodenum of dogs with indwelling portal and hepatic vein catheters. The tricaprylin was uniformly labeled at the carboxyl carbon. The MCT contained 98% C8 and C10 fatty acids. Blood was sampled repeatedly from both veins for periods lasting 7 to 30 hours. At the end of each experiment, intraluminal contents and mucosal tissue from the upper portion of the small intestine were taken for analysis. Radioactivity was measured in aqueous and organic solvent extracts from all samples. Lipid classes were separated by thin layer silicic acid chromatography, and their radioactivity was measured. Individual fatty acids were identified by gas-liquid chromatography (GLC). Results indicate that extensive hydrolysis of MCT occurred in the intestinal lumen before absorption into the portal vein. In the lipids of portal venous blood, radioactivity was almost exclusively confined to the free fatty acid (FFA) fraction. The presence of C8 and C10 acids as FFA in portal plasma also was shown by GLC. In hepatic venous blood all the radioactivity was in the aqueous extract. We conclude that, after absorption, medium chain fatty acids are transported as FFA in the portal vein. The liver appears to metabolize these acids rapidly into nonlipid products, presumably ketones and carbon dioxide.

An Immunologic Difference between Rheumatoid and Normal Hyaluronateprotein. JOHN SANDSON AND DAVID HAMERMAN,* New York, N. Y.

The hyaluronateprotein isolated from synovial fluids in rheumatoid arthritis is different from normal. Rheumatoid hyaluronateprotein contains more bound protein (about 10%) than normal hyaluronateprotein (2%) and forms a gel after dialysis in acetate buffer, pH 4.5. An immunologic difference is also present, as the following studies show. Rabbits were immunized with either normal (NHP) or rheumatoid (RHP) hyaluronateprotein mixed with Freund's complete adjuvant. RHP did not form precipitin lines with anti-RHP on agar double diffusion or immunoelectrophoresis. RHP digested with testicular hyaluronidase formed a single precipitin line with anti-RHP. Immunoelectrophoresis showed that this line extended through both alpha globulin zones. Hyaluronidase-digested RHP did not react with anti-NHP, nor did digested NHP react with either anti-NHP or anti-RHP. Anti-RHP thoroughly adsorbed with NHP still formed a precipitin line with hyaluronidase-digested RHP, a finding which suggests that the antigenic component in RHP is either absent in NHP or present only in traces. After treatment with trypsin, hyaluronidase-digested RHP formed no precipitin line with anti-RHP, indicating that the antigenic component in RHP is associated with the protein moiety. This antigenic component does not appear to be a serum protein trapped within the domain of RHP, for when NHP and rheumatoid sera were mixed and the NHP reisolated, the NHP, both before and after hyaluronidase digestion, failed to form a precipitin line with anti-RHP. The antigenic component in RHP is closely

related to a serum protein, for anti-RHP formed a precipitin line with serum that on agar double diffusion fused completely with hyaluronidase-digested RHP. This serum component migrated in agar as an alpha$_2$ globulin but has not yet been further characterized. The protein moiety in RHP appears to be responsible for the immunological and physical properties distinguishing RHP from NHP.

Acriflavine Hydrochloride: An Immunosuppressive Agent without Apparent Toxicity to Lymphatic and Myeloid Tissues. RICHARD S. FARR,* JOEL S. SAMUELSON, AND P. BRIAN STEWART, La Jolla, Calif.

Because the diaminoacridines bind DNA and RNA in cell nuclei of lymphatic tissue *in vivo* without apparent harm to laboratory animals, the present study was instituted to determine what effect acriflavine hydrochloride might have on the immune response. Rabbits were given daily intravenous doses of acriflavine from day −2 to day +14 and again from days 19 to 26. On day 0 and day 21 they were immunized with 100 mg of bovine serum albumin (BSA) intravenously. The amount of antibody in the sera was measured by precipitating I^{131}-labeled BSA-antibody complexes with ammonium sulfate. Groups of rabbits treated with 4 to 5 mg per kg acriflavine per dose had a markedly depressed primary response and less than 2% as much anti-BSA during the secondary response as control rabbits after similar immunizations with BSA but without acriflavine treatment. The ammonium sulfate test results were corroborated by the hemagglutination test using tanned sheep red cells coated with BSA. The acriflavine-treated group showed marked immune unresponsiveness when given additional injections of BSA on days 28 and 35 without further acriflavine therapy. The same dose of acriflavine had little or no effect on the secondary response after a primary response to BSA was established in the absence of acriflavine. At the 4 mg per kg per dose level, rabbits gained weight and were histologically normal. After 10 mg per kg per dose, rabbits died with severe acidosis, uremia, and nephrocalcinosis, but the peripheral leukocyte counts were not suppressed, and there was no histologic evidence of damage to bone marrow or germinal centers in lymphatic tissue and spleen. Thus, the diaminoacridines are of considerable interest because they effectively interfere with antibody production and in a manner apparently different from other known immunosuppressive agents.

Investigations on Mechanisms of Normal and Defective Immunity. JOHN L. FAHEY,* WERNER BARTH, AND LLOYD W. LAW, Bethesda, Md.

Immune poverty, including poor or absent antibody response, has been stressed in reports concerning the effects of neonatal thymectomy. Our studies were undertaken to determine *a*) whether neonatal thymectomy in mice induced a failure to form normal immunoglobulin components, failure to form specific antibody, or functional failure of other types in immune cells and *b*) the relationship of mouse neonatal thymectomy to clinical disorders of immunity. Four major immunoglobulin groups (7 $S\gamma_2$-, 7 $S\gamma_1$-, $\gamma_{1A}(\beta_{2A})$-, and γ_{1M}-globulins) were identified in normal mouse serum and measured quantitatively. All were present in neonatally thymectomized C_3H mice, but the serum β_{2A}-globulin levels were usually increased. Turnover studies with I^{131}-labeled immunoglobins showed normal or increased rates of immunoglobulin synthesis. Immunochemical evaluation indicated that H and L polypeptide chains of γ-globulins from thymectomized mice resembled those in normal γ-globulin. Antibody (hemolysin) response to sheep erythrocytes was uniformly depressed, but response to other antigens was normal or reduced. Antibody response in thymectomized mice depended on the antigen and mode of administration. These findings indicate that normal immune response requires co-ordinated function of multiple steps in the immune mechanism. Antibody deficiency, when it is present after neonatal thymectomy, is not due to inability to form H and L polypeptide chains but may result from failure to produce specific antibody configuration in the polypeptide chains. This failure is not absolute, however, for many aspects of the immune mechanisms can function normally in neonatally thymectomized mice. The observations on immunoglobulins and antibody response set apart the neonatal thymectomy syndrome from clinical syndromes of agammaglobulinemia, dysgammaglobulinemia, and related clinical disorders of immunity.

The Effects of Antimetabolites on Cell Renewal in the Colon of Man. MARTIN LIPKIN, BERTRAND BELL, AND PAUL SHERLOCK, New York, N. Y. (introduced by Thomas P. Almy).

To provide detailed measurements of the effects of antimetabolites on the proliferative cycle of intestinal epithelial cells *in vivo,* H^3 thymidine and antimetabolites were administered simultaneously to selected human subjects with limited life expectancy. Specimens of normal colonic mucosa from colostomies, removed by biopsy tube at frequent intervals before and during administration of the agents, were studied. Microradioautographic and cytologic analyses were correlated with alterations occurring in various phases of the proliferative cycle. 5-Fluorouracil (5-FU) was administered until clinical toxicity and depression of the leukocyte count were noted. A total dose of 3,750 mg was given. At this time, cytologic abnormalities in the colonic epithelial cells were observed. Nuclei were enlarged, stained lightly, and contained irregularities in chromatin pattern. Kinetic analysis revealed a simultaneous delay in the onset of mitosis, and the G_2 premitotic interval was prolonged. The number of mitoses per crypt also declined. After cessation of 5-FU, the abnormalities disappeared, and the mitotic rate returned to normal. 5-Fluorodeoxyuridine (FUDR) was administered in a total dose of 11 g to clinical and hematologic toxicity. Marked changes occurred in the renewal cycle

of some cells without occurring in others. In some epithelial cells, the G_2 premitotic interval was prolonged to 8 to 10 hours, and the onset of mitosis was greatly delayed. This was shown by the presence of both a normal wave of labeled mitosis and a very large secondary wave, the latter derived from cells whose G_2 intervals were markedly prolonged. Although the administration of both antimetabolites resulted in clinical and hematologic toxicity, these dosage schedules were insufficient to cause an increase in the mean duration of the DNA synthesis (S phase) of the normal colonic epithelial cells and caused only a transient and incomplete delay in cell renewal. Further measurements will reveal in detail the comparative effectiveness of various dosage schedules of different chemotherapeutic agents on the growth of both normal and neoplastic tissues in man.

Pathways of Iron Metabolism in the Intestinal Mucosa and the Regulation of Iron Absorption. JAMES MANIS AND DAVID SCHACHTER,* New York, N. Y.

The molecular events underlying the regulation of iron absorption across the duodenal mucosa of the rat were studied with everted gut sacs *in vitro* and duodenal loops *in vivo*. Methods were developed to separate and estimate specifically Fe^{++}, Fe^{+++}, Fe^{59++}, and Fe^{59+++} in mucosal tissue. In the course of active transport *in vitro*, Fe^{++} is absorbed into a mucosal ferrous pool that turns over relatively rapidly by *a*) transport of Fe^{++} to the underlying intestine and serosal surface, resulting in high concentration gradients serosal/mucosal, and *b*) diversion of Fe^{++} to a mucosal Fe^{+++} pool that turns over relatively slowly. The trivalent pool increases markedly *in vivo* and *in vitro* when increasing loads are presented for absorption and is therefore a depot for excess iron. When rats were given 0.2 to 4.0 mg Fe (as $FeSO_4$) by gastric tube and duodenal loops or gut sacs were studied 3 hours thereafter, three changes in iron transport were found. Fe^{++} uptake at the mucosal surface was decreased. The absolute quantity of mucosal Fe^{++} transferred to the mucosal Fe^{+++} pool was increased markedly, accounting frequently for the entire uptake at the mucosal surface. Finally, transport of mucosal Fe^{++} to the underlying intestine and serosal surface, or to the bloodstream *in vivo*, was greatly reduced. All three effects tend to limit iron absorption in the face of excess mineral. A sensitive hemagglutination-inhibition method was developed to estimate apoferritin in mucosal tissue, in order to study the mucosal trivalent pool. Ferritin iron could account for only 8.0% of the trivalent pool and was insignificant in these studies.

Studies on Hemolysis of Red Cells from Patients with Paroxysmal Nocturnal Hemoglobinuria. STANLEY YACHNIN, Chicago, Ill. (introduced by Murray Rabinowitz).

Polyinosinic acid (poly I) is a potent inhibitor of hemolytic complement (C′) activity in immune red cell lysis. However, when tested in the paroxysmal nocturnal hemoglobinuria (PNH) hemolytic system, poly I was found to enhance PNH red cell lysis both at pH 6.5 (twofold stimulation) and at pH > 7.4 (tenfold stimulation), in a manner analogous to thrombin. Poly I alone, or in heat-inactivated serum, was nonlytic to PNH red cells. Other polynucleotides were incapable of enhancing PNH hemolysis; Poly I previously complexed in a hydrogen-bonded helical structure with polycytidylic acid was also ineffective. Pre-exposure of PNH erythrocytes to poly I, followed by washing, did not alter their subsequent hemolysis in acidified serum. Since poly I is a purified biosynthetic substance, enhancement of PNH hemolysis cannot be attributed to contamination with heteroantibodies or properdin. Detailed study of the effects of poly I on C′ has shown that poly I, having an affinity for the C′1q subcomponent (11 S portion) of the first component of C′(C′1), binds C′1 and prevents its attachment to sensitized red cells. The poly I-C′1 complex in turn leads to inactivation of C′4. No inhibitory effect by poly I upon C′2 or C′3 has been demonstrated. In contrast, antigen-antibody aggregates and heat-formed γ-globulin aggregates, which "fix" C′1, C′4, C′2, and some C′3, inhibit PNH hemolysis. These findings, together with the known dependence of PNH hemolysis on Mg^{++} but not Ca^{++}, suggest that the ability of poly I to enhance PNH cell lysis may be due to activation via fluid phase C′1 and C′4 of the later steps in C′ activity, which in turn lyse the presumably defective stroma of PNH cells. Thombin and reduction in pH may enhance PNH cell hemolysis through a similar mechanism.

Renal Excretion of Amino Acids. DAVID BERNSTEIN, DAVID KAPLAN, STANLEY L. WALLACE, AND DAVID HALBERSTAM, Brooklyn, N. Y. (introduced by L. W. Eichna).

As part of studies investigating amino acid metabolism in primary hyperuricemia, renal mechanisms for the excretion of amino acids were studied. *1*) Observations were made on 12 normouricemic and 14 hyperuricemic individuals, all with normal renal function as judged either by endogenous creatinine clearance or inulin and PAH clearances. Total amino acid clearance was found to be dependent on urine flow rate, and the relationship may be expressed by the equation $UV/P = 12.6 + 2.00V$, with a coefficient of correlation of +0.84. *2*) Clearance was dependent on flow rate for each of the 14 amino acids measured individually. The slope (increase in clearance with each milliliter increase in rate of urine flow) varied from 0.013 to 0.458. *3*) In three individuals, clearance measurements at multiple flow rates confirmed the dependence of clearance on rate of urine formation. *4*) When the neutral amino acids were ranked in order of increasing percentage of reabsorption, the sequence was the same as the order of increasing rates of uptake of the amino acids at low concentration by the isolated rat intestine (Finch and Hird, Biochem. biophys. Acta (Amst.) 1960, **43**, 278). *5*) Chlorothiazide

was administered to patients to determine whether the drug had a similar effect on urate and amino acid clearances. In four normal individuals who had 4-hour amino acid clearances before and after taking 1 g of chlorothiazide a day for 7 days, the total amino acid clearance after the drug fell to 9.6 ml per minute from a control value of 14.7 ml per minute (mean values). (Urine flow rates were similar before and after the drug; diets were identical on the day preceding each clearance determination.) When 0.5 g of drug was given intravenously to 5 subjects, there was no immediate increase in amino acid clearance, despite an immediate increase in urine flow and in uric acid clearance. During the 30- to 40- and 50- to 60-minute post-drug collection periods, amino acid clearance decreased by 33%, despite maintaining the high flow rate. Thus, no paradoxical effect was noted, as occurs with uric acid. The data indicate that both passive reabsorption and active transport play a role in amino acid excretion.

Decreased Circulating Free Fatty Acid Turnover after Ethanol Ingestion in Man. DON P. JONES, EINAR S. PERMAN, AND CHARLES S. LIEBER, Boston, Mass. (introduced by Charles S. Davidson).

Ethanol produces an immediate fall in circulating free fatty acids (FFA) in man, which is considered to reflect either increased FFA uptake by the liver or other tissues or decreased FFA release from adipose tissue. Differentiation between these two mechanisms is important for the understanding of the pathogenesis of the alcoholic fatty liver. Therefore, the effect of ethanol on FFA turnover was measured in seven subjects by a constant intravenous infusion of palmitic acid-1-C^{14} bound to albumin (0.17 μc per minute). After a 30-minute equilibration period and a control period of 30 to 40 minutes, four doses of ethanol (0.25 g per kg per 15 minutes) were given orally during 1 hour followed by 0.1 to 0.5 g per kg per 30 minutes for a second hour, resulting in blood ethanol levels of 150 to 230 mg per 100 ml. Plasma FFA concentration was determined by the Trout modification of the Dole procedure, and after separation by a modified Borgström technique, FFA radioactivity was determined by liquid scintillation counting. In all seven subjects, FFA concentration decreased 42 to 61% from normal control values after ethanol, but, despite this, the FFA radioactivity per milliliter of plasma remained constant, resulting in 1.7- to 2.6-fold increases of FFA specific activity. With unchanged fractional turnover rates, as shown by the stability of plasma FFA radioactivity, comparison of steady-state FFA concentrations before and after ethanol indicates a 37 to 58% decrease of over-all FFA turnover after ethanol. These data, as well as measurements of peripheral arteriovenous FFA differences in other subjects, show that the fall in circulating FFA after ethanol is not produced by increased hepatic FFA uptake but reflects a decreased influx of FFA into the vascular space, most likely resulting from decreased FFA release from adipose tissue.

Sols, Gels, and the Mechanism of Action of Colchicine. STEPHEN E. MALAWISTA, New Haven, Conn. (introduced by Paul B. Beeson).

This work was motivated by the hypothesis that colchicine suppresses inflammation in acute gouty arthritis by interfering with sol-gel equilibria, leading to a decrease in cytoplasmic viscosity of polymorphonuclear leukocytes. The effect of this would be to impair motility and phagocytosis of leukocytes, thus inhibiting their ability to participate in the inflammatory response to urate crystals. Such a mechanism of action has been inferred from elegant experiments on the mitotic spindle, sarcoblast fibers, and cytoplasm, in systems which are technically complex and slow. In the present work the frog melanocyte has been employed to study the effect of colchicine. In this cell, transformations of a sol-gel type are effected rapidly and reversibly and are easily controlled and measured. Frog skin in Ringer's solution darkens (maximal in 1 hour) when melanocyte-stimulating hormone (MSH) is added. The darkening, measured by reflectance, represents dispersion of melanin granules in melanocytes and is thought to be accompanied by a gel-to-sol cytoplasmic transformation. When resuspended in fresh Ringer's solution, the skin lightens, with aggregation of melanin granules and gelation. Preincubation of skin with colchicine had the following effects. *1)* Darkening by MSH was potentiated, and on removal of MSH, lightening was inhibited. Inhibition was a function of both concentration (10^{-5} M to 0.9×10^{-4} M) and exposure (2 to 30 minutes). *2)* The same effects were noted when other darkening agents (adenosine triphosphate 10^{-3} M, caffeine 5.2×10^{-3} M, ethyl acetate 0.8×10^{-2} M) were used instead of MSH. *3)* Colchicine alone produced gradual, irreversible, dosage-dependent darkening over several hours. The most likely explanation for these results is that colchicine alters the dynamic equilibrium between sol and gel in melanocytes by maintaining the sol state. Such an action, if confirmed in leukocytes, allows a common formulation for the hitherto apparently unrelated effects of colchicine in gouty inflammation and on the mitotic spindle.

Enzyme and Coagulation Activity of Subcellular Platelet Fractions. AARON J. MARCUS* AND DOROTHEA ZUCKER-FRANKLIN, New York, N. Y.

A correlation was made between the structure, enzyme content, and clotting function of "subcellular" platelet particles. Fresh human platelets were homogenized to an extent that the membranes were disrupted, but the majority of granules and mitochondria remained intact as confirmed by electron microscopy. With ultracentrifugation in a continuous sucrose gradient (30 to 65%), a band containing membranes was separated from one containing granules and mitochondria. Cytochrome-c oxidase activity was limited to the granule fraction and attributed to the mitochondria. The granules were also the major site of acid phosphatase and beta-glucuronidase

activity, which is consistent with the hypothesis that they may be lysosomes. The total phospholipid content of the membranes and granules was similar, and thin-layer chromatography revealed the individual phosphoglyceride classes in both fractions to be qualitatively identical. Despite this, differences in clot-promoting properties of the membranes and granules were found. In the thromboplastin generation test (TGT) the specific activity of the membranes was comparable to the crude homogenate as well as to brain cephalin, whereas the granules showed only 20% of this activity. These differences were even more apparent in the "Stypven" coagulation system. However, when extracted lipids from either the granules or membranes were studied, their coagulation activity was similar. Apparently the lipid or (lipoprotein) in the platelet membrane is more "available" for interaction with plasma clotting factors than is the lipid from the granules. This hypothesis concurs with the observation that platelet granules disappear after the initial stages of coagulation have taken place. We postulate that the phospholipid fraction required for the formation of the intrinsic prothrombin activator is derived principally from the platelet membrane rather than from other sources of similar phosphatides in the platelets or other cells.

Determination of Digitoxin in Plasma by Double Isotope Dilution Derivative Assay. DANIEL S. LUKAS AND RALPH E. PETERSON,* New York, N. Y.

Currently available methods for measuring the cardenolides and their glycosides in biologic materials lack sensitivity and chemical specificity. To study the disposition and metabolism of digitoxin in man, we devised a double isotope dilution derivative method for measuring the steroid in plasma, urine, and other body fluids. The following steps are essential to the assay: *1*) addition of a quantity of tritium-labeled digitoxin of high specific activity to the sample, *2*) extraction followed by preliminary purification of digitoxin by liquid-liquid partition and paper chromatography, *3*) acetylation to digitoxin triacetate with acetic anhydride-1-C^{14}, *4*) isolation and purification of the triacetate by descending paper chromatography in four different solvent systems including a reversed phase system, and *5*) measurement of tritium and C^{14} activity in the pure digitoxin triacetate by liquid scintillation spectrometry. With tritium activity in the counting vial to correct for losses in the above procedures, and with knowledge of the specific activities of the H^3-digitoxin and C^{14}-acetic anhydride, the C^{14} activity in the vial not attributable to H^3-digitoxin triacetate-C^{14} was used to calculate the amount of digitoxin originally present in the sample. Assay of normal plasma to which 0.01 to 10 μg of digitoxin was added yielded values for digitoxin content and concentration that were $99 \pm 4\%$ (mean $\pm$ SE) of the expected values. The plasma of twelve patients maintained on varying amounts of digitoxin by mouth contained the glycoside in concentrations of 1.04 to 5.36 μg per 100 ml. Concentration varied directly with the size of the daily dose. Most of the concentrations approximated 2 μg per 100 ml, but two patients manifesting toxicity (gastrointestinal) had levels greater than 4 μg per 100 ml. Diurnal variation in concentration ranged from 0.11 to 0.32 μg per 100 ml. Over 90% of the digitoxin in plasma is bound to albumin; erythrocytes contain less than 5% of the steroid in the blood. Previous investigations have failed to demonstrate digitoxin in the blood of patients receiving the drug by mouth. This study indicates that the plasma of such patients contains a reservoir of 50 to 70 μg of the steroid, which must be considered in schema defining tissue kinetics, metabolism, and renal excretion of the compound.

Hypothalamic Regulation of Growth Hormone Secretion. ROBERT L. ABRAMS, MARY PARKER, SANTANDER BLANCO, SEYMOUR REICHLIN,* AND WILLIAM H. DAUGHADAY,* Rochester, N. Y., and St. Louis, Mo.

To investigate neural control of growth hormone (GH) secretion, the blood sugar response to insulin injection, fasting growth hormone levels, and the plasma growth response to insulin-induced hypoglycemia were determined in male squirrel monkeys with chronic lesions of the hypothalamus. GH concentration was measured by radioimmunoassay. Electrolytic lesions were produced under stereotaxic control, and tolerance tests were performed in unanesthetized animals with chronically implanted intra-atrial Teflon catheters. Ten monkeys with lesions and seven control-operated animals underwent nineteen insulin tolerance tests using 0.15 U regular insulin per kg body weight. Marked insulin hypersensitivity was noted in nine of ten animals with lesions (mean maximal percentage glucose depression, 72.3 ± 4.5 versus 39.9 ± 2.8 in controls; $p < .001$). The plasma GH response to hypoglycemia was significantly reduced in six of nine insulin hypersensitive animals (mean rise above base line, 2.0 ± 0.9 mμg per ml versus control group value of 7.1 ± 1.8 mμg per ml; $p < .02$). GH rise above base line was completely abolished in four animals. Base-line GH levels were not significantly reduced by the lesions. In certain animals, diabetes insipidus or hypothyroidism or both were also observed (low PBI, low thyroid-I^{131} uptake). Appropriate replacement therapy (Pitressin tannate, L-thyroxine) did not alter resting GH levels or the abnormal response to insulin injection. In brains thus far examined, lesions effective in blocking normal GH response are located in the anterior ventral hypothalamus. These results indicate that the hypothalamus is involved in GH regulation and forms an essential part of the mechanism by which hypoglycemia triggers secretion of the hormone.

Prevention of Pyelonephritis by Water Diuresis: Evidence for the Importance of Medullary Hypertonicity in Promoting Renal Infection. VINCENT T. ANDRIOLE AND FRANKLIN H. EPSTEIN,* New Haven, Conn.

The special susceptibility of the renal medulla to certain infections has been ascribed to its chemical composition, in particular, to its hypertonicity. To alter the

normally hypertonic environment of the medulla, chronic water diuresis was induced in male rats by adding 5% glucose to their drinking water. Water intake and output increased several fold as urinary osmolality dropped below 100 mOsm per kg. Sodium concentration in inner medullary tissue fell from 215 ± 21 to 123 ± 23 mEq per kg H_2O, and urea concentration from 489 ±127 mmoles per kg H_2O to a tenth of that value. Tissue concentrations of NH_4^+ and K^+ were unchanged by diuresis. Glucose did not appear in the urine. Water diuresis protected rats against two types of experimental pyelonephritis, that produced by intravenous injection of *Candida albicans* and of *Staphylococcus aureus*. An inoculum of *Candida* that induced gross infection in 16 of 17 control rats sacrificed 8 days after injection produced counts of over 50,000 organisms per kidney in only 3 of 18 rats undergoing water diuresis and abscesses in none. Incidence and severity of staphylococcal pyelonephritis were likewise strikingly diminished by diuresis begun a day before or a day after the intravenous inoculum. Rats begun on glucose as late as 3 days after injection of staphylococci showed significantly lower bacterial counts and fewer renal lesions, when killed on the eighth day, than control animals excreting a hypertonic urine. Extracts of renal medulla from animals undergoing diuresis did not inhibit bacterial growth. Water diuresis did not alter the number of organisms initially deposited in the kidney after intravenous injection. These data strongly support the view that the hypertonic environment of the renal medulla is important in the development of pyelonephritis and thus provide some experimental rationale for the time-honored practice of "forcing fluids" in patients with urinary infection. When hypertonicity is overcome by water diuresis, experimental renal infections may be prevented or greatly ameliorated.

Passage of Tris across the Peritoneum. G. G. NAHAS, J. GJESSING, M. VEROSKY, AND E. M. PAPPER,† New York, N. Y.

Intraperitoneal injection of Tris in rodents delays the appearance of the convulsions of hyperbaric oxygenation that may be caused, in part, by CO_2 retention. The use of Tris for dialysis in dogs also more than doubles the extraction of pentobarbital, phenobarbital, and salicylate. Therefore, the passage of Tris and CO_2 across the peritoneum was studied during dialysis in 5 dogs. The animals were mechanically ventilated and given by intraperitoneal administration 30 ml per kg of a solution (pH 9.8) containing 150 mmoles per L Tris (and tracer amounts of C^{14}-labeled Tris), 80 mmoles per L NaCl, 4 mmoles per L KCl, and 40 mmoles per L glucose. In the first 30 minutes arterial P_{CO_2} fell by 6 mm Hg and returned to control levels after 1 hour, whereas HCO_3^- increased 1.4 mEq per L, and pH rose 0.05 U. Plasma Tris levels reached a peak of 3.6 mmoles per L after 30 minutes and then slowly decreased to 1.6 mmoles per L after 7 hours. Urine pH increased from 6.7 to 7.4 in 1 hour and remained at that level or higher. Five to 14 ml per hour of urine was produced containing 226 to 310 mmoles per L of Tris. In 6 hours 45% of the Tris administered had been recovered in the urine. The concentration of Tris in the dialysate decreased in the first hour from 150 to 85 mmoles per L, pH fell from 9.8 to 8.2, and CO_2 (HCO_3^-) increased from 0 to 28 mmoles per L (9 mmoles per L higher than in plasma). Subsequently, as pH in the dialysate tended to approximate plasma pH (third hour) and the major fraction of Tris was ionized, the rate of disappearance of Tris from dialysate was much slower. After 7 hours Tris had not equilibrated between dialysate (12 mmoles per L) and blood (1.6 mmoles per L). Consequently, to maintain a high pH gradient between the Tris dialysate and plasma for optimal removal of weak acids or their salts, exchange should not exceed 1 hour. Histological examinations showed the omentum to be similar to that of animals dialyzed with standard solutions. Growth of bacteria is also inhibited at pH 9.0. Peritoneal dialysis (and possibly hemodialysis) with Tris might have clinical applications for the removal of exogenous or endogenous acids.

The Effect of Chronically Increased Ventricular Pressure on the Electrical Forces of the Heart. PAUL G. HUGENHOLTZ AND RAUL GAMBOA, Boston, Mass. (introduced by W. H. Abelmann).

Attempts to relate electromotive forces of the heart to its hemodynamic function have thus far been unsuccessful. Nevertheless, the concept of "systolic overloading" is widely used. Its hemodynamic basis was investigated by left and right heart catheterization in 50 patients with congenital aortic and 40 with pulmonic stenosis (ages, 3 to 22). The sum of magnitudes of selected spatial vectors (SMSV) recorded during the apogee of the QRS loop by the corrected lead system of Frank was correlated with ventricular peak pressure, ventricular work, pressure time, and valve area. In aortic stenosis, increased left ventricular peak pressure is related in a linear fashion to SMSV ($r = 0.90$; SE = 15 mm Hg). Similar correlation existed with right ventricular peak pressure ($r = 0.89$; SE= 24 mm Hg) in pulmonic stenosis. An inverse relationship existed between SMSV and the logarithm of the aortic valve area ($r = -0.72$) and the pulmonary valve area ($r = -0.68$). Correlation with left or right ventricular stroke work was insignificant ($r = 0.24$, 0.32). No significant change in SMSV occurred during normal cardiac growth (weight from 15 to 300 g). Thus the linear increase in SMSV, once an elevated resting pressure is produced, shows that this vectorcardiographic measurement is capable of reflecting accurately the full range of resting peak pressure delivered by either ventricle. Furthermore, the less significant relationship with valve area, or stroke work, indicates that ventricular pressure by itself is the chief factor governing SMSV. These findings parallel Linzbach's histological studies and suggest that SMSV reflects the number of cells constituting the actively contracting myocardial syncytium required for the production of the resting ventricular systolic pressure.

Effect of Rapid Fructose Infusion in Man. WILLIAM C. ELLIOTT, LAWRENCE S. COHEN, MICHAEL D. KLEIN, FRANCIS J. LANE, AND RICHARD GORLIN,* Boston, Mass.

Rapid intravenous fructose infusion caused marked, immediate lactic acidemia. Administration of 10% fructose (0.5 g per kg in 5 minutes) resulted in elevations of average arterial lactate from 0.5 mmoles per L to 3.0 mmoles per L with pyruvate increase from 0.09 mmoles per L to 0.34 mmoles per L. This response was not seen with glucose infusion. From peak arterial levels between 120 and 200 mg per 100 ml, fructose was removed rapidly ($t_{\frac{1}{2}} = 15$ minutes) due to accelerated utilization by liver and adipose tissue. A 600% increase in arterial lactate compared to 340% pyruvate rise shifted the lactate:pyruvate ratio (L:P) from 5.5:1 to 9:1, largely due to splanchnic production. Hepatic vein levels even exceeded rising arterial lactate and pyruvate levels and were concurrent with increased hepatic venous oxygen saturation. Myocardial fructose utilization was minimal. Even with increased myocardial lactate and pyruvate extraction, L:P rose from artery to coronary vein (9:1 to 15:1). Average coronary venous oxygen saturation rose from 27 to 36%. Infusion was immediately followed by development of subxiphoid pressure pain with migration into the substernal area. The discomfort was usually severe, lasting up to 20 minutes, without electrocardiographic alteration or nitroglycerin relief. Fructose metabolism is not under usual glycolytic controls either when phosphorylated by nonspecific hexokinase or by fructokinase. Thus, glycolysis may proceed without inhibition at a rate in excess of oxidation, with apparent accumulation of cytoplasmic DPNH. This would appear to occur in the liver and to a lesser extent in cardiac muscle. The increase in hepatic and cardiac venous oxygen saturation may be due either to vasodilatation from lactic acidemia or to inhibition of oxidation. Fructose infusion is similar to hypoxia, with both incurring excessive reduction of DPN with resulting lactic acidemia. A remarkably similar chest pain occurs with both fructose administration and with myocardial ischemia. The interrelationships, if any, remain speculative at this time.

Effects of Cytopropane on Splanchnic Blood Flow and Metabolism in Normal Man. HENRY L. PRICE,* STANLEY DEUTSCH, LEE H. COOPERMAN, ANTHONY J. CLEMENT, AND ROBERT M. EPSTEIN, Philadelphia, Pa.

Since the splanchnic circulation is believed capable of an intense degree of vasoconstriction, measurement of the effect of agents that can precipitate this change is important. Of the anesthetic agents that have been examined in man, cyclopropane appears to have the most definite and consistent vasoconstrictor actions, and, therefore, its effects have been examined in the present study. The subjects were 10 healthy male fasting volunteers. Splanchnic blood flow was estimated using indocyanine green and circulating splanchnic blood volume using I^{131} albumin. Arterial P_{O_2}, P_{CO_2}, and splanchnic a-v differences of oxygen, lactate, pyruvate, and pH were also determined. After atropine (0.01 mg per kg, iv) control measurements were made over a 30-minute period, and anesthesia was then induced with cyclopropane in oxygen. All measurements were repeated after a steady anesthetic state had been attained. In the final period of study, all measurements were repeated 15 minutes after the intravenous administration of 10 mg of hexamethonium. No significant change in Pa_{O_2} occurred during the study, and Pa_{CO_2} was maintained within normal limits. The administration of cyclopropane consistently reduced blood flow and increased splanchnic vascular resistance. Oxygen consumption and hepatic venous oxygen tension were inconsistently reduced. Splanchnic blood volume was reduced in five of six cases. Excess lactate (Huckabee) was present in most cases, and there was a statistically significant inverse relation between splanchnic arteriovenous excess lactate and oxygen consumption. After the administration of hexamethonium splanchnic blood flow, vascular resistance, oxygen consumption, and excess lactate returned to, or toward, the initial levels. We conclude that cyclopropane induces neurogenic vasoconstriction of the splanchnic bed, thus reducing blood flow. Reduced oxygen consumption and increased lactate production may reflect local ischemia.

The Mechanism of Excretion of Bilirubin by the Kidney. MILFORD FULOP, JOHN SANDSON, AND PAUL BRAZEAU, New York, N. Y. (introduced by Irmin Sternlieb).

Renal function studies in dogs with obstructive jaundice have indicated that most of the urinary conjugated bilirubin is excreted by glomerular filtration. To determine the plasma source of urinary bilirubin, electrophoresis and dialysis of plasma and urine were performed. *1*) Plasma. Unconjugated bilirubin (up to 40 mg per 100 ml) was not dialyzable from human plasma and migrated entirely with albumin. Most of the plasma bilirubin in patients with conjugated hyperbilirubinemia (20 to 30 mg per 100 ml) migrated with albumin, but there was a small fraction that migrated with beta globulins. Dialysis of such plasma yielded faint yellow dialysates with maximal absorption at 435 to 445 mμ, and azo derivatives with absorption spectra typical of azobilirubin. This dialyzable plasma bilirubin had the solubility characteristics of conjugated bilirubin. In patients with normal renal function the ratio of dialyzable plasma bilirubin to total plasma bilirubin was similar to the ratio of urinary bilirubin clearance to endogenous creatinine clearance. In patients with renal failure there was an increase both in the dialyzable plasma bilirubin fraction and in the bilirubin associated with beta and alpha globulins. The bilirubin associated with these globulins decreased after dialysis of uremic jaundiced plasma. *2*) Urine. Urinary bilirubin was found to migrate mainly in the beta and alpha globulin zones and was dialyzable. These studies show that the dialyzable bilirubin in both plasma and urine is conjugated and is associated with beta and alpha globulins. They indicate that the dialyzable fraction of plasma conjugated bilirubin is the major source of urinary bilirubin. Plasma unconjugated bilirubin is not dialyzable and hence not available for renal excretion.

The Effect of Insulin on Lipolytic Activity in the Wall of Arteries. ROBERT MAHLER, Indianapolis, Ind. (introduced by William P. Deiss, Jr.)

The accumulation of fat in tissues is the result of their synthesis and deposition and of their rate of removal by lipolysis. Arterial tissue possesses a lipase capable of splitting triglycerides to glycerol and fatty acids. Since the glycerol so produced cannot be reused by arterial tissue, lipase activity can be estimated in terms of glycerol production from triglyceride. Aortas from male rats were incubated in a phosphate buffer, pH 7.4, containing a 0.4% emulsion of tributyrin. The addition of epinephrine (10 μg per ml) or norepinephrine (1 μg per ml) to the medium increased lipolytic activity by 100%. Insulin (100 μU per ml) by itself had no effect on glycerol production, but when insulin was present together with either of the catecholamines, the increase in lipolysis expected from their action did not occur. This effect of insulin is similar to its action on adipose tissue lipase. Rats were made diabetic with alloxan and maintained on insulin. Insulin deficiency was produced by withholding insulin up to 72 hours from the diabetic rats. Lipase activity in the aortas from these rats was significantly higher than in the blood vessels from diabetic rats that continued to receive insulin. These results show that insulin restrains the activation of lipase in arterial tissue just as it does in adipose tissue. It is possible that insulin, through restraining the activation of arterial lipase, permits the accumulation of fat in the arterial wall by causing an imbalance between the rate of fat synthesis and breakdown.

Leukokinetics Studies by Cross Circulation. E. D. THOMAS,* J. M. BURNELL, R. B. EPSTEIN, J. W. ESCHBACH, AND G. L. PLAIN, Seattle, Wash., and Cooperstown, N. Y.

The number of leukocytes transferred during cross circulation between a donor and a leukopenic recipient may be calculated from the volume of blood exchanged and the mean difference in leukocyte counts. In the first study a donor dog was cross circulated over a period of 7.6 hours with a series of 8 recipients made leukopenic by irradiation. The donor dog gave up 8.2×10^9 leukocytes per kg, of which 1.8 were PMN's, 4.9 were band forms, and 0.9 were lymphocytes. A second donor dog gave up 10.1×10^9 leukocytes per kg, of which 3.1 were PMN's, 5.4 were band forms, and 1.4 were lymphocytes. In the first 10 minutes of cross circulation the donor's leukocytes fell to 600 per mm^3 with quantitative transfer of circulating leukocytes without contribution from a marginal pool. Rapid mobilization then ensued, first of PMN's, then of band forms, but not of immature cells. After mobilization had been initiated, the donor's circulating leukocytes could not again be brought to low levels until exhaustion of leukocyte stores. The number of myeloid cells mobilized was 9 to 10 times the circulating pool; the number of lymphocytes, 5 to 6 times. In a third study a patient with marrow failure and a donor with renal failure were cross circulated for 2 hours daily for 13 days. During this time 14.7×10^9 leukocytes per kg were moved from donor to recipient. The recipient's leukocytes were elevated to 1,700 to 3,300 per mm^3 each day with decline at $t_{\frac{1}{2}}$ varying from 3 to 9 hours. Cross circulation was discontinued because of transient marrow hypoplasia and leukopenia in the donor, perhaps due to immunosuppressive therapy. The recipient died with marrow failure. These studies in dog and man establish the feasibility of a leukocyte transfer adequate to meet the needs of the leukocyte-depleted subject.

Pulmonary Capillary Blood Volume and Diffusing Capacity of the Pulmonary Membrane: Findings in Men with Emphysma Contrasted with Those in Men with Fibrosis. GEORGE N. BEDELL * AND ROBERT L. EGGERS, Iowa City, Iowa.

Pulmonary diffusing capacity (D_L) is reduced in patients with fibrosis and in patients with emphysema. The purpose of this study was to determine if the reduction in D_L is primarily a reduction in the volume of blood in the capillaries (V_c), or primarily a reduction in the diffusing capacity of the membrane (D_M), or an equal reduction in both in patients with these two disorders. Measurements of D_L, D_M, and V_c were made in 7 men with emphysema and in 7 men with diffuse fibrosis (Boeck's sarcoid, histoplasmosis, Hamman-Rich syndrome, and silo fillers disease) with the breath holding carbon monoxide technique at rest. In the patients with emphysema the mean D_L was 15 ml per mm Hg per minute (58% of predicted normal), the mean D_M was 41 ml per mm Hg per minute (56% of predicted normal), and the mean V_c was 37 ml per mm Hg per minute (38% of predicted normal). In the patients with fibrosis the mean D_L was 17 ml per mm Hg per minute (59% of predicted normal), the mean D_M was 25 ml per mm Hg per minute (34% of predicted normal), and the mean V_c was 74 ml (78% of predicted normal). Although the reduction in D_L was comparable in the two groups, the reduction in V_c in patients with emphysema was significantly ($p < 0.05$) greater than the reduction in V_c in patients with fibrosis. We conclude that in pulmonary fibrosis the reduction in D_L is produced primarily by a reduction in D_M and represents involvement of alveolar walls by the fibrotic process. In patients with emphysema one of the primary lesions is a destruction of the capillary bed, and this is manifested by a disproportionate reduction in V_c as compared to the reduction in D_L.

Force-Velocity Relations in the Human Heart. EDMUND H. SONNENBLICK, GERALD GLICK, ANDREW G. MORROW, AND EUGENE BRAUNWALD,* Bethesda, Md.

A fundamental mechanical property of skeletal and cardiac muscle can be expressed by the relationship between force generated and velocity of shortening, but the relevance of this concept to the function of the human heart has not heretofore been examined. Papillary muscles were obtained from seven patients under-

going mitral valve replacement and were studied *in vitro* with control of muscle length, afterload rate, and chemical environment with simultaneous measurement of developed force and velocity. The peak of the isometric length-tension curve occurred when the muscle was stretched 46% ± 4% (SEM) beyond initial length; further stretching produced a descending limb. Under afterloaded conditions, velocity of shortening varied inversely with load. Increasing muscle length augmented maximal isometric force (P_o) without altering maximal velocity of shortening (V_{max}). Raising contraction rate elevated only V_{max}, but norepinephrine and strophanthidin increased both V_{max} and P_o. Duration of contraction always varied inversely with V_{max}. In ten patients, radiopaque clips were sewn to the ventricular surface. Postoperatively, in the intact unanesthetized state, relative velocity of shortening was determined from cineradiographic analyses of clip motion, while intraventricular pressure was recorded simultaneously. The force-velocity relation could then be established by determining pressure and velocity of shortening at equal ventricular dimensions, thereby establishing a constant relationship between pressure and wall tension. When afterload was increased with methoxamine or angiotensin or decreased by impeding venous return, force and velocity of shortening varied inversely. Exercise, norepinephrine, isoproterenol, and increasing heart rate by electrical pacing augmented velocity of shortening at any given pressure. Thus, basic skeletal muscle mechanics have been extended to the human myocardium, studied both *in vitro* and in the intact state. The force-velocity relation has been shown to permit a useful characterization of the contractile properties of human heart muscle.

A Proposed Mechanism for the Waterhouse-Friderichsen Syndrome. JACK LEVIN AND LEIGHTON E. CLUFF,* Baltimore, Md.

The Waterhouse-Friderichsen syndrome is characterized by adrenal hemorrhage associated with sepsis, often due to the meningococcus. Although first described by Voelker 70 years ago, the mechanism of adrenal hemorrhage during septicemia remains unknown. A similarity between the Shwartzman phenomenon and mid-zonal adrenal hemorrhage in meningococcemia has been noted previously. While investigating mechanisms involved in the Shwartzman phenomenon, it was found that endotoxin injected intravenously into rabbits prepared with Thorotrast produced massive bilateral adrenal cortical hemorrhage, particularly when endotoxin was administered less than 10 hours after Thorotrast. Possible mechanisms responsible for the localization of hemorrhage to the adrenal cortex, induced by Thorotrast and provoked by endotoxin, were investigated. Thorotrast, sepsis, or endotoxin might stimulate adrenal cortical activity, predisposing the adrenal to the hemorrhagic reaction. Experiments revealed that ACTH induced a similar predisposition of the adrenal cortex to hemorrhage when endotoxin was injected within a few hours after injection of the tropic hormone. Furthermore, adrenal hemorrhage after intravenous injection of endotoxin into ACTH prepared animals was most prominent in the mid-zone of the adrenal cortex. Suppression of adrenal cortical function by prior daily injections of corticosteroid resulted in inhibition of the hemorrhagic adrenal response to endotoxin in Thorotrast or ACTH prepared animals. Administration of multiple doses of hydrocortisone soon after injection of Thorotrast or ACTH suppressed the adrenal hemorrhagic reaction following injection of endotoxin. The possible relation of adrenal medullary response to the hemorrhagic adrenal reaction is being investigated. Single injections of Thorotrast, ACTH, or endotoxin did not induce adrenal hemorrhage similar to that observed when endotoxin was given shortly after Thorotrast or ACTH. We conclude that adrenal hemorrhage observed during sepsis, as in the Waterhouse-Friderichsen syndrome, may be attributable to endotoxemia occurring during or shortly after stimulation of the adrenal cortex by infection.

Studies on the Mechanism of Action of Aldosterone. GEORGE A. PORTER, RITA BOGOROCH, AND ISIDORE S. EDELMAN,* San Francisco, Calif.

Based on studies of the time-course of uptake and elution of H^3-aldosterone, the nuclear localization of aldosterone by radioautography, and the inhibition of the action of aldosterone by actinomycin D and puromycin, we proposed previously that aldosterone stimulates active Na^+ transport by inducing *de novo* synthesis of enzymes coupling metabolism to Na^+ transport via an increased rate of DNA-mediated nuclear synthesis of RNA. Additional evidence supporting this hypothesis has been obtained from studies on incorporation of H^3-uridine, a precursor of RNA, in the isolated urinary bladder of the toad *Bufo marinus*. In hemibladders exposed continuously to H^3-uridine, addition of aldosterone to the medium (7×10^{-7} M) increased incorporation of uridine into the perchloric acid (PCA) insoluble phase of the whole hemibladder by 28% over control values at 90 minutes, by 43% at 180 minutes, and by 58% at 300 minutes. In separated epithelial cells prepared by treatment of hemibladders with EDTA, exposed continuously to H^3-uridine, aldosterone-stimulated hemibladders incorporated 33% more uridine at 60 minutes and 53% more at 240 minutes than the control hemibladders. With pulse labeling (30 minutes) with H^3-uridine, addition of aldosterone increased the incorporation of uridine into RNA (defined by ribonuclease treatment) by 20% over control values at 90 minutes and by 52% at 180 minutes. Radioautographs of hemibladders after a pulse of H^3-uridine showed a greater concentration of grains in the epithelial nuclei of aldosterone-treated hemibladders than in the nuclei of the controls at 90, 180, and 360 minutes. Simultaneous measurement of the rate of active Na^+ transport showed that the aldosterone-induced increase in RNA synthesis preceded the onset of action on Na^+ transport in accord with the hypothesis.

The Hepatic Gluconeogenic "Set" as a Regulator of the Effects of Alcohol upon Glucose Production. NORBERT FREINKEL,* ALEX K. COHEN, RONALD A. ARKY, AND ANGELA E. FOSTER, Boston, Mass.

Alcohol can cause both hypo- and hyperglycemia. The present studies examined whether these bidirectional effects may be conditioned, at least in part, by the intrahepatic gluconeogenic "set." Liver slices from fed or fasted rats were incubated in Krebs-Ringer-bicarbonate (KRB) or -phosphate (KRP). Effects of ethanol (10 mM) upon radioactive products from "tracer" (0.01 mM) or "substrate" (10 mM) alanine-U-C^{14} (AL*) were assessed under varying conditions of pyruvate availability and turnover. *1*) "Tracer" AL*, KRB, fasted rats. With amounts of AL* that did not alter endogenous 3-carbon pools, ethanol reduced $C^{14}O_2$ and glucose-C^{14}. Unlabeled 6-carbon compounds (10 mM) augmented the inhibition of glucose-C^{14} in accord with DPNH-generating capacities (sorbitol > fructose and glucose). Unlabeled pyruvate (10 mM) reversed the inhibition and boosted glucose-C^{14} above control values. *2*) "Substrate" AL*, KRP, all rats. With media that poorly supported pyruvate turnover, ethanol further depressed $C^{14}O_2$ and glucose-C^{14}. *3*) "Substrate" AL*, KRB, fed rats. With media that supported pyruvate turnover, and tissues rich in endogenous substrates, ethanol increased glucose-C^{14} despite modest reductions of $C^{14}O_2$. *4*) "Substrate" AL*, KRB, fasted rats. With tissues depleted of endogenous substances and adapted to fat oxidation, changes in glucose-C^{14} correlated linearly with $C^{14}O_2$. When $C^{14}O_2$ was depressed more than 50%, glucose-C^{14} was reduced. With lesser inhibition of $C^{14}O_2$, glucose-C^{14} was unaffected or augmented. *Conclusions:* The prevailing gluconeogenic set conditions ethanol actions upon hepatic glucose production. When low, gluconeogenesis may be further dampened by the necessity for intramitochondrial reoxidation of most of the cytoplasmic DPNH derived from alcohol oxidation. When high, the possibility for extramitochondrial hydrogen transfer via reductive biosynthesis (1,3-diphosphoglycerate⟶ glyceraldehyde-3-phosphate) may abet gluconeogenesis. The latter could contribute to the disparate preservation of body fat in certain alcoholics, since facilitation of gluconeogenesis from amino acids would pre-empt lean body mass and redistribute it as fat.

The Genetic Code of Mammalian Cells. MANUEL OCHOA, JR., AND I. BERNARD WEINSTEIN, New York, N. Y. (introduced by John V. Taggart).

The mechanism of protein synthesis is intimately related to both neoplastic and inherited metabolic diseases in man. The present study indicates that, as in *E. coli*, synthetic messenger RNA's can direct protein synthesis in extracts of human chronic lymphocytic leukemia, mouse L1210 leukemia, and normal rat liver cells. A group of synthetic RNA's, differing in their content of adenylic (A), cytidylic (C), guanylic (G), and uridylic (U) acids, have been tested with seven C^{14}-amino acids in the L1210 system. The results indicate that the minimal number of nucleotides necessary to code for each amino acid is as follows: for phenylalanine, U; for leucine, either U plus G, U plus A, U plus C, or U alone; for serine, U plus C; for tyrosine, U plus A; for isoleucine, U plus A; for valine, U plus G; and for lysine, A. Qualitatively similar results were obtained with the other mammalian systems. These data are consistent with the coden assignments for *E. coli* and provide evidence for the concept of "universality of the genetic code." Sucrose gradient fractionation of L1210 microsomes indicated that whereas native protein synthesis occurs predominantly on polyribosomes, both single ribosomes and polyribosomes can engage in protein synthesis directed by synthetic messenger RNA's. The leucine-phenylalanine "ambiguity," i.e., the response of both amino acids to a sequence of uridylic acids, was found to be a function of microsome size. Studies with fractionated microsomes also indicated that polyadenylic acid, in addition to coding for lysine, appears to enhance the function of endogenous messenger RNA's. Taken together, these findings indicate that malignant transformation of cells is not accompanied by major alterations in the genetic code or in the over-all mechanism of protein synthesis.

Studies of the Hemolytic Effect of Hyperoxia in Vivo. CHARLES E. MENGEL AND HERBERT E. KANN, Durham, N. C. (introduced by Albert Heyman).

The lytic effect of hyperoxia on erythrocytes is of current interest with use of 100% oxygen environments in space capsules and development of hyperbaric-oxygenation (OHP) for medical purposes. Erythrocytes of mice and humans were studied before and after *in vivo* OHP for *1*) hematologic index, *2*) components of oxido-reduction transformation systems, *3*) sensitivity to *in vitro* oxidant stress (H_2O_2), *4*) lipid peroxides, and *5*) glycolytic intermediates. Vitamin E-deficient mice exposed to 100% oxygen at 60 psia for 1.5 hours developed fall of hematocrit (50% to 28%), marked hemoglobinemia, and mechanical fragility of erythrocytes. Lipid peroxides were demonstrated in their erythrocytes after OHP (even with administration of vitamin E after OHP before exsanguination and collection of blood in quantities of vitamin E known to prevent *in vitro* lipid peroxidation). No hemolysis or lipid peroxide formation occurred during OHP in mice supplemented with vitamin E. No changes occurred in blood methemoglobin and reduced glutathione content or erythrocyte glucose-6-phosphate-dehydrogenase and catalase activities. Of twenty-one patients and normal subjects exposed to 100% oxygen at 30 to 45 psia for 20 to 40 minutes, one whose erythrocytes showed an abnormal increase of lysis and lipid peroxide formation during incubation with H_2O_2 developed hemolysis after OHP. Erythrocytes of six others showed increased autohemolysis after OHP. Three additional patients were exposed to 100% oxygen at 30 to 45 psia for 8 to 10 hours. After OHP their erythrocytes contained less ATP and more ADP,

fructose-1,6-diphosphate, and inorganic phosphate when compared to pre-OHP levels. The data demonstrate *in vivo* hemolysis, peroxidation of lipids, and changes in levels of glycolytic intermediates in erythrocytes during *in vivo* hyperoxia. We postulate that lysis may result either directly from peroxidation of erythrocyte lipid or indirectly from interference with glycolysis by lipid peroxides (known to be capable of inactivating sulfhydryl-bearing enzymes), or from both.

Steady-State Diffusing Capacity as a Measure of Pulmonary Emphysema. M. HENRY WILLIAMS, JR.,* AND MICHEL JANIS, New York, N. Y.

Previous studies have provided indirect evidence that reduction of the steady-state diffusing capacity for carbon monoxide (D_{CO}) is an indication of the extent of emphysema present within the lungs of patients with chronic obstructive pulmonary disease. To obtain direct evidence of this relationship, we studied at autopsy the lungs of 11 patients who had died of or with chronic obstructive pulmonary disease and had received extensive studies of pulmonary function during the year of life prior to death. The extent of emphysema present in these lungs was quantitated by dividing 3 sections of each formalin-inflated lung into 1-inch squares with a plastic grid and grading each square on a 0 to 3 scale. The average grade for each lung was then computed and compared to the previously measured D_{CO}. There was a highly significant negative correlation ($r = -.87$) between the D_{CO} and the grade of emphysema. Since previous studies of larger groups of patients had revealed no significant correlation between reduction of the diffusing capacity and other abnormalities of pulmonary function, and since in this small group of patients there was no significant correlation between the diffusing capacity and either the maximal mid-expiratory flow rate or the dead space/tidal volume ratio, reduction of the steady-state diffusing capacity appears to be an established index of the extent of emphysema present in the lungs of patients with chronic obstructive pulmonary disease.

The Release of SRS Activity from Guinea Pig Lung by Cobra Venom Phospholipase A and Lysolecithin. GERALD B. PHILLIPS* AND ELLIOTT MIDDLETON, JR., New York, N. Y.

Addition of specific antigen *in vitro* to sensitized guinea pig lung results in the release of histamine and a slow-reacting substance (SRS). This release can be blocked by enzyme inhibitors. Cobra venom (*Naja naja*) added to nonsensitized guinea pig lung also causes the release of histamine and a SRS. To determine which enzyme(s) in the venom is responsible for this release, a neutral aqueous solution of the venom was heated at 100° for 15 minutes before addition to the lung. This treatment apparently destroys all enzyme activity except for phospholipase A. The presence of phospholipase A activity in the heated preparation was demonstrated by its lytic effect on human red blood cells with an absence of such an effect on sheep red blood cells. Since this heated venom preparation added to nonsensitized guinea pig lung resulted in the release of histamine and a SRS, this action of venom appeared to reside in its phospholipase A. Since phospholipase A catalyzes the hydrolysis of lecithin to lysolecithin, the effect of lysolecithin on guinea pig lung was tested. Lysolecithin was shown previously to cause histamine release. In this study, it was found also to effect the release of a SRS with properties similar to that released by venom and antigen-antibody reaction. The possible role of phospholipase A and lysolecithin in tissue antigen-antibody reactions thus is suggested.

Mechanisms of Action of Low Molecular Weight Dextran upon Blood Viscosity during Flow Stasis. ROE E. WELLS, JR., Boston, Mass. (introduced by E. C. Eppinger).

Studies of the flow properties of blood have shown that the viscosity of blood under conditions of stagnant flow (approaching zero rate of shear) increases 5 to 10 times over that of normal flow. The strength of the static fluid structure of blood is a function of protein-red cell networks and cell aggregation. There is evidence that these changes are directly related to failure of nutrient capillary flow in low flow states such as shock and thromboembolism. Low molecular weight (LMW) dextran (average mol wt, 40,000) has been shown to significantly reduce blood viscosity by dilution during plasma volume expansion. The influence of LMW dextran upon the fluid structure of blood under stagnant flow was studied by means of low shear viscometry (shear rate, 0.1 to 21 inverse seconds) of blood freshly drawn from patients receiving dextran for therapy of major tissue or organ ischemia. Measurements were made of erythrocyte aggregation *in vitro* and *in vivo* (by photomicrography), hematocrit, erythrocyte sedimentation rates, serum protein fractions, and plasma fibrinogen and were correlated with viscometry. Immediately after rapid infusion of 500 ml of LMW dextran 200 (Rheomacrodex) in 30 minutes, blood viscosity values dropped to as low as 50% of control values. Viscosity dependence on shear rate was markedly reduced during the peak effect of dextran. Hematocrit and serum protein values decreased to the same relative degree as viscosity, but onset and peak of change were later than those of the viscosity changes. Erythrocyte aggregation in bulbar conjunctival vessels was considerably improved after dextran infusion. These data suggest that before the dilutional effects of LMW dextran the fluid structure of blood at very low rates of shear is significantly altered, due probably to reduction of interactions between erythrocytes and plasma proteins.

Feedback Inhibition of Deoxyribonucleotide Interconversion in Human Leukocytes. ROBERT SILBER, New York, N. Y. (introduced by Jonathan W. Uhr).

The control mechanisms of reactions leading to DNA synthesis in the human leukocyte are poorly understood.

The enzymatic interconversions of DNA precursor pyrimidine deoxyribonucleotides are summarized in the following scheme: deoxycytidine-5′-PO_4 (dCMP) $\xrightarrow{1}$ deoxyuridine-5′-PO_4 (dUMP) $\xrightarrow{2}$ thymidine-5′-PO_4 (TMP) $\xrightarrow{3}$ thymidine triphosphate (TTP). The presence of this pathway has been established in human leukocytes. The current investigation describes the regulatory control exercised by the end product TTP, through feedback inhibition of dCMP deaminase, the enzyme catalyzing reaction (1). Leukocyte dCMP deaminase was very labile in crude homogenates, over 90% of activity being lost after storage at 4° C for 16 hours or during fractionation with ammonium sulfate. No loss of activity occurred on storage when the enzyme was stabilized with the sulfhydryl reagent 2-mercaptoethanol or by deoxycytidine triphosphate (dCTP). TTP (10^{-4} M) completely inhibited dCMP deaminase activity without competing with the substrate. This inhibition could be prevented by dCTP, 2-mercaptoethanol, or Mg^{++} ion. dCMP deaminase was also inhibited by the sulfhydryl group inhibitor, *p*-hydroxymercuribenzoate (pHMB), in a concentration of 10^{-4} M. dCTP and Mg^{++} reverse the inhibitor action of pHMB. Apparently therefore, TTP, dCTP, and pHMB compete for a site on the enzyme which is not the active site, but is an "allosteric" site, sensitive to feedback control through conformational changes in enzyme structure. The sulfhydryl-sensitive, Mg^{++} stabilized, allosteric site on the first enzyme of this biosynthetic series offers a means of controlling the rate of the entire pathway through feedback inhibition by TTP. The enzyme from normal and leukemic leukocytes had identical control mechanisms.

Inhibition of Nucleic Acid Synthesis: A Possible Mechanism for Aminonucleoside Nephrosis in Rats. CARL S. ALEXANDER, NESTOR DICKIE, AND HERBERT T. NAGASAWA, Minneapolis, Minn. (introduced by Wendell H. Hall).

The biochemical basis for the induction of the nephrotic syndrome in rats by puromycin aminonucleoside (PA) is unknown. Our results using a model system of osmotically active spheroplasts of *E. coli* B indicate that PA inhibits *de novo* purine synthesis. Thus, a 5×10^{-3} M PA incorporation of C^{14} from glycine-2-C^{14}, $HC^{14}OONa$, and $NaHC^{14}O_3$ into nucleic acid adenine was reduced 50% in lysozyme-treated cells. Under the same conditions, incorporation of C^{14} from adenine-8-C^{14} and hypoxanthine-8-C^{14} into nucleic acid adenine was not inhibited, and incorporation of 5-NH_2-4-imidazole-carboxamide-2-C^{14} into the nucleic acid fraction was reduced only 30%. Inhibition in RNA and DNA synthesis was indicated by reduced incorporation of radioactivity from uracil-2-C^{14} (50%) and thymidine-H^3 (50%) into acid-insoluble form, and from $NaHC^{14}O_3$ into nucleic acid UMP (70 to 80%). Thus, *de novo* purine biosynthesis was blocked at a point before closure of the ring. Protein synthesis was not inhibited under conditions of the experiment. Since incorporation of radioactivity from PA-8-C^{14} into nucleic acids was minor (<1%), PA-induced inhibition in nucleic acid synthesis in *E. coli* and perhaps in the rat is interpreted in terms of false feedback inhibition of purine biosynthesis. Adenine, like PA, also inhibits *de novo* purine biosynthesis but, unlike PA, is incorporated into nucleic acids. This could explain its protective action in PA-induced nephrosis. Studies with acetylated derivatives of PA in this *E. coli* spheroplast system revealed that monoacetyl PA was somewhat less effective than PA and that the triacetyl derivative was least effective. Neither acetylated derivative was nephrotoxic in the rat.

Black Liver Disease in Corriedale Sheep: A New Mutation Affecting Hepatic Excretory Function. IRWIN ARIAS,* LESLIE BERNSTEIN, ROBERT TOFFLER, CHARLES CORNELIUS, ALEX B. NOVIKOFF, AND EDWARD ESSNER, New York, N. Y.

Mutant Corriedale sheep demonstrate photosensitivity, mild and intermittent conjugated hyperbilirubinemia, and a black liver. Photosensitivity results from retention of phylloerythrin, a porphyrin derived from chlorophyll and normally excreted in the bile. Phylloerythrin levels were increased in the blood and liver and were decreased in the bile in the mutants as compared to normal Corriedale sheep. The total serum bilirubin concentration was .5 to 2.2 mg per 100 ml (normal: 0 to .6 mg per 100 ml), and the serum bilirubin was mainly conjugated. The relative hepatic storage of sulfobromophthalein (BSP) was normal (30.2 ± 8.0 mg BSP stored per mg per 100 ml BSP in plasma); hepatic Tm BSP was 19 to 25% of normal (normal: 16.3 ± 2.5 mg BSP excreted per minute), and BSP was retained in plasma as a conjugate. The gall bladder failed to visualize radiographically after ingestion of twice the dosage of iopanoic acid required for successful cholecystography in normal Corriedale sheep. Serum alkaline phosphatase, glutamic oxaloacetic and glutamic pyruvic transaminase activities, and the concentration of albumin, globulin, and cholesterol were normal. Morphological studies revealed pigment-containing granules in centrolobular parenchymal cells and Kuppfer cells; the hepatic architecture was otherwise normal. Acid phosphatase and tyrosinase activities were two to three times greater in homogenates of mutant liver than in homogenates of normal sheep liver. Pigment granules were isolated by sucrose density gradient centrifugation and separated from acid phosphatase and tyrosinase activities. The pigment was partially purified by alkaline hydrolysis and acid precipitation and solubilized in alkali. UV absorption spectra were similar to those described for melanin with a peak at 265 to 267 mμ. The alkaline extract was dialyzed and the pigment extracted into cyclohexanone, dried, dissolved in ethyl acetate, and filtered. After addition of petroleum ether, black granules formed. The granules melted at 200 to 210° C, had solubility characteristics of melanin, and, in alkaline solution, demonstrated an absorption peak at 267 mμ. Histochemical staining reactions of the

pigment were also consistent with melanin. These functional and morphologic findings are similar to those observed in the Dubin-Johnson syndrome.

General Correlation of Serum Alkaline Phosphatase and Urinary Hydroxyproline in Metabolic Bone Diseases. LeRoy Klein, Cleveland, Ohio (introduced by Max Miller).

Albright interpreted serum alkaline phosphatase in the absence of liver disease to be high in those conditions where matrix (skeletal collagen) is being laid down in excess, e.g., during growth, rickets, osteitis fibrosa, Paget's disease, and osteomalacia. Several laboratories have reported increased levels of urinary hydroxyproline peptides in growth, rickets, osteitis fibrosa, and Paget's disease. Similar observations have been made in our laboratory in a patient with osteomalacia. The above data indicate an association between serum alkaline phosphatase and urinary hydroxyproline. It has been documented that serum alkaline phosphatase is elevated during pregnancy and healing of long bone fractures. The present report is concerned with directly correlating serum alkaline phosphatase and urinary hydroxyproline by serial studies during pregnancy and during fracture repair. Seven subjects during weeks 19 to 33 of pregnancy showed a simultaneous increase of serum alkaline phosphatase (from 4.2 to 8.3 King-Armstrong units) and urinary hydroxyproline (from 25.8 to 50.2 mg per 24 hours). Three patients with undisplaced femur fractures demonstrated during their first 3 weeks of fracture healing simultaneous increases of serum alkaline phosphatase (5 to 20, 6 to 12, and 4 to 18 King-Armstrong units) and urinary hydroxyproline (65 to 165, 55 to 129, and 37 to 97 mg per 24 hours). These changes were unrelated to the urinary calcium levels. The findings demonstrate the close correlation between serum alkaline phosphatase and urinary hydroxyproline and support the hypothesis that urinary hydroxyproline mainly reflects *de novo* skeletal collagen synthesis. The poor correlation of urinary hydroxyproline to urinary calcium levels suggests that most of the skeletal collagen is turning over without being proteolytically destroyed during bone resorption.

An Alteration in Depot Fat Composition Associated with Obesity. Herbert A. Haessler and John D. Crawford,* Boston, Mass.

To elucidate some of the mechanisms associated with fat accumulation, we have used gas liquid chromatography to study the fatty acid composition of epididymal fat from rats with electrolytic lesions in the ventromedial nuclei of the hypothalamus. In young adult male rats lesion placement has been followed by striking changes in fat composition. Similar alterations were absent in sham-operated, thyroidectomized, adrenalectomized, and hypophysectomized animals. The largest changes were in palmitic and linoleic acids. Whereas each ordinarily constituted 23% of the total fatty acids, in lesioned animals palmitic rose to 33% and linoleic fell to 15% of the total. The linoleic : palmitic ratio lay below 0.55 in lesioned animals and above 0.85 in normals. Serial biopsies showed changes in the linoleic : palmitic ratio as early as 3 days after lesion placement, and 70% of the ultimate change had occurred within 1 week, long before development of substantial obesity. With continued free feeding lesioned animals became very obese, their weight tending to stabilize after 10 weeks. At this time up to 63% of their dry weight was fat, controls averaging 20%, and an inverse linear relationship obtained between the linoleic : palmitic ratio and the total body fat content. Limited isocaloric feeding of intact and lesioned animals yielded a correlation between rate of weight loss and the linoleic : palmitic ratio. On 30 calories per day animals having ratios of 1.0 lost between 5 and 6 g, whereas those with ratios of 0.4 lost only 2 g daily. We conclude that these animals have not only hyperphagia but an alteration in metabolism predisposing to fat accumulation, retention, and change in composition. Other types of obesity may have metabolic abnormalities reflected in a similar manner.

Genetic Abnormalities in Hereditary Angioneurotic Edema. Patricia Charache, Virginia Donaldson, Jack Pensky, Philip Fireman, Fred S. Rosen, and Charles A. Janeway,† Boston, Mass., and Cleveland, Ohio.

Hereditary angioneurotic edema (HAE) is characterized by periodic local edema with abdominal pain and deficiency of an inhibitor of the esterase derived from the first component of complement. C'1 esterase hydrolyzes *n*-acetyl tyrosine ethyl ester. Donaldson and Evans showed that normal sera inhibit this hydrolysis but that sera of patients with HAE have no inhibitory activity. During attacks, serum complement is altered by decreased levels of C'1 associated with a rise in free esterase activity, and C'2 and C'4 are destroyed. The inhibitor is an acid-labile 3.0 S alpha globulin according to Pensky and Lepow. Antibody prepared against partially purified human C'1 esterase inhibitor not only precipitated with inhibitor but blocked its ability to inhibit esterase activity. Upon immunochemical examination with this antibody, sera of 18 HAE patients in 7 kindreds showed markedly reduced concentrations of inhibitor protein. Other studies in our laboratories indicate that these low levels of inhibitor are due to decreased synthesis. Since HAE is transmitted as an autosomal dominant characteristic, afflicted individuals are heterozygous. In this disease the heterozygous individual has significantly less than the expected 50% of the normal concentration of the deficient protein. In contrast to the majority of kindreds, 5 patients from another family, with no detectable inhibitor activity by enzymatic assay, showed normal levels of antigen when tested immunochemically. This protein deficiency disease therefore has two variants: the first and usual type due to diminished synthesis of inhibitor protein; the second, due to synthesis of abnormal protein. The abnormal protein appears to differ from normal inhibitor in certain properties.

Role of Carbon Dioxide in the Production of Reactive Hyperemia. HERMES A. KONTOS, H. PAGE MAUCK, JR., AND JOHN L. PATTERSON, JR.,† Richmond, Va.

The mechanism of reactive hyperemia in response to ischemia of ½ to 2 minutes, largely unknown, was investigated in the hind limb of 43 anesthetized dogs and in the forearm of 16 normal subjects. Blood flow was measured plethysmographically in man and with an electromagnetic flowmeter in the dog. During reactive hyperemia in the human forearm, the P_{CO_2} of venous blood showed an increase and subsequent decline that correlated well with the changes in blood flow. Similar results were obtained in the dog for femoral venous blood during reactive hyperemia of the hind limb. Reactive hyperemia was abolished in 5 dogs and markedly diminished in 3 during hypocapnia induced by hyperventilation. This effect was dependent upon the production of hypocapnia, since it did not occur when sufficient CO_2 was added to the inspired air to prevent fall in arterial P_{CO_2}. It was also not due to decrease in the reactivity of the vessels, since the vasodilation due to intra-arterial acetycholine or bradykinin was increased during hypocapnia. Similarly, reactive hyperemia in the human forearm of 8 subjects was decreased by an average of 43% during hypocapnia induced by voluntary hyperventilation, but intravenous infusion of sodium bicarbonate in 5 subjects had no significant effect, although the alkalosis produced was comparable to that seen during hyperventilation. After acetazolamide administration in 4 dogs, reactive hyperemia increased by an average of 50%. Intra-arterial injection of nicotine abolished reactive hyperemia in 7 dogs and decreased it in 6. This effect of nicotine was accomplished by a decrease in CO_2 production by the tissues of the hind limb. We conclude that the accumulation of CO_2 in the ischemic tissues is the most important factor in the production of reactive hyperemia after short periods of ischemia in skeletal muscle and that this effect of CO_2 is not mediated by the production of acidosis.

Absorption of Radioactive Cholesterol and Turnover Rate of Cholesterol Esters in Human Subjects and Rats. KAROLY G. PINTER, J. G. HAMILTON, AND GRACE A. GOLDSMITH,† New Orleans, La.

Three male volunteers were hospitalized in a metabolic ward and given isocaloric diets containing 100 g of butter or cottonseed oil. After 3 weeks each individual received 200 μc of C^{14}-cholesterol in an emulsion containing 50 g butter or cottonseed oil according to the type of dietary fat. Each subject was studied with both kinds of fat. Blood was drawn at 0, 3, 6, 9, 12, 15, 24, and 48 hours after consumption of the emulsion. Twenty-six rats, conditioned to consume their food within ½ hour, were given diets containing 2 g of either butter or cottonseed oil and 4.5 μc of C^{14}-cholesterol. They were sacrificed in pairs at intervals up to 24 hours. All serum and liver samples were analyzed for cholesterol and cholesterol esters. In both humans and rats cholesterol was absorbed more rapidly and completely when cottonseed oil was administered; the specific activity of serum cholesteryl linoleate increased to high levels, cholesteryl palmitate and oleate to a lesser extent. When butter was given, serum cholesteryl palmitate and oleate rose moderately but with a smaller increase in linoleate. In rats fed cottonseed oil, specific activity of liver cholesteryl linoleate increased more than that of other esters. As a result of hydrolysis of the esters, specific activity of free cholesterol rose to a relatively high level. When butter was administered, specific activity of liver cholesteryl palmitate increased rapidly, whereas free cholesterol rose slowly. These observations indicate a marked and immediate influence of dietary fats on serum and liver cholesterol ester pattern and suggest that cholesteryl linoleate has a faster rate of hydrolysis, *in vivo*, than other cholesterol esters.

Metabolic Control of Cell pH. SHELDON ADLER, ARLENE M. ROY, AND ARNOLD S. RELMAN,* Boston, Mass.

We recently reported that the mean $[H^+]$ inside resting skeletal muscle (calculated from the distribution of C^{14}-DMO in rat diaphragm *in vitro*) is normally three times that of extracellular fluid and remains constant despite progressive reduction in external $[HCO_3^-]$ to 9 mEq per L or increase in P_{CO_2} to 70 mm Hg. Beyond this degree of acidosis, and at all significant degrees of extracellular alkalosis, cell $[H^+]$ varies linearly with external $[H^+]$. We have now found that the normal cell $[H^+]$, as well as its characteristic responses to changes in P_{CO_2} or external $[HCO_3^-]$, is critically dependent upon cell metabolism. Inhibition of respiration and glycolysis by anoxia plus iodoacetate increases cell pH when external $[HCO_3^-]$ is normal and furthermore renders muscle freely permeable to external H^+ or HCO_3^- ions. When muscle is incubated at 4° C, thus suspending metabolism but preventing deterioration of the membrane, the pH at normal $[HCO_3^-]$ rises to the same degree. The membrane, however, is virtually impermeable to HCO_3^- ions because cell pH is hardly affected by very wide variations in external $[HCO_3^-]$. The normal shape of the CO_2 titration curve of muscle also disappears at 4° C, and cell $[H^+]$ becomes a simple linear function of P_{CO_2} throughout. Cold has no significant effect on cell $[H^+]$ when metabolism has been previously inhibited by anoxia plus iodoacetate. These data suggest that the control of cell pH is a metabolic function, rather than the simple result of the interposition of a semipermeable membrane between two buffer systems. Under physiologic stress there may be variations in the permeability of the membrane to HCO_3^- or H^+ ions or in the internal production or disposition of acid. Both of these phenomena appear to be closely linked to endergonic cellular reactions.

Glomerular Lesions Induced by Intravenous Injection of Streptococcal M Protein. FRED S. KANTOR, New Haven, Conn. (introduced by Elisha Atkins).

Crude M protein extracts of group A streptococci have been previously shown to contain a fibrinogen precipitating factor. The present report concerns the nature of

this factor, the stoichiometry of the reaction with fibrinogen, and the biological effects of M protein injection into mice and rats. Type I streptococcal M protein was partially purified and precipitated with human fibrinogen. The precipitate was repeatedly washed and then used to immunize rabbits by injection in complete Freund's adjuvant. The resulting sera were shown to contain bactericidal and mouse protective antibodies against type I streptococci, indicating the identity of the M protein and fibrinogen precipitating factor. The stoichiometry of the reaction between purified M protein and fibrinogen was studied by trace labeling M protein with I^{131}. Precipitants demonstrated no constant proportion between M protein and fibrinogen. Within 24 hours after injection of 3 mg of M protein into mice and 10 mg into rats, renal lesions were observed consisting of eosinophilic, hyaline depositions of material within the glomerular capillary bed. This material was identified as M-fibrinogen complexes by immunofluorescent technique. One day after injection, rats developed proteinuria and urea retention, but both anomalies disappeared by the sixth day. However, on the eighth to tenth days animals developed secondary proteinuria and blood urea retention, at which time circulating anti-M antibodies were demonstrable. This model is compared to and contrasted with human poststreptococcal glomerulonephritis.

The in Vitro Effect of Bacterial Metabolites and Antibiotics on Human Pancreatic Lipase Activity. SATISH K. MEHTA, ELLIOT WESER, AND MARVIN H. SLEISENGER,* New York, N. Y.

Bacteria are known to play a role in some forms of steatorrhea, although the mechanisms involved are poorly understood. Interference with normal digestion or absorption of fat, or both, are possible mechanisms. In the present study the *in vitro* effect of bacterial metabolites on hydrolysis of long chain triglyceride by human pancreatic lipase was investigated. Human pancreatic juice, free of bile, was obtained from a patient with a fistulous communication from the pancreas to the exterior, and lipase activity was determined by a turbidimetric method. Human pancreatic lipase was active over a pH range of 7.4 to 10.0, with a maximal activity at pH 8.8. Cholic acid, deoxycholate, taurocholate, taurodeoxycholate, and glycodeoxycholate all lowered the optimal pH. Tryptamine (3×10^{-3} M) and sodium lactate (2.8×10^{-4} M) produced 40% inhibition. Preincubation of lipase with indole at room temperature increased the degree of inhibition proportional to the duration of incubation. No inhibition was found with pyruvic acid, 5-HIAA, or indolelactic acid. Neomycin (2 mg per 100 ml), bacitracin (2 mg per 100 ml), polymyxin (.05 mg per 100 ml), streptomycin (.45 mg per 100 ml), and kanamycin (3 mg per 100 ml) markedly inhibited lipase activity, whereas tetracycline, chloramphenicol, and sulfisoxazole had no effect. The results indicate that some products of bacterial metabolism are capable of inhibiting pancreatic lipase activity *in vitro*. Whether this also occurs *in vivo* must await further study. Bacteria may also inactivate lipase by lowering intestinal pH. An *in vivo* effect of neomycin, similar to that demonstrated *in vitro*, may possibly explain the steatorrhea induced by this drug.

Isolation and Characterization of a Cholesterol-Lecithin Complex from Bile. DEWEY H. NEIDERHISER, HAROLD P. ROTH, AND LESLIE T. WEBSTER, JR.,* Cleveland, Ohio.

Cholesterol, an important constituent of gallstones, is transported in bile in an unknown form. Lecithin, bile salts, bilirubin, and protein have been proposed individually and in various combinations as complexing agents. Some investigators have attributed the differing electrophoretic mobilities of cholesterol in gallbladder and hepatic bile to different complexes. We purified the cholesterol component of bile to study its characteristics. When human or swine gall-bladder bile or human hepatic bile was chromatographed on Sephadex-G-75, essentially all of the cholesterol emerged with lecithin in opalescent fractions near the solvent front. After the opalescent material from swine bile was recycled twice through Sephadex, the initial fractions again contained cholesterol and lecithin in constant ratio and were essentially free of bile salts, bilirubin, and protein. Free cholesterol is eluted subsequently. Thus, cholesterol and lecithin appeared to be in a large molecular complex. Whether derived from human gall bladder or hepatic bile, the purified cholesterol-lecithin fraction showed no electrophoretic mobility at pH 8.6. After taurocholate was added, the complex migrated towards the anode (a characteristic of cholesterol in native gall-bladder bile). Free cholesterol-C^{14} was incorporated into the swine complex. Addition of bile salts was not required. Net incorporation of free cholesterol occurred also; after incubation the reisolated complex showed a lecithin:cholesterol ratio of 1:1, whereas it was 9:1 before incubation. We conclude that cholesterol in bile is complexed predominantly with lecithin; bile salts and bilirubin are not essential. Whether derived from gall-bladder or hepatic bile, the complex shows an electrophoretic mobility relating primarily to its degree of association with bile salts. The complex affords available binding sites for free cholesterol, which is consistent with a "carrier" function. Experiments in progress with lecithin-C^{14} and lecithinase should yield further information concerning the structure and function of this lipid complex.

The Effects of Colchicine on RNA Synthesis in Cultured Blood Leukocytes. AVERY A. SANDBERG,* ROBERT N. HOLDSWORTH, AND HERBERT WEINFELD, Buffalo, N. Y.

The synthesis of RNA, and the effects of colchicine thereon, in normal blood leukocytes have been studied with the aid of tritiated uridine and cytidine. The leukocytes were grown *in vitro* for 72 hours in the presence of phytohemagglutinin. Radioautographic studies, after the addition of uridine-H^3 (or cytidine-H^3) during the last 6 hours of culture, have revealed a definite sequence for

cellular RNA synthesis in the leukocytes; nuclear (chromosomal) RNA is synthesized during the initial 1 to 2 hours of that period, followed by nucleolar RNA (2 to 4 hours) and then by cytoplasmic RNA (4 to 6 hours) synthesis. The presence of colchicine in the culture medium, at concentrations of 1.2×10^{-7} to 1.2×10^{-3} M, resulted in greatly decreased evidence of incorporation of uridine or cytidine into cytoplasmic and, often, into nucleolar RNA. Colchicine did not seem to affect the chromosomal (nuclear) RNA synthesis to the same extent as that exhibited for cytoplasmic and nucleolar RNA. The degree of RNA synthesis inhibition was related to the level of colchicine in the culture medium and to the length of exposure to the alkaloid. An interesting finding was that RNA synthesis was evident in cells arrested in metaphase by colchicine, indicative that such synthesis does take place during some phases of metaphase. The data indicate that in all probability RNA synthesis in cultured leukocytes is initiated by the chromosomes, from which the RNA is transferred to the nucleolus and thence to the cytoplasm. Colchicine would appear to interfere with the transfer of RNA from the nucleus to the nucleolus and, in particular, from the latter to the cytoplasm.

The Varying Susceptibility of Man to Alcohol Hepatotoxicity. GILBERT R. CHERRICK, MACEO M. HOWARD, WILLEM TENHOVE, AND CARROLL M. LEEVY, Jersey City, N. J. (introduced by Harold Jeghers).

Considerable variation in susceptibility to hepatotoxic effects of alcohol occurs in man. Experiments were designed to test the hypothesis that such variability may in part derive from differences in capacity to oxidize alcohol. Ethanol-induced liver cell damage (mitochondrial degeneration or necrosis) was demonstrated to be time and dose related in adult Sprague-Dawley rats; 3 g per kg given twice daily for 14 days was the minimal requirement. Fifteen chronically alcoholic patients with equivalent alcohol intake and diet habits were studied; their liver function and histology had been followed for 5 to 15 years. Five had never shown liver cell damage (group I); 4 previously had liver cell damage but were normal when studied (group II); 3 had liver cell damage without cirrhosis (group III); and 3 had liver cell damage with cirrhosis (group IV). Ethanol, 1.5 g per kg, was given iv and its serum disappearance rate followed. DPN, DPNH, and alcohol dehydrogenase (AD) levels were measured in liver tissue before and 4 hours after ethanol. Mean serum ethanol 1 hour after infusion was 142 ± 9 (SEM), 187 ± 10, 218 ± 4, and 255 ± 26 (mg per 100 ml) in groups I, II, III, and IV, respectively. DPNH accumulation was not found in group I; mean DPNH increase of 269 ± 10, 375 ± 46, and 446 ± 33 (μmoles per kg protein) occurred in groups II, III, and IV, respectively. Mean AD activity was normal and did not vary significantly in groups I, II, and III; it was 60% less in group IV. We conclude that a population (group II) without liver cell damage, having normal hepatic AD activity, oxidizes ethanol relatively slowly and accumulates hepatic DPNH abnormally. Such persons appear unusually susceptible to hepatotoxic effects of alcohol. Liver cell damage causes a decrease in ethanol oxidation rate that renders the diseased liver especially vulnerable to further alcohol-induced injury.

Mechanisms of Pyrogenic Tolerance to Bacterial Endotoxin in Man: Blood Clearance of Cr^{51}-labeled Pseudomonas Endotoxin during Tolerance and Induced Typhoid Fever. SHELDON E. GREISMAN,* HENRY N. WAGNER, JR.,* MASAHIRO IIO, RICHARD B. HORNICK, AND WILLIAM E. WOODWARD, Baltimore, Md.

Pyrogenic tolerance to bacterial endotoxins has been postulated to result from nonspecific stimulation of reticuloendothelial system (RES) phagocytic activity that brings about accelerated endotoxin clearance and destruction. Recently, striking pyrogenic tolerance in man was shown to develop without a generalized increase in RES phagocytic activity as measured by plasma clearance rates of I^{131}-labeled aggregated human albumin. The present studies concern blood clearance rates of Cr^{51}-labeled *Pseudomonas* endotoxin (Piromen) during pyrogenic tolerance. Initial studies in rabbits indicated that pyrogenicity correlated inversely with clearance rates of this preparation; the mean blood clearance $t_{\frac{1}{2}}$ in minutes of 25 μg in normal rabbits was 3.13 compared to 1.92 in tolerant rabbits ($p < 0.05$); in normal rabbits after RES blockade by Thorotrast, $t_{\frac{1}{2}}$ was 5.47 compared to 4.08 in tolerant rabbits with comparable Thorotrast treatment ($p < 0.05$). These latter findings indicate that RES blockade slows blood clearance of endotoxin in both normal and tolerant rabbits but does not abolish the characteristic differences in clearance between these groups. In 10 healthy volunteers, the initial mean blood clearance, $t_{\frac{1}{2}}$, of 25 μg Cr^{51}-*Pseudomonas* endotoxin was 8.1 minutes. With daily intravenous injections, clearance accelerated progressively. By day 7, during maximal pyrogenic tolerance, mean $t_{\frac{1}{2}}$ was 3.3 ($p < 0.005$). Four of the tolerant volunteers were then infected with viable *Salmonella typhosa*. For 24 hours before, during, and several days after overt typhoid illness, pyrogenic responses to the Cr^{51}-*Pseudomonas* endotoxin consistently exceeded the initial control responses. Despite such hyperreactivity, endotoxin clearance rates remained accelerated. The data indicate that pyrogenic tolerance in man, as in rabbits, is associated with activation of mechanisms that specifically accelerate blood clearance of endotoxin and suggest that augmented pyrogenic responses can be induced either by inhibiting endotoxin blood clearance (RES blockade) or by increasing reactivity of the fever producing mechanisms to circulating endotoxin (typhoid fever).

Steroid Conversion by Pheochromocytoma Tissue. ANDRES CARBALLEIRA AND ELEANOR H. VENNING, Montreal, Quebec, Canada (introduced by J. S. L. Browne).

Slices and homogenates of a pheochromocytoma were incubated with C^{14}-labeled substrates: progesterone, corticosterone, 11-desoxycorticosterone, 17α-hydroxyproges-

terone, and cholesterol. The remnant of the normal adrenal gland was also incubated. Extracts were subjected to fractionation by a paper chromatographic scheme involving a variety of solvent systems that allow an excellent resolution of steroids. In the absence of exogenous substrates (pooled preincubation media) pheochromocytoma slices failed to release ultraviolet-absorbing or blue tetrazolium-positive materials. After the addition of precursors, numerous conversion products were detected. The criteria adopted for the characterization of radioactive steroids were these: identical behavioral chromatographic characteristics to unlabeled carriers, a constant H^3/C^{14} ratio of acetylation and oxidation products after successive chromatographic runs, and a constant specific activity after 3 or more crystallizations. Scarcity of material precluded attempts at identification of several unknowns. The following compounds were well characterized: cortisol, corticosterone, and 11-desoxycorticosterone (from progesterone-4-C^{14}); 18-hydroxycorticosterone (from corticosterone-4-C^{14}); 18-hydroxycorticosterone and corticosterone (from 11-desoxycorticosterone-4-C^{14}); and 11-desoxycortisol and Δ4-androstenedione (from 17α-hydroxyprogesterone). Qualitative differences were observed when the same exogenous substrate was incubated with fresh slices or with homogenates, the latter failing to produce cortisol from appropriate precursors. The tumor was a uniform, well-differentiated pheochromocytoma with no obvious cortical tissue. The cells showed a positive chromaffin-granule reaction throughout. The catecholamine content was as follows: adrenalin, 8.65; noradrenalin, 8.64; and dopamine, 0.18 mg per g of tissue. The patient showed the classical features of the disease. Urinary excretion of VMA was 37 mg per g creatinine. Steroid excretion was within normal range. Evidence has been obtained for the capacity of pheochromocytoma tissue to introduce hydroxyl groups at different sites of the steroid nucleus *in vitro*. Under the same conditions it failed to effect cleavage of the side chain of cholesterol but did produce side-chain scission of C21 precursors.

The Role of Bile Salts in Neomycin-induced Malabsorption. WILLIAM W. FALOON, DONALD WOOLFOLK, HOWARD NANKIN, KELLEY WALLACE, AND EXPEDITO N. HARO, Syracuse, N. Y. (introduced by Eugene L. Lozner).

Neomycin-induced malabsorption is associated with increased fecal excretion of fat, bile acids, and sterols and with lowering of serum cholesterol. Accordingly, the role of bile salts in such malabsorption has been studied *in vitro* and *in vivo*. A clear neomycin solution (3 to 10 mg per ml) added *in vitro* to clear human common duct bile produced a precipitate at pH 7.5 or less (the pH of upper small intestinal contents). Bile salt concentration, analyzed in two samples, fell after neomycin (10 mg per ml), in one sample from 5.5 to 2.4 mg per ml, and from 2.5 to 1.1 in the other. When neomycin (10 mg per ml) was added to increasing concentrations of sodium taurocholate, glycocholate, or deoxycholate solutions (1 to 60 mg per ml), precipitates occurred. Supernatant fluids of such neomycin-bile salts mixtures were unable to form stable emulsions with triglycerides or oleic acid, whereas the original bile salt solutions formed stable emulsions easily. Neomycin added to a micellar solution of bile salts, phosphate buffer, oleic acid, and glycerol-1-monoleate produced a precipitate and a surface lipid layer. Sudan III added to micellar solution yielded diffuse coloring, but after neomycin addition, color appeared only in the precipitate and in the surface globules. In two subjects homogeneous clear jejunal aspirate became cloudy 30 to 60 minutes after 3 g of neomycin orally. In 3 subjects given constant moderate fat diets (80 to 100 g daily) steatorrhea (fecal fat, 13 to 38 g per day) induced by neomycin (12 g daily) was significantly reduced (20 to 66%) when sodium taurocholate (900 to 2,000 mg daily in divided doses) was added. These observations indicate that precipitation of bile salts by neomycin may be a major mechanism in neomycin-induced malabsorption and also suggest a new technique for the study of bile salt physiology.

Antagonistic Effects of Etiocholanolone and Cortisone on Lysosomes. GERALD WEISSMANN AND LEWIS THOMAS,† New York, N. Y.

Neutral 5β-H steroids, such as etiocholanolone, or pregnanedione, provoke fever and induce local inflammation in man. These effects of etiocholanolone are known to be suppressed by cortisol, the pharmacologic action of which we have previously attributed, at least in part, to its stabilization of lysosomal membranes. It therefore appeared possible that pyrogenic steroids might disrupt lysosomes. Thirty-eight neutral steroids and bile acids were tested *in vitro* for their ability to release acid hydrolases from lysosome-rich fractions of rabbit liver, spleen, and leukocytes, in sucrose. At concentrations above 2.5×10^{-4} M, such 5β-H steroids as etiocholanolone, pregnanedione, pregnanolone, lithocholic and glycolithocholic acids, and progesterone (Δ4,5) released 78 to 85% of the granules total content of lysosomal enzymes (acid phosphatase, beta-glucuconidase, and cathepsins) at 37° C; 42 to 51% at 20° C. Metabolic precursors of etiocholanolone, i.e., testosterone, dehydroepiandrosterone, or 11-desoxycortisol, released hydrolases at 37° C, but not at 20° C. No more enzymes were released by nonpyrogenic (5α-H) steroids, such as androsterone, allopregnanolone, and so forth, than by solvent controls (10 to 14% at 37° C; 6 to 9% at 20° C). Estrone, estradiol, and estratriol were also inactive; an oxygen function at C 11 reduced the activity of 5β-H steroids. Preincubation of liver and spleen lysosomes with cortisone acetate, cortisol acetate, or chloroquine ($> 2.5 \times 10^{-4}$ M) significantly retarded the release of hydrolases by etiocholanolone or progesterone. The turbidity of pure suspensions of lysosomes isolated from peritoneal leukocytes was reduced by etiocholanolone and progesterone, but not by androsterone; beta-glucuronidase was solubilized from the granules. Chloroquine (1×10^{-4} M) also prevented the effects of etiocholanolone and progesterone upon the granules. The

results indicate that 5β-H steroids and progesterone render the membranes of lysosomes more permeable and that cortisone and choloroquine antagonize this effect.

Studies on the Control of Hemoglobins A and F Synthesis in Man. EDWARD R. BURKA AND PAUL A. MARKS,* New York, N. Y.

These studies investigate the mechanism of *1*) conversion from fetal to adult hemoglobin (Hg) synthesis in newborns and *2*) the increased levels of Hg F in adults with certain anemias. Factors controlling Hg F and A synthesis were examined by determining rates of formation of these proteins in cell populations in which both are synthesized. Four cord bloods and blood from 5 thalassemia major (thal), 3 sickle cell anemia (SS), and 2 acquired hemolytic anemia (AHA) subjects were incubated with C^{14}-amino acids (aa) for 80 minutes. After incubation, ribosome content of the cells, class of ribosomes active in protein synthesis (sucrose gradient density centrifugation), and aa incorporation into Hg A and F (Amberlite IRC-50 chromatography) were determined. In all groups, polyribosomes (sedimentation coefficient >110 S) were the primary site of aa incorporation. Fetal (cord) blood had high rates of Hg F formation, averaging 488 (range, 305 to 695) mμmoles aa incorporated per mg ribosomes recovered (unit for all values), whereas incorporation into Hg A averaged 116 (32 to 162). Among adults studied, thal cells had very low rates of Hg A synthesis, but Hg F formation was similar to nonthal cells. Average values and range for aa incorporation into Hg A (or S in SS) was 18 (10 to 24) in thal, 236 (124 to 316) for SS, and 396 (396 to 398) in HA. The aa incorporation into Hg F was 20 (8 to 28) in thal, 36 (10 to 62) for SS, and 34 (32 to 36) for HA. Polyribosomes of fetal blood, compared with thal, had a tenfold capacity for Hg F synthesis, although there is a selective defect in Hg A formation in thal. Adult thal, SS, and HA bloods had comparable rates of Hg F synthesis. Thus, in adult cells, capacity for Hg F synthesis appears limited. Hg F formation in adults may reflect an altered cell population associated with erythropoietic stimulation and not the specific fetal-adult Hg switch mechanism occurring in newborns.

Steroids Secreted by the Fetal Adrenal Cortex. WALTER R. EBERLEIN,* Philadelphia, Pa.

To study the secretory function of the fetal adrenal cortex, umbilical vein blood was collected from 54 placentas at delivery. Plasma (780 ml) was processed by the transesterification method previously described. The crude extracts after saponification, digitonin precipitation, and Girard separation were fractionated by paper chromatography. The recovery steroids were identified by mobility on paper and thin layer chromatograms, specific staining reactions, derivative formation, spectra in ethanol-sulfuric acid, and where amount permitted, by infrared analysis. The following steroids were isolated; 3β-hydroxy-Δ5-pregnene-20-one; 3β-hydroxy-Δ5-androstane-17-one; 3β17α-dihydroxy-Δ5-pregnene-20-one; 3β,17α,21-trihydroxy-Δ5-pregnene-20-one; 3β,16α-dihydroxy-Δ5-pregnene-20-one; 3β,21-dihydroxy-Δ5-pregnene-20-one; 3β,11β,17α,21-tetrahydroxy-Δ5-pregnene-20-one; Δ5-pregnene-3β,17α, 20ε,21-tetrol; Δ5-pregnene-3β,17α,20ε-triol; and Δ5-androstene-3β, 17β-diol. With the exception of 3β-hydroxy-Δ5-androstene-17-one, none of these steroids has previously been detected in human blood. The presence of this steroid with others hydroxylated at C-17, C-21, and C-11 indicates their origin from the adrenal cortex. The predominance of Δ5-3β-hydroxy steroids in cord blood indicates that the fetal adrenal cortex is characterized by a relatively severe deficiency of 3β-ol-dehydrogenase. The resultant interference with hydrocortisone synthesis leads to compensatory hyperplasia of the adrenal cortex *in utero*.

The Skeletal Contribution to the Hypercalcemia of Hyperparathyroidism in Man. GEORGE NICHOLS, JR.,* AND BARRY FLANAGAN, Boston, Mass.

Mechanisms responsible for the constancy of the serum calcium concentration, as distinct from those governing calcium balance, are confined in the steady state to the bone-ECF equilibrium and the factors that influence it. The purpose of this investigation was to study this equilibrium isolated *in vitro* in normal and hyperparathyroid states. Fresh bone samples from normal and hyperparathyroid human subjects were incubated *in vitro* in calcium-free Krebs-Ringer bicarbonate buffered medium with glucose at 37° C under 95% O_2/5% CO_2. The calcium concentration in the medium was measured at steady state (after 6 hours incubation) in a fresh and a heat-inactivated sample from each patient. Concentrations supported by heat-inactivated bone were ascribed to the solubility of the bone mineral, whereas the higher concentrations supported by fresh samples were considered to represent the sum of the mineral solubility fraction and an additional fraction dependent on the metabolic activity of the cells. The latter, measured by the difference in the concentrations maintained by fresh and inactive samples, has been termed the "metabolic" fraction. The results indicate that the medium concentrations supported by fresh bone and the "metabolic" fraction were significantly elevated ($p < 0.05$) in hyperparathyroid samples, indicating a shift in equilibrium toward the ECF to be present in hyperparathyroidism. Although the degree of elevation of medium Ca concentration was reflected by the *in vivo* serum Ca level in primary hyperparathyroidism, no such correlation was observed in secondary hyperparathyroidism or when severe renal disease was present. Thus the ability of bone in hyperparathyroidism to support higher Ca concentrations *in vitro* is a function of changes in bone cell metabolism. Hypercalcemia—its reflection *in vivo*—may be masked by extraskeletal factors.

Cl^- and Glucose Transport in the Human Erythrocyte Ghost. EUGENE D. ROBIN* AND JAMES THEODORE, Pittsburgh, Pa.

The red cell ghost is widely accepted as a model of cellular plasma membranes. The ability of isolated

ghosts to "pump" Na^+ is accepted as strong evidence that this function resides in the cell membrane and is independent of cellular contents. In the present study, Cl^- and glucose transport in isolated ghosts was compared with transport in the intact erythrocyte. $[Cl^-]_i/[Cl^-]_e$ in the intact red cell averaged 0.73 ± 0.09 ($pH_e = 7.42$) over a wide range of $[Cl^-]_e$ values. $[Cl^-]_i/[Cl^-]_e$ in ghosts averaged 1.08 ± 0.1 over the same range of $[Cl^-]_e$ values. Calculated transmembrane potential assuming thermodynamic equilibrium fell from approximately -8.4 mv in the erythrocyte to essentially 0 mv in the ghost. Cl^- distribution ratios appear to depend on Gibbs-Donnan equilibrium, and presumably Cl^- transport occurs by simple passive diffusion. Glucose transport in the intact human erythrocyte exhibits saturation kinetics and is therefore widely believed to be carrier mediated. In the ghost, saturation kinetics was not found. There is an increase in $[glucose]_i$ with increasing $[glucose]_e$ over a wide range of $[glucose]_e$ values. The above findings do not seem to be related to anatomical disruption of the membrane with a general increase in permeability, since the ghosts, under appropriate conditions, are able to maintain K^+ gradients and function as osmometers. The normal steady-state value of $[Cl^-]_i$ appears to be dependent on factors related to the intracellular erythrocyte milieu and independent of specific membrane transport processes. Glucose transport in the ghost differs from that found in the intact erythrocyte. The basis of this difference is not clear.

Enterohepatic Circulation of Urobilinogen-C^{14} in Rats and Humans. ROGER LESTER, WILLIAM SCHUMER, AND RUDI SCHMID,* Chicago, Ill.

Earlier attempts to provide direct proof that urobilinogens are absorbed from the intestine, and re-excreted in bile and urine, have been marred by lack of an isotopically labeled urobilinogen. The problem has now been investigated employing a crystalline radioactive urobilinogen, mesobilirubinogen-C^{14}, prepared by sodium amalgam reduction of bilirubin-C^{14}. Radiochemical purity was established by recrystallization to constant specific activity, melting point determination, and thin layer chromatography. Mesobilirubinogen-C^{14} in bile or aqueous taurocholate was infused into the duodenum or terminal ileum of rats with an external bile fistula. Absorption and biliary re-excretion of the isotope began during the subsequent 30 minutes and continued for 15 to 40 hours. Enterohepatic circulation equaled about 60% of the dose when mesobilirubinogen-C^{14} was infused into the duodenum but amounted to only 10 to 20% after infusion into the terminal ileum. A significant fraction of the mesobilirubinogen-C^{14} was absorbed and re-excreted in the bile intact. Urinary excretion of isotope invariably was less than 5% of the dose, unless bile flow was obstructed. Mesobilirubinogen-C^{14} was administered by duodenal tube to two patients with external biliary drainage. Forty-five and 55% of the isotope was excreted in bile collected over a 24-hour period, and 14 and 2% of the radioactive dose appeared in the urine. In a third patient with an intact biliary tree, urinary excretion of radioactivity was comparable. The findings indicate that in man and rats a representative urobilinogen is absorbed from the intestine and re-excreted predominantly in the bile. Since under physiologic conditions, urobilinogen is formed only in the large bowel, the magnitude of its enterohepatic circulation may be limited by the absorptive surface of the colon by mixing with semisolid fecal material and possibly by the presence of urobilinogen in conjugated form.

The Role of the Parathyroids in the Phosphaturia of Vitamin D Deficiency. CLAUDE ARNAUD, JAN FISCHER, AND HOWARD RASMUSSEN,* Madison, Wis.

The exact role of the parathyroid glands in the hypophosphatemia and phosphaturia of osteomalacia remains unsettled. Two points of view have been expressed: *1*) The parathyroid glands are overactive and are directly responsible for the phosphaturia, and *2*) the parathyroid hormone is ineffective in the absence of vitamin D. A corollary of the second is that vitamin D promotes the renal tubular reabsorption of phosphate. The present experiments resolve this controversy. Parathyroidectomized rats were maintained for several days with a constant infusion of glucose, NaCl, KCl, $MgCl_2$, and $CaCl_2$. An indwelling catheter allowed the accurate collection of frequent urine samples. Perfusion of purified parathyroid hormone (5 μg per hour) into a D-fed parathyroidectomized rat led to a tenfold increase in phosphate excretion and a concomitant, but less dramatic, twofold decrease in calcium excretion. Perfusion of the same dose of hormone into a parathyroidectomized D-deficient rat resulted in the same initial rise in phosphate excretion with inconsistent changes in calcium excretion. Long term perfusion of hormone did not sustain phosphaturia in the D-deficient animal, whereas it did in the D-fed animal. This suggested that mobilization of phosphate from bone was produced by the hormone in the D-fed but not the D-deficient animal. By prelabeling a rat with calcium^{45} several weeks before the study, it was possible to determine the time course of bone mobilization during a constant infusion of hormone. Sustained hormone infusion (5 μg per hour) into parathyroidectomized rats led to a gradual but progressive increase in the mobilization of calcium (as measured by urinary radiocalcium) from the bones of the D-fed animal but very little increase in the calcium from the bones of the D-deficient animal. These results indicate that vitamin D is needed for the effects of the parathyroid hormone upon bone mobilization but not for its effects on the renal excretion of phosphate.

Observations on the Repeated Administration of Viruses to a Patient with Acute Leukemia. E. FREDERICK WHEELOCK AND JOHN H. DINGLE,† Cleveland, Ohio.

Recent demonstrations by electron microscopic and epidemiologic methods indicate that human acute leukemia may be a viral disease. Experiments performed in mice with virus-induced leukemia show that inocula-

tions of other viruses can ameliorate the disease. These findings suggest, therefore, that viruses may be used to modify leukemia in man either by a direct oncolytic effect or through the phenomenon of viral interference. Temporary remissions in human leukemia have been reported following a variety of infections, including those due to viruses. Consequently the decision was made to administer active viruses to a patient with acute myelogenous leukemia who could not derive further benefit from conventional forms of therapy. After intravenous administration of each of 6 different viruses, there occurred a marked reduction in the number of myeloblasts in the peripheral circulation and, with the exception of one virus, a reduction in the size of lymph nodes, together with clinical improvement. These changes lasted for brief periods of time and were followed by a rise in myeloblasts in the peripheral blood, enlargement of lymph nodes, clinical deterioration, and ultimate death of the patient. Terminally he developed staphylococcal sepsis. During the periods of temporary hematologic and clinical improvement, the bone marrow remained unchanged, myeloblasts constituting approximately 95% of the cellular composition. Post-mortem sections, however, revealed a marrow almost completely devoid of myeloblasts. Administration of these viruses was associated with fever but no other unusual sign or symptom, and with only one virus were there subsequent viremia and evidence of infection. Interferon was detected in the patient's sera on two separate occasions and in post-mortem samples of lymph nodes, spleen, and bone marrow. This case is being reported because the administration of viruses apparently stimulated a mechanism which, if understood and augmented, might lead ultimately to effective treatment of leukemia.

Micropuncture Studies of Renal Tubular Excretion of Chloride during Hypercapnia. MICHAEL KASHGARIAN, YVES WARREN, AND HOWARD LEVITIN, New Haven, Conn. (introduced by Philip K. Bondy).

Hypercapnia induces an increase in the urinary excretion of chloride. The electrochemical gradient of chloride was studied in individual proximal renal tubules of rats that were made hypercapnic by breathing 12% CO_2 in air for 18 to 21 hours and compared to values obtained in rats breathing compressed air. By interruption of a previously injected column of oil with a solution containing 150 mmoles of raffinose and 75 mEq of NaCl, a state closely approximating zero net flux was achieved. Transtubular electrical potentials were measured, and the intratubular perfusate was analyzed for chloride using the microelectrometric titration of Ramsey. Net transtubular efflux was measured in saline-perfused tubules using sequence photomicrography. Independent of the site of puncture, a mean potential of 23 mv, lumen negative, was found in hypercapnic rats and a mean of 24 mv in controls. The mean tubular fluid/plasma ratio (TF/P) of chloride was 1.12 during hypercapnia and 1.01 in controls ($p < 0.001$). The half volume reabsorption time of sodium chloride in the proximal tubule was 9 seconds and was unchanged by hypercapnia. The calculated electrochemical gradient of chloride was 26 mv during hypercapnia and 24 mv in normals. A gradient of zero would be expected if chloride were distributed in a simple passive fashion. The data suggest that chloride is handled by an active process in the proximal tubule of the rat.

Repression of Alkaline Phosphatase in Cultured Human Cells. RODY P. COX, New York, N. Y. (introduced by Colin M. MacLeod).

Alkaline phophatase in certain human cells in culture is controlled by both inductive and repressive mechanisms. In cultures of skin fibroblasts, the enzyme is regulated in part by the presence of L-cyst(e)ine, a constitutent of the medium. Although L-cysteine is known to be an inhibitor of alkaline phosphatase, present evidence shows it affects the level of enzyme rather than its activity. Even though certain analogues and isomers of L-cysteine inhibit enzyme activity as well as or better than L-cysteine, several of these sulfhydryl compounds are ineffective in repressing the level of enzyme in skin fibroblasts. On the other hand, there are analogues that do not inhibit enzyme activity but produce good repression. Certain molecular requirements for inhibition of alkaline phosphatase and for its repression will be described. Inhibition of enzyme activity by L-cysteine is reversed by adding zinc. However, zinc does not increase enzyme activity of lysates prepared from cysteine-repressed cultures. Kinetic studies show no difference at 6 hours in the enzyme activity of cultures incubated in maintenance medium with or without L-cysteine even though cyst(e)ine enters the amino acid pool within 30 minutes and is concentrated to approximately twice the medium concentration. At 24 hours enzyme activity of cultures incubated with L-cysteine is the same as at 6 hours. In contrast, replicate cultures maintained in the absence of cysteine show a moderate increase in enzyme at 24 hours. Enzyme level at 48 hours in the presence of L-cysteine is 20 to 30% of the 6-hour level, whereas in the absence of L-cysteine there is a further rise. The data are more compatible with repression of enzyme synthesis than inhibition of its activity.

A Disease of Steroid Stereoisomerism: Gallstone Formation in the Cholestanol-fed Rabbit. ALAN F. HOFMANN AND ERWIN H. MOSBACH, New York, N. Y. (introduced by Jules Hirsch).

When cholesterol is converted into bile acids, an important change in steric configuration occurs: the double bond of cholesterol is saturated stereospecifically, and the insertion of a hydrogen in the 5β orientation results in the transformation of a planar molecule into an L-shaped molecule. In cholesterol, the A/B ring juncture is essentially trans; in the 5β bile acid molecule, the A/B ring juncture is cis. It might be predicted that if the saturated homologue of cholesterol, cholestanol (5α, A/B trans), could be converted into bile acids,

these bile acids would of necessity retain the 5α or A/B trans configuration. Such A/B trans ring juncture stereoisomers of normal bile acids are termed "allo" bile acids. The rabbit absorbs cholestanol efficiently, and cholestanol-fed rabbits rapidly develop gallstones composed largely of bile acids, which are present as the calcium and sodium salts of glycine conjugates. In this study, the major component of these gallstones was identified as allodeoxycholic acid, the A/B trans isomer of deoxycholic acid. The 5α configuration of this bile acid was proved by optical rotatory dispersion measurements and by the reduction of its diketo- derivative to allocholanic acid. The position and orientation of the hydroxy substituents were proved by a synthesis of the gallstone acid from cholic acid. The pathogenesis of gallstone formation was investigated by comparing the solubility of the calcium salts of synthetic glycoallodeoxycholate (5α) and glycodeoxycholate (5β). In the presence of Na^+ ions, the solubility of calcium glycoallodeoxycholate was strikingly lower than that of calcium glycodeoxycholate, suggesting that the type of A/B ring juncture has an important influence on the solubility properties of bile salts and affording a possible explanation for gallstone formation. Cholestanol-induced cholelithiasis occurs because of an elevated synthesis rate of a stereoisomer of a normally occurring steroid, deoxycholic acid. Conceptually, the process is related to etiocholanolone fever, a disease in which there is an elevated synthesis rate of the A/B cis isomer of androsterone, etiocholanolone. Both may be considered diseases of steroid stereoisomerism.

Relationships of Bence Jones Proteins to Each Other and to Normal Gamma Globulin. RALPH L. NACHMAN, RALPH L. ENGLE, JR., STEFAN STEIN, AND LOIS COPELAND, New York, N. Y. (introduced by Paul Reznikoff).

Recent studies of Bence Jones proteins have helped to clarify some features of the molecular nature of the human immunoglobulins. Thus, the two major antigenic groups of Bence Jones proteins, groups 1 and 2, have immunologic counterparts in normal $gamma_2$, $gamma_{1A}$, and $gamma_{1M}$ globulins. We have studied the immunologic and structural relationships of individual Bence Jones proteins within each major antigenic group. Group specific antisera made in rabbits to Bence Jones proteins were absorbed with normal serum and with pooled gamma globulin at levels greater than equivalence. The absorbed antisera retained reactivity against the immunizing protein and in addition cross reacted with Bence Jones proteins of the same antigenic group but from different patients. There was also immunologic evidence of subgroups or families within each major group. These immunologic relationships have been further studied by comparing the peptide maps obtained from the tryptic digests of four purified group 1 and eight purified group 2 proteins. Ten peptides were shared by all the group 1 and nine peptides by all the group 2 proteins. Most of these peptides were present in normal pooled gamma globulin. There were no peptides in common between the two groups. Within each antigenic group there appeared to be structural evidence of at least two subgroups or families. Thus the immunologic and structural studies suggest that Bence Jones proteins of the same group share a common core of antigenic determinants that differ either qualitatively or quantitatively from normal pooled gamma globulin. The existence of subgroups of Bence Jones proteins suggests that normal gamma globulin may also be composed of families of molecules within the two major antigenic groups.

Human Antibody to Bence Jones Proteins. WALLACE V. EPSTEIN AND DALE GROSS, San Francisco, Calif. (introduced by Ernest Jawetz).

Much of our current information concerning the antigenic structure of human γ_2- and γ_{1M}-globulin derives from studies using antisera prepared by the immunization of animals with antigenic group 1 or group 2 Bence Jones proteins. To date there has been no evidence that naturally occurring antibodies to these antigens may be found in man. In our present study, tannic acid-treated sheep erythrocytes, coated with purified group 1 or group 2 Bence Jones proteins or with the light polypeptide chain or human γ_2- or γ_{1M}-globulin, have been used to detect naturally occurring, highly specific agglutinators in human sera. Such antibodies were found in sera from apparently healthy persons, individuals with rheumatoid arthritis, and those with a variety of bacterial infections. Results of hemagglutination and hemagglutination inhibition studies using human antisera were similar to those reported with animal antisera in that the group 1 and group 2 antigenic groupings were reactive in Bence Jones proteins and in the L-polypeptide chains of γ_2- and γ_{1M}-globulin. In contrast, when using human antisera, these antigenic groups appear inaccessible in intact γ_2- and γ_{1M}-globulin and in all products obtained by proteolysis and reduction-alkylation of the intact molecule before the actual chromatographic separation of heavy from light polypeptide chains. By means of human antisera, Bence Jones protein of antigenic group 2 as defined by rabbit antisera was divisible into highly specific subtypes. Antigenic differences between individual group 1 Bence Jones proteins were also discernible. The presence of circulating antibody in man directed toward a portion of the human γ-globulin molecule that is inaccessible in the intact molecule suggests that antigenic stimulation may occur at a time when the heavy and light polypeptide chains of the γ-globulin antigen are disassociated.

Isolation and Characterization of the Long-acting Thyroid Stimulator of Graves' Disease. JOSEPH C. MEEK, A. ERIC JONES, URBAN J. LEWIS, AND WILLARD P. VANDERLAAN,† La Jolla, Calif.

After the discovery of the long-acting thyroid stimulator (LATS) in the blood of patients with Graves' disease, attempts were made to isolate the active prin-

ciple with limited success. The following study outlines a method of partial purification of LATS, which affords insight into the characteristics of this abnormal substance. Plasma was obtained from a patient with active Graves' disease that contained a high titer of LATS when assayed by the McKenzie method. The lyophilized plasma was treated with 40% ammonium sulfate, and the resulting precipitate contained the major activity. The precipitate was dissolved in phosphate buffer and passed through a diethylaminoethyl cellulose column. The activity was found to reside exclusively in the unabsorbed fraction, which was identified by polyacrylamide gel electrophoresis as gamma globulin. The assay response at this stage indicated a tenfold purification based on protein weight. The active gamma globulin fraction was then subjected to reduction with mercaptoethanol in a neutral aqueous solution. The two resulting polypeptide chains (Porter A and B) were identified by electrophoresis in polyacrylamide-8 M urea and were separated on a G75 Sephadex column. No loss of activity occurred after reduction, and the activity resided entirely in the A polypeptide chain. At this stage a 25-fold purification had been achieved. Throughout the early isolation stages the active extracts were "long acting"; however, after reduction and identification of the A chain of the gamma globulin fraction, the activity became "short acting" and resembled thyrotropin. These studies suggest, then, that the long-acting principle of this abnormal protein is related to sulfhydryl linkages. In addition, the finding of this material in the same protein fragments that contain antibody indicates that the elaboration of the long-acting thyroid stimulator may be related to a specific antigen-antibody response.

Studies of Oxidative Hemolysis in Hereditary Acatalasia. HARRY S. JACOB, SIDNEY H. INGBAR,* AND JAMES H. JANDL,* Boston, Mass.

Hereditary acatalasia presents a unique opportunity to assess the role of H_2O_2 in red cell (RBC) metabolism, aging, and drug-induced hemolysis. Three acatalasic siblings, lacking catalase in RBC, leukocytes, and liver, were studied. As judged by the formation of methemoglobin and Heinz bodies, acatalasic RBC were inordinately sensitive to the oxidant action of H_2O_2 vapor and H_2O_2-generating compounds such as ascorbate. Acatalasic RBC were moderately hypersusceptible to hydroquinone and preincubated primaquine, implying H_2O_2 generation by these drugs. However, their reactivity to acetylphenylhydrazine and fresh primaquine was normal. Despite sensitivity *in vitro,* acatalasic RBC survived normally *in vivo* before and during primaquine or ascorbate ingestion. This stability was investigated. In untreated acatalasic RBC, the hexosemonophosphate shunt was accelerated to approximately thrice normal and increased twelvefold with doses of H_2O_2 that had little (1.3-fold) effect on normal cells. Selective blockade of glutathione prevented this shunt-stimulating effect of H_2O_2 but not that of methylene blue. After glucose deprivation low levels of H_2O_2 oxidized glutathione in acatalasic but not in normal RBC. Thus, with catalase absent, peroxidation of glutathione provides for disposal of H_2O_2. This in turn accelerates the shunt through oxidation of TPNH. The shunt stimulation by H_2O_2 is specifically mediated by glutathione, whereas that by methylene blue is not. Whenever catalase and shunt activity were both deficient, oxidative injury by H_2O_2 was markedly potentiated. These findings indicate that H_2O_2 is not involved in oxidative hemolysis induced by acetylphenylhydrazine and its congeners, although it is generated on standing by primaquine. In acatalasia the oxidative challenge of H_2O_2, whether spontaneously generated or drug-induced, both stimulates and is offset by hexosemonophosphate shunt activation. In the normal cell, this mechanism and catalasic decomposition combine to prevent oxidative injury; both must be overcome before H_2O_2 induces cell damage.

The Effect of Nephrectomy and Hypertransfusion on Neonatal Erythropoiesis. GUIDO LUCARELLI, DONALD HOWARD, BRIGID LEVENTHAL, AND FREDERICK STOHLMAN, JR.,* Boston, Mass.

After nephrectomy but not ureteral ligation, adult rats have almost total suppression of erythropoiesis. From this it has been generally inferred that the kidney produces erythropoietin; the absence of erythropoietin in the anephric leads to erythroid aplasia. Hypertransfusion produces a similar effect, thought to result from decreased erythropoietin production due to increased tissue oxygenation. In the bone marrow of the newborn rat, erythroid precursors increase from $\sim$ 6% on the first day to $\sim$ 60% on the fourth day. After the tenth day there is a gradual decrease in the proportion of erythroid cells, until the adult value of $\sim$ 25% is seen by days 40 to 50. Bilateral nephrectomy of the rat during the first 3 weeks of life does not importantly alter the proportion of erythroid cells in the bone marrow, the differential, or the mitotic index. The possibility that milk provides a source of erythropoietin was excluded by similar studies on nephrectomized newborns nursing from hypertransfused or nephrectomized mothers. Although there was the usual shutdown of erythropoiesis in the mother, erythropoiesis was not affected in the newborn. Hypertransfusion to hematocrits of 60 to 70 of 13- to 17-day-old newborns decreased erythropoiesis but not to the magnitude seen in adults; newborns $\sim$ 2% reticulocytes, adults 0 to 0.2% reticulocytes. The 18-day-old hypertransfused rat responded to exogenous erythropoietin. The results on the nephrectomized newborns suggest that either erythropoietin is formed in sites other than the kidney or that neonatal erythropoiesis is governed to a significant extent by other regulators. The failure to observe a comparable degree of suppression of erythropoiesis in the newborn and adult rat after hypertransfusion points to regulators other than oxygen-dependent erythropoietin.

Renal Tubular Bicarbonate Resorption in the Dog during Acute Changes in Filtered Load. JOHN I. LEVITT, ARTHUR G. GOLDMAN, AND JACOB GROSSMAN, New York, N. Y. (introduced by Louis Leiter).

Renal tubular bicarbonate resorption has been shown to be dependent upon several factors including plasma P_{CO_2}, plasma chloride and potassium concentrations, and renal carbonic anhydrase activity. A maximal bicarbonate resorptive capacity (bicarbonate Tm), the value of which is determined by these variables, has been demonstrated during elevation of the systemic plasma bicarbonate concentration. In the present experiments, bicarbonate resorption in the dog was studied during acute alterations in filtered bicarbonate load while controlling the above variables. Two procedures were used. In the first, filtered load was lowered by increasing the ureteral pressure of one kidney, thereby causing a unilateral fall in GFR. Although the absolute amount of bicarbonate resorbed fell, resorption relative to GFR rose progressively as the latter decreased, without achieving a maximal value. In the second, acute increases in filtered load were produced by infusion of hypertonic sodium bicarbonate into one renal artery. Bicarbonate resorption, both absolute and relative to GFR, increased markedly, exceeding both that of the control side and previously determined "maxima." This increase was associated with increased sodium and decreased chloride resorption by the perfused kidney. Resorption correlated with the filtered bicarbonate, and even at the very high bicarbonate loads produced, no resorptive maxima could be demonstrated. These data indicate that local renal factors in addition to those mentioned above strongly influence bicarbonate resorption. The high figures obtained support previous suggestions that given the proper composition of tubular fluid, the kidney's ability to resorb bicarbonate is considerably in excess of previously stated tubular maxima.

The Significance of Erythrocyte Crenation in the Pathophysiology of Hemolytic Anemias. DAVID G. NATHAN, FRANK A. OSKI, VICTOR W. SIDEL, AND LOUIS K. DIAMOND,† Boston, Mass.

Normal red cells suspended in plasma between coverslip and slide crenate very slowly. Suspension of cells in a mixture of plasma and saline increases the rate of crenation to a limited degree. In contrast, the red cells of a child with a violent hemolytic anemia associated with homozygous pyruvate kinase (PK) deficiency assume both crenated and balloon shapes as soon as the wet preparations can be examined and are present to an equal extent in smear preparations. In addition to their abnormality of carbohydrate metabolism (virtually absent glucose consumption), these cells have a profound loss of ATP during incubation and a markedly elevated rate of potassium leakage. The extensive leak of potassium is not adequately compensated by an increased potassium influx. The cells are removed from the circulation of a normal individual within one day and are predominantly sequestered in the liver. Examinations of the erythrocytes of other patients with PK deficiency, G6PD deficiency, paroxysmal noctural hemoglobinuria, and immune hemolytic anemia also reveal an abnormal tendency of these cells to crenate within minutes of their application to the coverslip-slide interface. Such cells also exhibit abnormal instability of ATP and an increased leak of potassium. The severity of the metabolic abnormalities may be predicted from the extent and rapidity of the morphologic disturbance produced in the wet preparation. Enhancement of crenation in wet preparations of erythrocytes is therefore a sign of erythrocyte membrane injury. It occurs in leaky cells in which ATP levels rapidly fall due to the increased ATP consumption that is associated with "hyperpumping" of potassium.

Mechanism of Renal Sodium Retention in Experimental Edema: Response to Acute Sodium Loading after Vena Caval Constriction. NORMAN G. LEVINSKY* AND RICHARD C. LALONE, Boston, Mass.

Previously we demonstrated that in normal dogs a "third factor," other than decreased aldosterone and increased glomerular filtration rate (GFR), accounts for part of the sodium diuresis after acute saline infusions. A possible role of this factor in the sodium retention that occurs in dogs with thoracic inferior vena cava (TVC) constriction is suggested by the demonstration by other workers that aldosterone is not responsible. To evaluate the relative importance of "third factor" and possible changes in GFR, we compared the response to acute sodium loading in 14 normal dogs to that in 9 dogs studied 2 to 5 days after TVC constriction. Sodium loading was accomplished by infusing 1,800 to 2,800 ml of isotonic saline. To eliminate changes in endogenous secretion of aldosterone as a factor, large amounts of mineralocorticoids were infused throughout all experiments. In control dogs, GFR increased 4 ml per minute and $U_{Na}V$ 909 μEq per minute after saline infusion. Although GFR increased 17 ml per minute after saline in dogs with TVC constriction, $U_{Na}V$ was virtually unchanged, increasing only 35 μEq per minute. Six dogs were studied with and without TVC constriction. In 5, GFR, both before and after saline loading, was higher during TVC constriction. The decrease in plasma protein concentration during saline infusion was equal before and after TVC constriction, implying that the saline infused during TVC constriction remained in the intravascular compartment. We conclude: *1*) Neither decreased filtered sodium nor increased aldosterone accounts for the profound sodium retention caused by TVC constriction. *2*) The "third factor" involved in normal response to sodium loads is not manifested during saline infusion with TVC constriction. *3*) Our data could be explained if the "volume receptor" which triggers increased GFR during TVC constriction is different from that which activates "third factor."

Protective Effect of Antibody in Staphylococcal Infections in Chick Embryos. WILLIAM R. MCCABE, Boston, Mass. (introduced by Chester S. Keefer).

A previous report of experimental infections in embryonated eggs demonstrated that allantoic injection of pooled human gamma globulin afforded significant protection against subsequent challenge with virulent strains of *Staphylococcus aureus*. The potential value of this system of elucidation of the characteristics of staphylococcal immunity prompted further investigation of the role of antitoxic and antibacterial immunity and studies of the specificity of the protection. Since both antibacterial antibody and antitoxin (> 15 units) were present in significant amounts in gamma globulin, either could have been responsible for the protection that was observed. Adsorption of gamma globulin with alpha (α) hemolysin completely removed antibody against α toxin but failed to diminish the protective effect ($p < 0.02$) of the gamma globulin. Commercial staphylococcal antitoxin that contained agglutinating antibody (1 : 640) in addition to anti-α hemolysin (4,000 units) also afforded significant protection ($p < 0.01$) against staphylococcal infection. Adsorption of antitoxin with formalin-killed bacteria completely removed cell reactive antibody without materially reducing antitoxin titers. Adsorbed antitoxin failed to protect against lethal infection with the adsorbing strain although it was still capable of completely neutralizing α hemolysin produced *in vivo* (< 10 units) as compared to levels of 640 to 10,240 units in the controls. Protection against lethal infection with 10 strains of staphylococci (nontypable and phage groups I, II, and III) paralleled the titer of agglutinating antibody. A similar relationship between agglutination titers and protection with individual strains was also observed with serial dilutions of gamma globulin. Adsorption of gamma globulin with viable staphylococci completely abolished the protective effect of gamma globulin against homologous strains but did not affect its protection against heterologous strains.

An Aldosterone Biosynthetic Defect in a Salt-wasting Disorder of Infancy. STANLEY ULICK,* KATHRYN K. VETTER, EMILE GAUTIER, GIORGIO L. NICOLIS, JAMES R. MARKELLO, AND CHARLES U. LOWE,* New York and Buffalo, N. Y.

A 6-month-old child with a salt-losing syndrome was found to have a unique abnormality of steroid secretion confined to the mineral corticoid series. When the child was sodium-depleted, the secretory rate of aldosterone (8.3 μg per day) was normal for the basal state but was low in relation to the degree of sodium depletion. The more striking abnormality was a secretory rate of 18-hydroxycorticosterone (2,420 μg per day) that was 100 times greater than normal. Corticosterone secretion (6,800 μg per day) was also markedly increased. Correction of sodium depletion with a high salt intake and desoxycorticosterone led to a decrease in the secretion of both corticosterone and 18-hydroxycorticosterone. The abnormal corticosteroid pattern can be explained by a defect in the final step of aldosterone biosynthesis and an increased production of aldosterone-stimulating hormone. Intensive stimulation by trophic factor would result in the maximal possible rate of aldosterone synthesis consistent with the degree of the defect and in hypersecretion of 18-hydroxycorticosterone and corticosterone. The degree of the biosynthetic defect is expressed by an increase in the secretory ratio of 18-hydroxycorticosterone to aldosterone, which normally does not exceed five. Two possible pathways of aldosterone synthesis from corticosterone are *1*) hydroxylation at C-18 followed by dehydrogenation or *2*) direct oxidation of the angular methyl group to an aldehyde without preliminary hydroxylation. The latter mechanism was supported by *in vitro* studies of zona glomerulosa tissue of several species in which aldosterone was synthesized almost 100 times faster from corticosterone than from 18-hydroxycorticosterone. However, the *in vitro* studies did not exclude the former pathway, since the incubations of necessity used the cyclic hemiketal of 18-hydroxycorticosterone, a tautomeric form that predominates in aqueous solution and is more resistant to oxidation.

The Role of Serum Factors in Reticuloendothelial Blockade. M. GLENN KOENIG, ROBERT M. HEYSSEL, M. ANN MELLY, AND DAVID E. ROGERS,* Nashville, Tenn.

The frequency of serious microbial infections in patients with reticuloendothelial disease has prompted numerous studies in which the ability of the RES to remove bacteria or other circulating particles has been experimentally altered. In such studies it has been generally assumed that the impaired blood stream clearance that follows large injections of particulate substances (RES blockage) results from saturation of the phagocytic capacity of reticuloendothelial cells. The present investigation indicates that this is not the case. Experiments in rabbits show that such RES blockage is specific for the particle under study. Injections of colloidal carbon, Thorotrast, aggregated albumin, or latex particles did not alter the subsequent blood stream clearance of radiolabeled colloidal gold stabilized with gelatin. However, when gelatin was used for the preliminary injection, subsequent clearance of the gelatin-stabilized radiogold was profoundly impaired. These observations suggested that changes in serum factors rather than changes in RE cell function might be operative. Studies performed with isolated perfused rabbit livers support this belief. Normal rabbit serum was required for hepatic uptake of gelatin-stabilized radiogold in such preparations. If serum obtained from a rabbit previously blockaded with gelatin was employed for perfusion, hepatic uptake of the gelatin-stabilized radiogold was markedly reduced. Conversely, livers from rabbits previously blocked with gelatin and normal livers both removed gelatin-stabilized radiogold equally well when the perfusate contained normal rabbit serum. Dissimilar particles (i.e., staphylococci) were removed with equal avidity from perfusion systems containing either normal or gelatin-blocked sera,

again indicating block specificity. Heating of normal serum to 56° C for 30 minutes did not impair its ability to promote liver uptake of suspended particles. These observations indicate that this type of RES blockade is produced by changes in specific serum factors rather than saturation of reticuloendothelial cells.

Genetic Influence upon Blood Lipids: An Unexpected Correlation with ABO and Lewis Blood Groups. DAVID H. BLANKENHORN,* JULIUS JENSEN, H. P. CHIN, AND PHILLIP STURGEON, Los Angeles, Calif.

Twin studies by others have shown that blood lipids are under genetic influence. This study presents the first thorough determination of the extent of genetic influence upon individual blood lipids. Thirty-one pairs of healthy adult monozygotic twins were typed for blood factors A, B; Le^a, Le^b; D, C, E, c, e; M, N; Fy^a; K; P_1; and Vel. Fasting serum total cholesterol, free cholesterol, phospholipid, and triglyceride were determined. Each twin was bled when first seen and again 2 days later. This experiment allows examination of genetic influence by determination of variance between twins of the same pair and distinction of this variance from variance associated with differences between pairs of twins, day to day variance, and residual variance. Genetic influence upon free cholesterol was greatest; influence upon total cholesterol and phospholipid followed in that order. When variances of free and total cholesterol between twins of the same pair were arranged in ascending order, they showed a logarithmic increase not correlated with weight, age, sex, level or variance of any lipid. Variance between twins of the same pair for free and total cholesterol of type O, Lewis a–b+ twins was significantly greater than that of all other Lewis a–b+ twins ($p < 0.01$). This was not true for phospholipid and triglyceride. Although the cause of this correlation is not known, the finding that monozygotic twins can be separated into subgroups having different abilities to maintain genetic similarity is important. More precise studies of genetic effects upon blood lipids should now be possible. The findings also suggest a previously unrecognized effect of blood group substances on blood lipids in man.

The Identification of Antigen E as the Principal Allergen of Ragweed Pollen. PHILIP S. NORMAN AND T. P. KING, Baltimore, Md., and New York, N. Y. (introduced by Dudley P. Jackson).

It has been postulated that a single substance is responsible for the sensitizing and hay fever producing potential of ragweed pollen. Earlier studies showed that a series of protein fractions from a Sephadex column had skin test activity in hay fever patients that paralleled their content of one antigen, designated as "E." Additional studies attempt to assign the importance of antigen E in human sensitivity to ragweed. Antigen E preparations gave positive direct skin tests in sensitive people and positive passive transfer tests in concentrations of 10^{-10} to 10^{-14} g per ml. Antibodies to antigen E were found by passive hemagglutination test in 45 of 47 untreated hay fever patients. Further separation of the fraction richest in antigen E on TEAE cellulose shows that antigen E exists as four closely related chemical congeners that differ in charge but are indistinguishable immunologically by gel diffusion and are equally active by skin test. Two of these congeners have been prepared 99% pure and used to make specific precipitating antisera in rabbits. By precipitation at the zone of equivalence these sera removed the antigen E from crude pollen extracts, leaving the other antigens in the supernatant fluid. Such supernatants lost 90 to 99.9% of the skin test activity of whole extracts in untreated ragweed-sensitive patients; hence, an antigen representing 6% of the protein in pollen is responsible for nearly all of the biological activity. The occurrence of antigen E in several biochemically separable but immunologically identical forms explains earlier experiments suggesting the presence of several allergens in ragweed pollen.

Effects of Norepinephrine and Methoxamine on Left Ventricular End-systolic and End-diastolic Volumes. JOHN W. ECKSTEIN,* MICHAEL G. WENDLING, AND FRANÇOIS M. ABBOUD, Iowa City, Iowa.

Cardiac output is reported to decrease with methoxamine and increase with norepinephrine. These experiments were performed to compare the effects of the two pressor agents on ventricular filling and emptying when heart rate was held constant. A cool solution of green dye was injected into the left ventricle (LV) of 10 chloralose-anesthetized dogs treated with decamethonium and ventilated artificially. The stepwise ejection of thermal indicator was recorded with a fast responding thermistor in the root of the aorta. Dye curves were recorded simultaneously at the femoral artery. Arterial (AP) and LV end-diastolic (EDP) pressures were measured continuously. Heart rate was held at 150 beats per minute by pacing the atrium electrically. Control observations were made after ganglionic blockade with pentolinium; experimental observations were made at the end of 5-minute infusions of equipressor doses of norepinephrine and methoxamine (0.2 μg and 0.025 mg per kg per minute, respectively). The interval between infusions was 30 minutes. Five dogs received norepinephrine, then methoxamine; the others received methoxamine first. Observations were made during 30 seconds of apnea produced by disconnecting the respiratory pump from the endotracheal tube. End-diastolic (EDV) and end-systolic (ESV) volumes were calculated by Holt's method. The ratio of ESV to EDV averaged 0.82 during control periods, 0.76 during norepinephrine infusion, and 0.87 during methoxamine infusions. Corresponding averages of EDV were 77.5, 88.0, and 98.9 ml; for ESV, 63.5, 67.3, and 85.7 ml; for EDP, 7.0, 8.2, and 12.3 mm Hg; and for AP, 106, 167, and 169 mm Hg. Stroke volume increased regularly with norepinephrine and decreased or remained unchanged with methoxamine. Failure of SV to increase with methoxamine as it did with norepinephrine when equipressor

doses were given was the result of reduced ventricular emptying from a greater end-diastolic volume.

Direct Assessment of Bone Formation and Resorption in Hyperparathyroidism by Quantitative Microradiography. B. LAWRENCE RIGGS, PATRICK J. KELLY, JENIFER JOWSEY, AND F. RAYMOND KEATING, JR.,† Rochester, Minn.

Specimens of posterior iliac crest were obtained from twenty-five patients with surgically proved hyperparathyroidism and from twenty-one control patients without metabolic bone disease. In the hyperparathyroid patients, serum calcium (normal, 8.9 to 10.1 mg per 100 ml) was 10.6 mg per 100 ml or less in six, between 10.7 and 12 in sixteen, and above 12 mg per 100 ml in three; serum alkaline phosphatase was elevated in three; and bone was abnormal by roentgenogram in five and by conventional histologic criteria in five. Bone specimens were studied by Jowsey's method of quantitative microradiography in which the linear extent of bone-forming and bone-resorbing surfaces is measured on an enlarged microradiograph and expressed as a percentage of total bone surface. Bone-forming surfaces are smooth and of low radiopacity; bone-resorbing surfaces are uneven and crenated. The method has been verified by *in vivo* labeling of bone-forming sites with tetracycline (correlation = 0.995) in humans and by labeling of bone-resorbing sites with Y^{91} (correlation = 0.990) in monkeys. In necropsy studies, this method gave similar formation: resorption ratios in different bones from the same individuals. All hyperparathyroid cases demonstrated increased bone resorption by quantitative microradiography (clinical diagnosis unknown): mean resorbing surface = 13.5% (range, 8.5% to 24.3%) compared with mean = 3.8% for controls (range, 1.8% to 6.0%). Bone formation was elevated in seven cases ($p = 0.05$). Preoperative serum calcium values correlated roughly with the extent of resorption. Results in two patients with pseudohypoparathyroidism resembled those in hyperparathyroidism. We conclude that almost all patients with hyperparathyroidism have demonstrable bone involvement when studied by this method.

The Carnitine-dependent Distribution of Fatty Acyl CoA's into Cellular Compartments. R. BRESSLER AND R. I. KATZ, Durham, N. C. (introduced by W. Nicholson).

Carnitine derivatives of fatty acids have been shown to serve as carriers of fatty acyl CoA's between extramitrochondrial and intramitochondrial sites. Microsomal transferases convert long chain fatty acyl CoA's to carnitine derivatives, which enter the mitrochondria, are reconverted to acyl CoA's, and are oxidized. Mitrochondrial transferases convert short chain acyl CoA's formed inside to carnitine derivatives that are transported out and reconverted to acyl CoA's. These short chain acyl CoA's are converted to long chain fatty acids in fed animals, whereas they are deacylated to acetoacetate in fasted animals. The effects of exogenous carnitine on the distribution of substrates in liver homogenates were studied in control and 24-hour fasted guinea pigs. Carnitine increased the production rate of acetoacetate in the fasted (F) preparation (F, 13; F + carnitine, 36 μmoles per g protein per hour), whereas it decreased the rate in the fed (C) (C, 7.6; C + carnitine, 4.2). Carnitine increased the rate of $C^{14}O_2$ production from palmitate-1-C^{14}, but decreased the rate of $C^{14}O_2$ production from pyruvate-2-C^{14} in both fed and fasted preparations. Both palmitate oxidation and acetoacetate production were markedly inhibited by γ-butyrobetaine, a competitive inhibitor of carnitine. This effect was reversed by carnitine. The *in vivo* synthesis of long chain fatty acids from pyruvate-2-C^{14} in the liver and epididymal fat pad was augmented five- to tenfold by the administration of carnitine to the fed but not the fasted animals. Carnitine effects the translocation of long chain fatty acids into mitochondria and short chain fatty acids out. This results in the augmentation of ketosis in fasting and decreases it in the fed animals, wherein fatty acid synthesis from acetyl CoA occurs primarily via pyruvate. This hypothesis is consistent with increased oxidation of long chain fatty acids (increased transport rate into mitochondria) and decreased oxidation of pyruvate associated with an increased extramitochondrial synthesis of acetoacetate or long chain fatty acids (active pumping of acetoacetyl CoA or acetyl CoA out of the mitochondria).

Mechanism of Biocarbonate Reabsorption in the Proximal Tubule of the Rat Kidney. F. C. RECTOR, JR.,* AND N. W. CARTER, Dallas, Texas.

Reabsorption of filtered HCO_3^- is thought to be mediated by secretion of H^+, resulting in the following intratubular reaction: $H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightarrow H_2O + CO_2 \rightleftharpoons CO_2$ (plasma). Since dehydration of H_2CO_3 is not instantaneous, excess H_2CO_3 would accumulate in tubular fluid and would not be in equilibrium with plasma CO_2. Theoretically, intratubular pH (pH_{TF}) should be at least 1 pH unit lower than that calculated by the Henderson-Hasselbalch equation, using plasma P_{CO_2} and $[HCO_3^-]_{TF}$ in tubular fluid. To test this hypothesis, we infused rats with 0.15 $NaHCO_3$ to assure maximal rates of HCO_3^- reabsorption; pH_{TF} was measured by puncturing proximal tubules with pH sensitive glass microelectrodes; $[HCO_3^-]_{TF}$ was measured in fluid aspirated from the same puncture site, using quinhydrone electrodes; plasma P_{CO_2} was calculated from blood pH and CO_2 content. In 25 measurements (15 rats) pH_{TF} was virtually identical to the calculated equilibrium pH, the difference averaging +0.05 pH unit. Therefore, there was no excess H_2CO_3 in the tubular fluid. This suggests either that HCO_3^- is reabsorbed by some process not involving the formation of H_2CO_3 (i.e., no H^+ secretion) or that H_2CO_3 is rapidly broken down by the action of carbonic anhydrase in the luminal membrane. These two possibilities were examined by repeating the measurements after complete inhibition of carbonic anhydrase (CA) with the inhibitor C 11,366. In 12 measurements (6 rats) pH_{TF} was consistently lower than the calcu-

lated equilibrium pH, the difference averaging −0.84 pH unit. After CA inhibition, therefore, excess H_2CO_3 does accumulate in proximal tubular fluid. These results establish first, that proximal reabsorption of HCO_3^- is mediated by secretion of H^+, and second, that CA is functionally located in the luminal membrane of the tubular cell, where it catalyzes the dehydration of H_2CO_3 generated in the tubular fluid. This important physiologic function of CA facilitates H^+ secretion by markedly reducing the pH gradient against which H^+ is transported.

Pacemaker Periodicity in Atrial Fibrillation. EDWARD J. BATTERSBY, Nashville, Tenn. (introduced by Elliot V. Newman).

Atrial depolarization in atrial fibrillation has been interpreted in the past as either the "heterorhythmic" result of a rapid ectopic focus or an equally aperiodic consequence of an irregular circus movement. Present studies demonstrate a definite underlying regularity to this atrial rhythm. Periodicity in an apparently disordered waveform may be detected by the generation of certain correlation functions. Particularly, a cross-correlation function formed employing a known periodic function may be used to demonstrate the presence, magnitude, and phase constancy of information of that frequency in a waveform being studied. The atrial waveform electrocardiograms from patients with atrial fibrillation were analyzed in this manner. A bipolar transthoracic lead was recorded at high gain, and 11- to 22-second segments of the record were digitized at 0.02-second intervals and the data transferred to IBM cards. An IBM 7072 digital computer was programmed to remove ventricular deflections and to generate the correlation function from "pure" atrial waveforms. Autocorrelation functions from the records of nine of eleven patients showed definite periodicity with progressive attenuation characteristic of narrow frequency band signal. Cross-correlation functions were generated from each of these nine records and a known periodic function and "tuned" to maximal amplitude. The root mean square deflection of the harmonic component of the atrial waveform ranged between 8 and 37% of that of the total waveform. Atrial rates by this method had an average of 440 beats per minute with a range of 388 to 528. The persistence of a strong fundamental periodicity with phase constancy is evidence that spontaneous atrial fibrillation in man is produced by a rapid persistently periodic pacemaker. The "randomness" of the waveform is likely the result of varying pathways of atrial conduction.

Influence of the DPN Content on the Rate of Reduction of Methemoglobin in Normal Human Erythrocytes. ERNEST R. JAFFÉ* AND GERTRUDE NEUMANN, New York, N. Y.

The major pathway for the reduction of methemoglobin to hemoglobin in normal human erythrocytes probably involves a methemoglobin reductase that preferentially utilizes DPNH. The present investigation was designed to determine whether increasing the intracellular concentrations of DPN, which can be reduced to DPNH by glyceraldehyde-3-phosphate dehydrogenase or lactic dehydrogenase activity, would increase the rate at which methemoglobin was reduced. After incubating washed, normal human erythrocytes for 16 hours at 37° C with glucose, glutamine, inorganic phosphate, and nicotinic acid, the concentration of DPN was increased by about three times. Little or no change in DPN concentration occurred in the absence of nicotinic acid or when nicotinamide replaced nicotinic acid. These preincubated erythrocytes, treated with sufficient sodium nitrite to convert essentially all of the hemoglobin to methemoglobin, were washed and then incubated without added substrate or with 10 μmoles of glucose or inosine or 20 μmoles of sodium lactate per ml. Significant, almost linear reduction of methemoglobin occurred during 10 hours of incubation without added substrate, presumably from endogenous substrate that accumulated during preincubation. The rate of reduction was about two times greater in erythrocytes with an increased content of DPN than in control suspensions. The rate with added substrates was two to five times greater than without their addition. The most rapid rate occurred with inosine, and this rate was not influenced by the DPN concentration. The rate with inosine may represent the maximal rate. The rate with glucose was about 1.5 times greater in cells with increased DPN concentrations than in erythrocytes with unchanged DPN concentrations. The rate with lactate was similar to that with inosine and was only slightly greater in cells with elevated DPN concentrations than in those with unaltered concentrations. These findings indicate that DPNH-dependent methemoglobin reductase activity can be influenced by availability of DPN.

The Effect of Changes in Boundary Layer Fluids on Osmotic Flow across the Isolated Turtle Bladder. WILLIAM A. BRODSKY,* T. P. SCHILB, H. WYSSBROD, AND C. GONZALEZ, Louisville, Ky.

Osmotic flow of solvent was studied in sacs of turtle bladders filled with sucrose solutions, 0 to 220 mM, and immersed in oxygenated Ringer's solution. The rate of solvent flow per unit of transmembrane gradient increased gradually for gradients of 0 to 175 mOsm and sharply for gradients of 175 to 220 mOsm. Such a curvilinear pattern could be due to changes in the geometric configuration of the tissue path available for solvent flow. This was confirmed by concurrent observations on changes in water content of the bladder wall. Assuming that geometric changes were restricted to boundary layer fluids subtending the membranes but not to the membranes per se, one could estimate geometric changes in the "unstirred" or boundary layer fluids from simple laws of diffusion and mass flow. Plots of theoretical resistance or length/area ratio of the diffusion path versus transmembrane gradients were similar in shape to plots of measured content of tissue water versus transmembrane gradients. An underlying assumption is that the rate of solvent flow versus effective os-

motic gradient is linear across the membranes, whence effective transmembrane gradients are less than experimentally imposed ones. In summary, consideration of boundary layer phenomena obviates the need for invoking driving forces other than the transmembrane osmotic gradient to account for a wide range of osmotic flow rates across a membrane that can perform osmotic work.

Effect of Amphotericin B on Cellular Membranes. WILLIAM T. BUTLER, DAVID W. ALLING, AND ERNEST COTLOVE, Bethesda, Md. (introduced by Robert M. Chanock).

Freshly drawn heparinized human blood, freed of its buffy coat, was incubated at 37° C with the polyene antibiotic, amphotericin B. During the first 2 hours there was a rapid increase in plasma K and a rapid decrease in plasma Na. Rate of change depended on the concentration of drug, and the kinetics were consistent with an increase in cell membrane permeability without hemolysis. Two-hour plasma K values of 6.1, 9.6, 16.0, 18.4, and 23.2 mEq per L were observed with concentrations of amphotericin B of 10, 30, 60, 100, and 150 μg per ml, respectively. During the next 30 hours the rate of increase of plasma K slowed and became constant; it was approximately the same (0.5 mEq per L per hour) for all concentrations of amphotericin B. Decreases in plasma Na followed the same pattern as that for the increases in K but were of lesser magnitude. Slight hemolysis occurred when incubation time exceeded 2 hours or when drug concentrations exceeded 150 μg per ml. Dog erythrocytes, which normally have a low-K, high-Na content, were incubated with amphotericin B, and only a slight increase in plasma K and decrease in plasma Na occurred. When the drug was injected intravenously into dogs, however, serum K rose an average of 1.7 mEq per L within 2 minutes. This rise considerably exceeded the estimated leakage from erythrocytes, indicating loss of K from other cells also. Other investigators have shown that the drug causes loss of K and other constituents from yeast cells. Thus, damage to cellular membranes by amphotericin B appears to be a general phenomenon and may be the basis for the toxicity of the drug in man.

The Lysis of Artificially Induced Intravascular Clots in Man by Intravenous Infusions of Urokinase: A Simplified Method for Clinical Use. ALAN J. JOHNSON,* W. ROSS MCCARTY, J. NEWMAN, AND HENRIETTE LACKNER, New York, N. Y.

Previous experiments with highly purified urokinase (UK), a plasminogen activator from human urine, indicated UK was nontoxic and nonantigenic in man. Unlike streptokinase, the endogenous inhibitor of UK was found to be competitive; therefore, the initial or priming dose was relatively predictable and constant. Lysis of artificially induced intravascular thrombi was readily produced by urokinase. However, the method was complicated and impractical, and the previously lysed thrombus tended to re-form during and after the period of infusion. In the present studies, the technical biochemical details required to produce a thrombolytic system *in vivo* have been simplified for practical clinical use. Thus, reformation of experimental thrombi was prevented during the period of infusion by infusing UK at an optimal rate. The rate chosen was large enough to produce an excess of circulating activator *in vivo* in the presence of endogenous UK inhibitors and small enough to prevent depletion of endogenous plasminogen to levels less than 20% of normal. Re-formation of thrombi after the infusion period was prevented by the administration of heparin in anticoagulant amounts for 24 to 48 hours after the urokinase infusion (heparin did not prevent thrombus re-formation due to excessive plasminogen depletion). Since the optimal infusion rate of UK was especially critical with respect to endogenous plasminogen, it was of interest to determine whether this rate might be constant from individual to individual. Persistent thrombolysis was obtained in each of 12 individuals with a single uniform sustaining dose of UK.

Specificity of Sodium Transport and the Biologically Active Form of Sodium Ion. HOWARD S. FRAZIER, Boston, Mass. (introduced by Paul C. Zamecnik).

Mucosal entry, the initial step in active transport of sodium by the toad bladder, is energetically passive but involves an interaction with the mucosal membrane. The specificity of mucosal entry of sodium has been investigated by examining the effects on it of the presence of other alkali metal ions and a series of organic monovalent cations. Among the alkali metal ions, an eight to one preponderance of Li or K in the mucosal medium significantly reduced ($p < 0.05$) the transbladder flux of Na^{24}, although only Li significantly depressed the labeling of the tissue pool of Na by Na^{24} in the mucosal medium. Of the organic ions tested, guanidinium, aminoguanidinium, 1,1-dimethyl guanidinium, and hydroxylamine reduced the transbladder flux of Na^{24}, but only guanidinium, aminoguanidinium, and hydroxylamine depressed tissue labeling with Na^{24} from the mucosal medium. The combination of reduction in Na^{24} flux from mucosal to serosal media and decrease in tissue labeling with Na^{24} from the mucosal medium indicates that it is the process of mucosal entry of Na that is affected. The specificity of the Li and guanidinium effects is shown by their failure to depress transbladder movement of K^{42} or alter that of Cl^{36}. In addition, the inhibition of transbladder transport of Na^{24} by Li and guanidinium is competitive. The guanidinium ion in aqueous solution is known to be a trigonally symmetrical resonance hybrid. The results of the present studies suggest that the form in which Na penetrates the mucosal cell boundary consists of the ion and three water molecules symmetrically arranged in the first hydration shell.

Hgb A_4, a Naturally Occurring Hemoglobin Possessing Only α Chains. AMOZ I. CHERNOFF,* Knoxville, Tenn.

Hgb A_4, a minor fraction of human hemoglobin solutions, has been isolated from all human hemolysates studied. This material elutes from DEAE cellulose before Hgb A_2 using 0.003 PO_4 buffers at pH 8.8. Hgb A_4 comprises from 0.05 to 0.3% of the total hemoglobin mass in normal adults. Preliminary studies suggest that increased amounts are present when hemoglobins with β chain abnormalities are being produced. Purified material has been prepared by sequential elution from DEAE cellulose and CMC. Its spectral properties are identical to those of Hgb A except for a flatter inflection at 288 to 292 mμ, suggesting that fewer tryptophan residues are present in this compound than are found in normal hemoglobin. Further evidence that the substance is a form of hemoglobin is provided by its reactivity with heme and protein stains and by peptide analysis. The latter has been carried out by a technique of starch gel electrophoresis, using urea-Veronal buffers at pH 8.0, and by peptide mapping of the tryptic digest of the isolated globin. The patterns thus obtained are indistinguishable from those observed with isolated α chains of Hgb A. The material has a sedimentation velocity of 2.86 consistent with a molecular weight of approximately 34,000. Molecular weight determinations at sedimentation equilibrium, however, indicate a molecular weight of slightly over 60,000, suggesting that the material exists as a tetramer, α_4. To rule out artifacts of preparation and handling as a cause for the presence of Hgb A_4, a number of different parameters were studied. Although my conclusion is that Hgb A_4 may be produced *in vitro,* particularly when dealing with certain abnormal hemoglobins, the evidence suggests that Hgb A_4 also occurs *in vivo* in the small amounts noted above. The implication of these observations relative to hemoglobin synthesis and its genetic control will be discussed.

Possible Protective Activity of Antilysosomal Autoantibodies in Patients with Hepatitis. P. A. MIESCHER,* G. WIEDERMANN, R. HIRSCHHORN, AND G. WEISSMANN, New York, N. Y.

Patients with hepatitis often exhibit a number of anticytoplasmic antibodies. Among 71 patients, 32 gave positive complement fixation reactions (serum titers, 1:40 to 1:640) with various cytoplasmic constituents (fractions rich in mitochondria, lysosomes, microsomes). The serological specificity of the serum factors was proven by absorption studies. Different antibodies react with a number of differing antigenic determinants localized in various cell fractions. These reactions are without organ or species specificity, i.e., similar results are obtained with cytoplasmic fractions of various species and organ origin. Most of these antibodies are 7 S globulins, although some belong to the 19 S globulins (saline gradient ultracentrifugation). Their presence may also be demonstrated by fluorescent technique. No pathogenic activity of these serum factors could be detected: they do not penetrate living cells and are without cytotoxicity on living cells *in vitro* (human carcinoma cells, human leukocytes, monkey kidney cells). Among these antibodies, antilysosomal factors appear of special interest. When they react with isolated lysosomes in the presence of complement, no lysis occurs. On the contrary, the vitamin A induced release of β-glucuronidase from lysosomes is significantly reduced by pretreatment of the granules with serum containing these antibodies (titers against lysosomes, 1:160 to 1:640). This "protective" effect is abolished by absorption of antibody containing serum with lysosomes. The possible protective activity of antilysosomal factors was further investigated by phagocytosis experiments: lysosomes isolated from rabbit leukocytes are readily phagocytized by human polymorphonuclear leukocytes. Upon phagocytosis of the lysosomes, leukocytes undergo extensive cell damage finally resulting in the death of the cell. When rabbit lysosomes were incubated with human antilysosomal serum before their phagocytosis by human leukocytes, little or no cell damage resulted from phagocytosis of lysosomes. This "protective" effect of human hepatitis serum containing antilysosomal activity is also readily removed by absorption with lysosomes. These results are compatible with the hypothesis that some autoantibodies act to preserve the integrity of cellular structures.

Studies on Placenta and Brain Thromboplastins. WILLIAM J. WILLIAMS,* Philadelphia, Pa.

Previously it was demonstrated that the clot-promoting activity of aqueous extracts of bovine lung resides in subcellular particles behaving as microsomes that function as enzyme in catalyzing the development of coagulant activity from a bovine serum fraction containing Factors VII and X. Added phospholipid is required for full activity of the product, which appears to be activated Factor X. These studies have now been extended to extracts of human placenta and brain. Placenta thromboplastin was found to reside in microsomes prepared by differential centrifugation, density gradient centrifugation, and extraction with butanol-benzene mixtures. Brain thromboplastin was prepared from a powder of acetone-dried brain by extraction with saline at 50° C, followed by differential centrifugation and treatment with butanol-benzene. The placenta preparations contained phospholipid, cholesterol, and protein; the brain preparations contained phospholipid and protein. Both placenta and brain preparations sedimented as a single band on isopycnic density gradient centrifugation, the placenta with average density about 1.15 and the brain with average density about 1.09. Both placenta and brain preparations functioned as previously described for the lung microsomes: the particles acted as enzyme in catalyzing the development of coagulant activity from the bovine serum fraction, calcium was required for the reaction, added phospholipid was necessary for full activity of the product, and part of the activity that developed was sedimented with the tissue particles. Brain preparations bound more activity than either lung or placenta. Brain particles recovered after incubation with serum fraction

and calcium showed a change in density from about 1.09 to 1.15, presumably due to binding of protein from the serum fraction. Apparently thromboplastins of various tissues differ somewhat in chemical composition but function in essentially the same manner in blood coagulation.

Effects of Structural Deletions on the Activity of Lysine Vasopressin. I. L. SCHWARTZ * AND L. LIVINGSTON, Cincinnati, Ohio, and Upton, N. Y.

The synthesis and study of desamino-oxytocin by Du Vigneaud and associates first showed the importance of removing functional groups of neurohypophyseal hormones and the need in general for caution in interpreting data on derivatives of biologically active peptides. Therefore, in continuing our survey of the relation of chemical structure to antidiuretic activity of vasopressin, we have compared the effect of addition and deletion of substituent groups of lysine vasopressin (LVP). *N*-acetyl Cys^1Lys^8 vasopressin, an analogue of LVP in which the terminal amino group is acetylated, was found to have less than 1% of the mammalian antidiuretic and uterotonic activities of LVP, to inhibit competitively the pressor activity of synthetic LVP and highly purified natural aginine vasopressin (AVP), and to have reduced affinity for neurophysin, the presumptive physiologic carrier protein of vasopressin and oxytocin within the hypothalmo-neurohypophyseal system. In sharp contrast, desamino Cys^1Lys^8 vasopressin (1-β-mercaptopropionic acid lysine vasopressin), the analogue of LVP in which the terminal amino group is deleted, has higher mammalian antidiuretic activity than its parent hormone although its mammalian pressor potency and its effect on amphibian membrane permeability are less than those of LVP. Its affinity for neurophysin is lost completely. An even more striking deletion effect is encountered in the case of Asp^4Lys^8 vasopressin, an analogue in which the glutaminyl residue of the natural hormone is replaced by an asparaginyl residue, thus shortening the amino acid side-chain in position 4 by one methylene group. This analogue has a higher antidiuretic/pressor activity ratio than LVP or AVP in rat, dog, and man; moreover it has greater absolute antidiuretic potency than AVP, heretofore the most potent known natural or synthetic mammalian antidiuretic principle. Thus, as a practical consideration, removal of the α NH_2 group in position 1 or of a $-CH_2$ group of the side chain of position 4 affords the therapeutic advantage of increasing antidiuretic activity relative to pressor activity; theoretically, the effects of these deletions suggest the importance of factors such as molecular complementarity, orientation, and, perhaps, hydrophobic bonding, as major determinants of the affinity of hormone for receptor and ultimately, therefore, of hormone action.

Changes in Tubular Function Produced by Saline Loading. LAURENCE E. EARLEY AND ROBERT M. FRIEDLER, Boston, Mass. (introduced by Laurence B. Ellis).

Evidence has been accumulated that regulation of sodium excretion involves changes in tubular reabsorption independent of mineralocorticoid. The effects on tubular function of infusions of isotonic saline (containing potassium and bicarbonate) were studied in fifteen dehydrated anesthetized dogs infused with vasopressin and desoxycorticosterone. Mild mannitol diuresis was employed in some experiments. Flow from one kidney was controlled by partial occlusion of the ureter or the aorta between the renal arteries. Urine flow was maintained at control rates while the animals received 1,000 to 1,500 ml saline. The controlled flow was associated with decreased glomerular filtration rate (GFR), as GFR increased in the contralateral kidneys undergoing diuresis. Despite unchanged urine flow during saline loading, urinary sodium and chloride concentration increased (when initially low), and urinary osmolality always decreased. Aortic or ureteral constriction without saline loading produced similar decreases in GFR, but sodium and chloride concentrations always decreased, and osmolality always increased. Decreased reabsorption was demonstrated by *1*) increased sodium excretion with decreased filtered load, *2*) decreased GFR with unchanged urine flow, and *3*) frank diuresis with reduced GFR as ureteral constriction was partially released during loading. This decreased tubular reabsorption was accompanied by decreased urine osmolality (despite vasopressin infusion), which was not associated with increased solute excretion or urine flow. Ureteral or aortic constriction alone did not account for these changes. These studies indicate that saline loading may decrease tubular reabsorption in a manner affecting the concentrating mechanism. We suggest that increased medullary blood flow may lower medullary hypertonicity and reduce water reabsorption from the descending limb of Henle's loop. Reduced tubular fluid sodium concentration may then influence net transport at more distal sites.

Serum α_2-Macroglobulin and the Transport of Insulin. GASTON R. ZAHND AND JEAN-JACQUES SCHEIDEGGER, Geneva, Switzerland (introduced by Albert E. Renold).

The biochemical nature of serum insulin-like activity (ILA) as measured with rat adipose tissue is as yet unknown. This study was undertaken *1*) to obtain further data concerning I^{131}-insulin binding to α_2-macroglobulin and *2*) to investigate the possible relationship between α_2-macroglobulin-bound insulin and serum ILA. Human sera were equilibrated with porcine, bovine, and human I^{131}-insulin (15 to 60 mμg per ml), and microelectrophoretograms of these mixtures were analyzed by means of various antihuman sera followed by radioautography. In addition, preparative fractionation of sera was performed by ultracentrifugation, with and without sucrose density gradient. Purified I^{131}-insulin preparations (specific activity between 5.0 and 10 μc per μg) with full biological activity were used. At concentrations below 30 mμg per ml, radioinsulin combined preferentially with α_2-macroglobulin as identified with anti-α_2-macroglobulin rabbit serum. After separation of high

molecular weight serum proteins by ultracentrifugation, the radioactivity of I^{131}-insulin, added both *in vitro* or *in vivo,* remained associated with the precipitin arc corresponding to α_2-macroglobulin. The formation of the insulin-macroglobulin complex occurred rapidly, and reversibility of the binding was demonstrated when unlabeled insulin or rat liver slices were added to the incubation mixture. Upon addition of anti-insulin guinea pig serum to normal sera, the radioactivity shifted from the α_2-macroglobulin to the insulin antibody. Finally, in serum fractions obtained by ultracentrifugation, the more dense layers, enriched with α_2-macroglobulin, contained more than 75% of the total serum ILA. These results indicate that α_2-macroglobulin may serve as a physiological carrier of insulin in blood and thereby contribute to the biochemical characteristics of ILA.

Dialyzable, Ultraviolet Absorbing Substances from the Serum of Patients with Renal Failure and of Nephrectomized Dogs. R. I. HENKIN, P. A. SMALL, JR., F. C. BARTTER,† AND G. E. SCHREINER,* Bethesda, Md., and Washington, D. C.

Gel filtration on Sephadex G50 of serum from patients with renal failure shows three separate peaks with UV absorption at 280 mμ; that of normal serum shows only two peaks. With serum from chronically ill subjects without renal disease and from normal volunteers, a mean of 95% of the optical density (OD) at 280 mμ was recovered in the first peak (serum molecules greater than mol wt 10,000), 0.5% in the second, and 4.5% in the third (both of mol wt less than 10,000). Corresponding values for five patients with renal failure are 79.2%, 7.4%, and 13.4% OD in the first, second, and third peaks, respectively. Hemodialysis of the patients did not change the first peak but significantly decreased OD in the second and third peaks; values progressively returned to predialysis levels within 7 days. In patients with various types of renal disease, OD in the second and third peaks correlated with degree of their renal failure. One patient with dehydration and "prerenal" BUN elevation to 92 mg per 100 ml showed a normal pattern, without change after rehydration (BUN, 28). Two patients without kidneys, maintained by dialysis, showed a renal failure pattern; dialysis of the patients decreased OD of the second and third peaks significantly. Normal dogs show the pattern of normal man; after bilateral nephrectomy, OD in the second and third peaks increases progressively, reaching a maximum immediately before death. The second peak is composed of small molecules, dialyzable *in vitro,* which are eluted at the column volume. The third peak is composed of small aromatic polycyclic compounds that adsorb to Sephadex. Lyophilized eluates of the second peak from nephrectomized dogs and patients in renal failure have mass far in excess of corresponding eluates from normal dogs and human volunteers. This is far greater than that expected on the basis of OD.

Single Peptide Differences between γ-Globulins of Different Genetic (Gm) Types. E. C. FRANKLIN,* M. MELTZER, H. H. FUDENBERG,* AND B. FRANGIONE, New York, N. Y., and San Francisco, Calif.

7 S γ-globulin consists of two pairs of polypeptide chains known as A (H) and B (L). Synthesis of Gm factors, which are on the part of the A chain recovered in the F fragment after papain digestion, is controlled by two major codominant alleles known as Gm (a) and (b). Gm (a) and Gm (b) positive and negative proteins differ in that only positive proteins inhibit specific agglutination systems of Rh+ cells coated with incomplete antibody of the same Gm type by selected rheumatoid factors. We attempted to discover structural differences between molecules of different Gm types by comparing peptide patterns of trypsin hydrolysates by two dimensional chromatography and electrophoresis (fingerprinting). Peptide maps were prepared from F fragments and A chains of 22 Caucasians of the three genotypes [Gm (a+b+), Gm (a+b−), Gm (a−b+)]. All sixteen Gm (a+) maps contained a dark spot that was absent in twelve maps of Gm (a−) proteins. The difference was more striking in F fragment maps due to faint staining in the adjacent area in all A chains. Peptide maps of thirteen Gm (b+) F fragments had another distinct dark spot equal in intensity to a constant reference spot. This spot was absent in one Gm (b−) map and faint in another. Each of ten Gm (b+) A chains showed the same dark spot. Three Gm (b−) A chains, although distinctly different from the Gm (b+) maps, were more difficult to recognize, since they lacked the dark spot but had faint diffuse staining in the same region, due either to peptides from the slow fragment, further genetic heterogeneity based on minor differences at this locus, or the presence of Gm (b) activity in only a small fraction of the molecules. Apparently the serologic specificity of human gamma globulin groups reflects differences in single peptides. This genetic variability must be considered in studies of the structure of purified antibodies.

The Skin as an Excretory Organ for Iron. L. R. WEINTRAUB, D. J. DEMIS, M. E. CONRAD, AND W. H. CROSBY,* Washington, D. C.

After the intravenous administration of radioactive iron to a normal human subject, the loss of radioactivity, measured in a whole-body liquid scintillation detector during a two months' period, was greater than the recovery of radioactivity in complete stool collections. This led to the investigation of the skin as another organ for iron loss. Trace quantities of $iron^{59}$ citrate were injected intradermally in the forearm of normal volunteers. Thereafter the radioactivity was determined by placing the subject's arm in a small-animal whole-body liquid scintillation detector. During the first day there was a rapid disappearance of radioactivity from the injected arm and a concomitant increase of radioactivity in the blood. In the subsequent weeks there was a

second, slow loss of radioactivity. Radioautographs prepared from sections of skin excised from the area of injection revealed selective localization of the $iron^{59}$ in the epithelial cells of the epidermis, sweat glands, and hair follicles. In glands and follicles there was radioactivity in old as well as new epithelial cells, indicating that iron could be accepted by such cells after they were formed. $Iron^{59}$ was also detected in collections of sweat and keratin. These findings suggest that external loss of iron was responsible for at least a part of the second phase of the decrease in radioactivity of the arm; the skin as well as the small intestine may function as an excretory organ for iron through loss of iron-loaded epithelial cells.

Normalization of the Oxyhemoglobin Dissociation Curve in Sickle Cell Disease by Fetal Hemoglobin. PHILIP A. BROMBERG AND WALLACE N. JENSEN,* Pittsburgh, Pa.

It has been reported that the oxyhemoglobin dissociation curve of whole blood in SS disease is shifted to the right. This observation was reinvestigated with simultaneous measurements of intraerythrocyte pH and of fetal (F) hemoglobin content. All equilibrations were carried out *in vitro* at 37° C. The curves are characterized by the Po_2 required for 50% saturation ($P_{50}O_2$) and the value of n defined by the Hill equation. Intraerythrocytic pH was measured in each tonometer on freeze-thaw hemolysates. All $P_{50}O_2$ values were adjusted to an arbitrary intraerythrocytic pH of 7.20 with a Bohr effect correction of $\Delta \log P_{50}O_2 = -0.5\ \Delta$ pH. A hemolysate of each blood was analyzed for hemoglobins A, A_2, S, and F. No hemoglobin A was detected in the abnormal bloods. In 18 normal blood samples from 5 subjects, mean $P_{50}O_2$ was 25.4 mm Hg $\pm$ 1.8 mm (SD); n ranged from 2.0 to 2.9. In 16 sickle blood samples from 11 patients with SS disease, $P_{50}O_2$ ranged from 23.4 to 35.2 mm; n ranged from 2.1 to 3.0. There was a significant inverse correlation between the percentage of F hemoglobin and the degree of rightward shift of the curve. Nine sickle bloods contained less than 10% F hemoglobin. All their $P_{50}O_2$ values exceeded 29.5 mm. The remaining seven sickle bloods contained 10 to 19% F hemoglobin. Their $P_{50}O_2$ values were less than 29 mm in five samples (29.6 and 30.6 mm in two). The apparent effect of increasing concentrations of F hemoglobin in normalizing the oxyhemoglobin dissociation curve in SS disease may help explain the correlation of elevated F hemoglobin levels with milder clinical manifestations in SS disease.

ATEe Esterase Activity of Human Serum. Assay, Properties, and Presence of an Inhibitor. METIN YUCE AND MARIO STEFANINI,* Toledo, Ohio, and Chicago, Ill.

When native human serum is diluted 1/20 with distilled water and the pH of the solution is then adjusted at various levels with the gradual addition of 0.5% acetic acid solution, protein precipitates are formed which, when resuspended in water, are able to digest a variety of synthetic substrates when tested with the Brown modification of the Hestrain reaction. The fraction precipitated when the pH of the diluted serum is adjusted at 7.0, after resuspension in a volume of water equal to that of the serum used originally, digests tosyl arginine methyl ester, benzoyl arginine methyl ester, and benzoyl arginine ethyl ester. These activities are enhanced by the addition of streptokinase to the reaction mixture. The fraction digests also acetyl tryptophan ethyl ester, acetyl phenylalanine ethyl ester, and acetyl tyrosine ethyl ester (ATEe; 0.02 M in 10% cellosolve). The latter activities are not enhanced by the addition of streptokinase. Lysine methyl ester is not digested. Several physical and chemical properties suggest that ATEe esterase activity of human serum is identical to the C^1 1 esterase component of human complement. Human serum contains between 18 and 26 U per ml ATEe esterase activity, with an average value of 22.1 U and $\sigma = \pm 0.33$. Human serum contains also an inhibitor of ATEe esterase activity, which is precipitated from diluted human serum at pH 5.2 and which combines reversibly and, possibly, in stoichiometric proportion with ATEe esterase. ATEe esterase activity of human serum is depleted in severe parenchymal liver disease and in patients with diabetes mellitus showing liver involvement or fatty changes of liver.

The Replication Time and Pattern of Hepatoma Cells. JOSEPH HOFFMAN AND JOSEPH POST,† New York, N. Y.

The method of pulse-labeling of DNA and radioautography has been employed to study the replication of rat hepatoma cells *in vivo*. The tumor was induced by 5 months of feeding 3'methyl-4-dimethylaminoazobenzene, dissolved in corn oil, begun when rats were 3 weeks old. Tumors reach great sizes and may metastasize at this time. Animals were sacrificed 0.5 to 72 hours after the administration of labeled tritiated-thymidine (H^3TDR), 1 μc per g with SA of 0.36 c per mmole. The following time intervals have been estimated: replication time, −31 hours; DNA synthesis, −17 hours; G_2 plus mitosis, −2 hours; G_1, −12 hours. Only about 8% of the tumor cell interphase population is flash labeled after a single injection of H^3TDR. This group of cells has been followed through three cycles of division. The regular rhythm of tumor cell multiplication resembles that previously reported from this laboratory for liver cells in the normal growing rat (J. Cell Biol. 1963, **18**, 1). However, the tumor cell population differs from these normal cells in that the replication time is slower and there is more variation from cell to cell in the several time intervals studied. These findings indicate that hepatoma cell replication is under controls and is not chaotic. Two other autogenous tumors are under study to determine whether this pattern is unique to hepatoma cells. These findings on the kinetics of tumor cell replication *in vivo* are basic to the study of cytotoxic antitumor agents and

their competitive effects upon normal and tumor cells within the host.

Genetic Control of Lactate Dehydrogenase in Testis. ANTONIO BLANCO AND WILLIAM H. ZINKHAM,* Baltimore, Md.

Most mammalian and avian tissues exhibit five molecular forms (isozymes) of lactate dehydrogenase (LDH). Information currently available indicates that each isozyme is a tetramer formed by random association of two polypeptides, A and B. LDH-1 through LDH-5 have been designated A^0B^4, A^1B^3, A^2B^2, A^3B^1, and A^4B^0. If A and B synthesis is controlled by separate genes, then the isozymic composition of tissues would depend on the relative activity of these genes. Electrophoresis of testicular homogenates from some mature animals revealed the presence of one or more LDH isozymes ("band X") in addition to the usual five, suggesting that more than two genetic loci were responsible for LDH synthesis in testes. Further evidence for a third genetic locus was obtained by studying LDH in pigeon testes. Localization of LDH activity in starch gel with lactate and alpha-hydroxybutyrate as substrates revealed three types of isozyme patterns: types I and III exhibited one new major isozyme, and type II, five new isozymes. Dissociation and reassociation studies indicated that the band X complex in type II represented recombinants of the polypeptides from the major band X in types I and III. The frequencies of types I, II, and III in three different randomly mating populations containing 210, 312, and 204 pigeons agreed with those expected according to the Hardy-Weinberg law for a single pair of alleles. These observations indicate that three genetic loci control LDH synthesis in postpubertal testis: A, B, and C. In animals with a single band X, the genes at the C locus are alike, whereas in pigeons they may differ, so that three genotypes are possible: ABC/ABC, ABC/ABC′, and ABC′/ABC′. Previous studies on the ontogeny and localization of band X indicate that genes at the C locus are active in testis only after pubescence.

Impairment of Urinary Concentrating Mechanism Early in Essential Hypertension. DAVID S. BALDWIN,* HERBERT CHASIS, AND ERVIN A. GOMBOS, New York, N. Y.

The finding of renal functional defects early in essential hypertension would support the thesis that the kidney plays a causal role in the genesis of this disease. We have observed reduced sodium excretion in single kidneys of a majority of hypertensive patients and have localized excessive sodium reabsorption proximal to the diluting segment. In the present study the site of increased sodium reabsorption is further localized by demonstration of a defect in urinary concentrating mechanism. Renal hemodynamics and solute clearance (C_{osm}) were determined in the separate kidneys of 8 normotensives and 14 hypertensives during hydropenia and during mannitol osmotic diuresis with continuous administration of Pitressin. At each urine volume (V) mean values for C_{osm} and osmol U/P ratio were lower in the hypertensive than in the normotensive group. At a V of 0.25 ml per minute, U/P ratio averaged 2.60 in the hypertensive patients as compared to 3.28 in the normotensives. $T^c_{H_2O}$ at a C_{osm} of 6.0 ml per minute averaged 2.15 ml per minute per kidney in hypertensives and 2.35 ml in normotensives. To compare the two groups at the same solute load per nephron, the data were examined after factoring by filtration rate (GFR). Mean values for osmol U/P ratio and $T^c_{H_2O}$ remained lower in the hypertensive patients at solute clearances below 7 ml per minute per 100 ml GFR but were equal in the two groups at higher solute loads. Decreased U_{osm} and $T^c_{H_2O}$ at lower solute loads in hypertensives suggest impairment in Na transport by the ascending limb. However, normal $T^c_{H_2O}$ during osmotic diuresis when Na supply to the limb is increased indicates that maximal transport of Na is not affected. We conclude that impairment of urinary concentrating capacity is present early in the course of essential hypertension and that excessive proximal Na reabsorption is probably responsible for this defect.

Effects of a New Inhibitor of Cholesterol Biosynthesis on Adrenal Cortical Secretion in the Dog. PAUL CUSHMAN, JR., CAROL DICKSON, AND JAMES G. HILTON,* New York, N. Y.

Trans 1,4-bis,2-chlorobenzylaminomethyl cyclohexane dihydrochloride (AY 9944), a sterol Δ^7 reductase inhibitor that presumably blocks the conversion of lanosterol into zymosterol, was acutely perfused into isolated adrenal glands of dogs. Three dosage levels were administered to preparations undergoing sustained maximal ACTH stimulation, and the following effects on adrenal venous secretion of cortisol were noted. *1*) At a dose of 5 μg per minute for 10 minutes, the post-drug mean cortisol secretion rate of 8.0 μg per minute did not differ from the control mean of 8.1 μg per minute. *2*) After a 50 μg per minute for 15 minutes dose, the mean cortisol secretion rate was 5.5 μg per minute versus the mean control rate of 8.1 μg per minute, or a 29% reduction ($p < 0.20$). *3*) In five other experiments after 15 minutes of a dose of 500 μg per minute a 57% decrease occurred in cortisol secretion, i.e., 7.1 μg per minute versus control of 12.3 μg per minute ($p < 0.02$). Thirty minutes after cessation of the drug, cortisol secretion recovered to a rate of 11.1 μg per minute. Corticosterone production underwent similar reversible inhibition of identical magnitude (to a mean of 55% control). These results indicate a significant, but reversible, inhibition of corticosteroid secretion from the isolated adrenals after acute perfusion with AY 9944. The studies suggest that in the maximally stimulated adrenal corticosteroid production may, in part, depend upon *de novo* synthesis of sterol precursors from mevalonic acid rather than solely from preformed cholesterol adrenal stores.

Intracellular Protein Denaturation: An Ultrastructural Analysis. RICHARD A. RIFKIND, New York, N. Y. (introduced by Donald F. Tapley).

This study was designed to investigate the mechanisms of cellular injury associated with intracellular protein denaturation. Phenylhydrazine-induced hemolytic anemia, characterized by oxidative denaturation of hemoglobin and the formation of Heinz-bodies, serves as a model to study the fate of cells injured in this manner. As observed by light, phase contrast, and electron microscopy, Heinz-bodies are readily induced in circulating rabbit erythrocytes, as well as in more mature reticulocytes (identified by the absence of polyribosomes). Nucleated cells of marrow are insensitive to the drug. Heinz-bodies first appear as small central aggregates of electron-dense material that may display a paracrystalline organization. These bodies enlarge, coalesce, and migrate to lie beneath the plasma membrane, which is distorted but not ruptured by them. Blood isolated *in vitro* or within intraperitoneal diffusion chambers undergoes similar changes. Despite severe deformation of cellular architecture, no evidence of spontaneous hemolysis is observed. Evidence of red cell destruction was, consequently, sought in the spleens and livers of treated animals. Numerous Heinz-body-containing hemolyzed erythrocyte ghosts are observed within splenic sinuses and cords. Phagocytosis of this debris is active, whereas ingestion of intact cells is less frequent. On the other hand, in hepatic sinuses intravascular hemolysis appears uncommon, and there is phagocytosis of intact cells. In either case native hemoglobin is extracted from red cells before dissolution of Heinz-bodies, and there is no "pitting out" mechanism. The following conclusions were drawn. *1*) Cells are sensitive to phenylhydrazine-induced injury as a function of their maturity; older reticulocytes and erythrocytes have a decreased reductive potential and capacity to protect hemoglobin from oxidative denaturation. *2*) Injured cells are severely deformed but not hemolyzed by the drug alone. *3*) During splenic passage injured cells undergo, in part, intravascular hemolysis, and *4*) there is a difference in the manner whereby spleen and liver sequester and destroy erythrocytes exposed to an identical primary injury.

Cell Wall Composition and Virulence in Escherichia Coli. DONALD N. MEDEARIS, JR., AND EDWARD C. HEATH, Baltimore, Md. (introduced by Robert E. Cooke).

The polysaccharide components of the cell wall lipopolysaccharide (LPS) of the *Enterobacteriaceae* are primary determinants of O antigen specificity; O antisera possess opsonins for these organisms. Our studies indicate that the presence or absence of specific sugars in the LPS of *E. coli* strikingly affects its virulence. *E. coli* 0111 produces LPS that contains lipid, phosphorus, hexosamine, 3-deoxy-octulosonate, heptose, glucose, galactose, and colitose. In contrast, a mutant (J-5) which lacks UDP-galactose-4-epimerase produces LPS that contains no galactose or colitose. However, when J-5 is grown in medium supplemented with galactose (J-5-gal), its LPS is identical to that of 0111. The virulence of log-phase 0111, J-5, and J-5-gal was investigated by determining the capacity to resist phagocytosis *in vitro*, the LD_{50} for weanling mice, and the O antigen specificity. The phagocytic index of mouse polymorphonuclear leukocytes for 0111 was 48%; for J-5, 73%. Conversely, when the mutant was grown under conditions that allowed it to produce complete LPS (J-5-gal), the phagocytic index was 45%. The LD_{50} of 0111 was $10^{4.0}$, whereas that of J-5 was $10^{7.3}$. If galactose was injected with J-5-gal, its LD_{50} was $10^{6.7}$. The LD_{50}'s of formalinized 0111, J-5, and J-5-gal did not differ, nor did the pyrogenicities of their LPS preparations differ. Mouse antisera against 0111 and J-5-gal were cross-reactive with their respective O antigens, and unreactive with J-5 O antigen; J-5 antiserum was unreactive with 0111 and J-5-gal. It is possible that the lack of UDP-gal-4-epimerase in J-5, in addition to altering the sugar composition of its LPS, may have affected J-5 in some other way. Our results, however, strongly suggest that the altered polysaccharide composition of the LPS of J-5 affected not only its O antigen specificity, but also its capacity to resist phagocytosis and to kill mice.

Isolation, Physicochemical and Physiological Characterization of Gastrin. STUART D. TAUBER AND LEONARD L. MADISON,* Dallas, Texas.

The purpose of the present study was to isolate and purify the antral hormone, gastrin, in order to define its physicochemical and physiological properties. After initial acetone extraction of porcine antral mucosa, a new isolation procedure consisting of calcium phosphate brushite chromatography, anion exchange chromatography, and gel filtration yielded a single histamine-free polypeptide that migrated rapidly as an anionic protein in an electrical field and ascended as a single spot with an R_f value of 0.38 in paper chromatography. Ultracentrifugal analysis in a synthetic boundary cell revealed a single symmetrical peak with a sedimentation coefficient of 0.97×10^{-13}. Acid hydrolysis of a 3-mg sample of the purified polypeptide yielded 106 amino acid residues consisting of 17 different amino acids from which a mol wt of 12,430 was calculated, a value in close agreement with the sedimentation coefficient. Defining a unit of gastrin activity as that amount which produces HCl secretion equal to $\frac{1}{2}$ maximal histamine response in chronic gastric fistula dogs, the purified polypeptide had a specific activity of 4,200 mU per mg, which represents a ninetyfold increase in potency over the initial partially purified acetone powder. As little as 20 μg (84 mU) administered intravenously produced a significant increase in HCl secretion, and increasing amounts resulted in a typical log-dose response. Although doses in excess of 2,500 mU resulted in HCl secretion (22.8 mEq per hour) greater than that produced by maximal histamine stimulation (8.8 mEq per L), the log-dose response remained linear despite administration of doses as high as 4,200 mU. The magnitude of this response in HCl secretion

induced by this single polypeptide can account for the massive HCl secretion that characterizes the Zollinger-Ellison syndrome.

The Surface Charge of Isolated Toad Bladder Epithelial Cells. RICHARD M. HAYS AND KENNETH M. LIPMAN, New York, N. Y. (introduced by Quentin B. Deming).

The control of ion transport by the epithelial cells of the renal tubule and toad bladder may depend to a considerable extent on the nature and density of fixed charges on the cell surface. We have observed that in the absence of calcium, large numbers of epithelial cells detach from the toad bladder. Taking advantage of this, and employing the microscope method of cell electrophoresis, we have obtained direct estimates of the net surface charge of single cells. At pH 7.3, in KCl-phosphate buffer, the cells possess a net negative charge (mobility -9.8×10^{-5} cm per second per v per cm). Mobility decreases slightly between pH 7.3 and 4.0, then drops sharply as surface acid groups are titrated. The cells are isoelectric at pH 3.4; at pH 3.0 they move rapidly toward the cathode. The effect of calcium on cell electrophoretic mobility has also been detemined. In the absence of calcium (pH 7.3, imidazoleacetic acid buffer) mobility is $-11.2 \pm 0.3 \times 10^{-5}$, and in the presence of 5 mmoles per L calcium, $-8.9 \pm 0.4 \times 10^{-5}$ cm per second per v per cm. The difference, though small, is significant ($p < 0.001$). The cell surface, then, is a mosaic of fixed negative and positive charges; the calculated excess negative charge is 33,000 electronic charges per μ^2 and is generated by acid groups with pK's near 3.0. Neuraminic acid, a common component of cell surfaces, is present in toad bladder epithelial cells (thiobarbituric acid assay); neuraminidase, however, does not decrease cell electrophoretic mobility. Calcium, which promotes cell adhesion in the intact bladder, appears to bind to a limited number of negatively charged surface sites. If the sites prove to be those involved in intercellular attachment, we would suggest that calcium regulates ion transport by toad bladder and other epithelia by altering the spatial relationships between cells.

Importance of the Adrenergic Nervous System for Sodium Conservation during Sodium Deprivation in Normal Man. JOHN R. GILL, JR., AND FREDERIC C. BARTTER,† Bethesda, Md.

Adrenergic blockade has been shown to facilitate sodium excretion during rapid infusion of saline in normal subjects. It has also been shown to facilitate escape from sodium-retaining steroids, diminishing the sodium retention and weight gain in normal subjects taking 250 mEq of sodium daily. The present study shows the effects of adrenergic blockade on sodium conservation in normal subjects on a 9 mEq sodium intake. In the control studies decrease of daily sodium intake to 9 mEq was followed by cumulative sodium loss which ranged from 15 to 78 mEq and weight loss which ranged from 0.29 to 0.94 kg, during 8 days of study. During adrenergic blockade produced in the same subjects with guanethidine, decrease of daily sodium intake to 9 mEq was followed by cumulative sodium loss which ranged from 90 to 306 mEq and weight loss which ranged from 1.01 to 2.25 kg, during 8 days of study. Mean creatinine clearances during sodium deprivation were unchanged with guanethidine. Treatment with guanethidine did not prevent the rise in aldosterone secretion with sodium deprivation. Thus, in normal man, adrenergic blockade increases sodium excretion during rapid or prolonged sodium loading and during sodium deprivation. Furthermore, the additional loss of sodium with adrenergic blockade can occur without change in glomerular filtration rate. The adrenergic nervous system exerts its important role in the control of sodium metabolism through means other than the adrenal cortex.

Bacterial Interference in Experimental Staphylococcal Infections. JOHN C. RIBBLE AND HENRY R. SHINEFIELD, New York, N. Y. (introduced by Edward W. Hook).

The presence of one actively multiplying bacterial strain may prevent superinfection or production of lesions by another strain. In the present study this phenomenon of infection-immunity has been demonstrated in chick embryos infected with staphylococci. Additional observations are concerned with factors influencing the development of the immune state. Ten-day-old chick embryos were injected intra-allantoically with sterile broth or 10^4 coagulase-negative staphylococci with low virulence for chick embryos despite active multiplication in allantoic fluid. One day later 100 viable units of coagulase-positive staphylococci (phage type 7) highly virulent for chick embryos was injected intra-allantoically into both groups. Embryos inoculated with broth before challenge showed 84% (155 of 185) mortality at 3 days. In contrast, embryos previously inoculated with living coagulase-negative staphylococci showed a mortality of 32% (72 of 224). Significant differences in mortality were evident as long as 9 days after challenge. Heat-killed staphylococci did not confer protection. Protection was associated with a decrease in growth of the challenge strain. In embryos previously injected with broth, the concentration of coagulase-positive staphylococci in allantoic fluid was 10^9 per ml after 48 hours, but in protected embryos the titer was only 10^5 per ml. Staphylococcal infection was apparently confined to the allantoic cavity. No inflammatory lesions or bacteria were seen in stained sections of the body of the embryo. Growth of the coagulase-positive staphylococcus to concentrations of 10^9 per ml resulted in the appearance of a substance in allantoic fluid which passed bacterial filters and killed other chick embryos injected intravenously. These studies have shown that infection with avirulent staphylococci protects chick embryos against infection with virulent staphylococci. Protection is associated with interference with growth of the challenge strain.

Failure of Cultured Lymphocytes of Patients with Acute Rheumatic Fever to Respond to Streptolysin S. KURT HIRSCHHORN AND ROCHELLE R. SCHREIBMAN, New York, N. Y. (introduced by Saul J. Farber).

Cultured human peripheral blood lymphocytes from subjects with acute rheumatic fever (ARF) show a markedly diminished response to streptolysin S (SLS), a nonantigenic streptococcal toxin. Cells from normal individuals respond to 50 hemolytic units of SLS per 10^6 cells to approximately the same degree as to phytohemagglutinin (PHA), a kidney bean extract. Both SLS and PHA are mitotic stimulants producing morphological transformation of small lymphocytes to large lymphocytes and plasma cells. They induce this response in 80 to 95% of cultured cells, whereas only 5 to 15% of cells in culture undergo such changes spontaneously. Lymphocytes from patients with untreated ARF, although responding normally to PHA, show increases in transformation and mitosis of only 3 to 14% above the spontaneous rate when exposed to SLS. Abnormal individuals showing a normal high response to SLS in culture include patients with streptococcal pharyngitis, glomerulonephritis, rheumatoid arthritis, inactive rheumatic heart disease, and ARF after 3 weeks or more of penicillin therapy. Occasional patients with severe streptococcal infections demonstrate diminished responses to SLS. To demonstrate that unresponsiveness to SLS was relatively specific for lymphocytes from patients with ARF, these cells were challenged with 50 hemolytic units per 10^6 cells of streptolysin O (SLO), an antigenic streptococcal toxin. There was no difference between their response and that of cells from normals (5 to 40%). This rate of response is similar to that seen with other antigens, such as tuberculin. SLS does not act as a specific antigen in this system since cells from a 10-month-old child, not responding to SLO, did respond to SLS. These results are compatible with the hypothesis that individuals susceptible to ARF lose their ability to react to, and to inactivate, SLS when exposed to this toxin *in vivo*. Noninactivated SLS may consequently remain free to induce the destruction of tissue.

Mechanism of Neuromuscular Block in Myasthenia Gravis. DAVID GROB,* HAJIMEI HIMEI, AND TATSUJI NAMBA, Brooklyn, N. Y.

Neuromuscular block can be produced or aggravated in myasthenic patients by the intra-arterial administration of acetylcholine, which is followed by inhibition of, or in severely ill patients refractoriness to, the normal depolarizing action of this compound. Injected or endogenous acetylcholine combines with a receptor substance in the motor end-plate; in myasthenic patients this combination is followed by inhibition of transmitter action. A ribonucleoprotein was isolated by *d*-tubocurarine precipitation from normal and myasthenic skeletal muscle which combined strongly and competitively with acetylcholine and *d*-tubocurarine. Chemical differences in this ribonucleoprotein were not identified in myasthenic patients, but 30 of 56 patients had in their serum complement-fixing antibodies to this ribonucleoprotein, in contrast to none of 77 normal subjects and 8 of 267 patients with other diseases. Complement was not fixed by ribonucleoprotein treated with ribonuclease or chymotrypsin, or by the isolated ribonucleic acids. The complement-fixing antibody in the serum of myasthenic patients, a 7 S gamma globulin, combined with the A bands, nuclei, and end-plates of normal and myaesthenic muscle, and with nuclei of muscle, thymus, liver, and mucous membrane cells, as demonstrated by direct and indirect immunofluorescent techniques. The binding sites of globulin corresponded to the localization of ribonucleic acid, as indicated by pyronine staining. Immunization of rabbits with the ribonucleoprotein obtained from normal human muscle by *d*-tubocurarine precipitation evoked complement-fixing antibody that had muscle-binding and other properties similar to the antibody present in the serum of myasthenic patients. The antibody could be absorbed from the serum of myasthenic patients or immunized rabbits preferentially by human *d*-tubocurarine-binding ribonucleoprotein. The ribonucleoprotein appears to be a muscle receptor which is present in normal subjects and which, for unknown reasons, evokes in myasthenic patients muscle, nuclear, and end-plate-binding antibody.

Simultaneous Determination of the Volume of Air, Distribution of Ventilation, and Distribution of Blood Flow in the Lung. G. EMMANUEL AND I. SAFONOFF, Brooklyn, N. Y. (introduced by David M. Kydd).

The turnover rates of air in the lungs, $\dot{V}A_{2,1}/L_{2,1}$, and the fractions of the cardiac output, perfusing groups of alveoli with different ventilation/perfusion ratios, $\dot{Q}_{2,1}/Q_T$, were determined from the corrected semilogarithmic plot of the changing oxygen tension of arterial blood during oxygen breathing. Oxygen tension was monitored continually *in vivo* with an intra-arterial platinum electrode. $\dot{V}A_{2,1}/L_{2,1}$ was assessed from the decay time of the semilogarithmically plotted exponentials and $\dot{Q}_{2,1}/Q_T$ from their extrapolation to zero time. From these data and the measured oxygen consumption, alveolar ventilation, $\dot{V}A_{2,1}$, was estimated. The volume of air in the lungs, L_2, L_1, L_T, was determined from the ratios, $\dot{V}A_2/\dot{V}A_2/L_2$ and $\dot{V}A_1/\dot{V}A_1/L_1$. Determinations were made in five emphysematous patients. Simultaneous estimates of the lung volume and distribution of ventilation were made from the semilogarithmic plot of the concentrations of nitrogen in the mixed expired gas. Estimates of the distribution of pulmonary blood flow were obtained by the arterial oxygen saturation method. Mean values for lung volume obtained by the intra-arterial platinum electrode method were: $L_2 = 4.5$ L, $L_1 = 1.3$ L, $L_T = 5.8$ L, $L_2/L_T = 76\%$. Respective values obtained from analysis of the mixed expired gas were: $L_2 = 4.4$ L, $L_1 = 1.1$ L, $L_T = 5.6$ L, $L_2/L_T = 78\%$. Values for the distribution of ventilation obtained by the platinum electrode method were: $\dot{V}A_2 = 0.86$ L per minute, $\dot{V}A_2/L_2 = 0.22$ L per minute per L, $\dot{V}A_1/L_1 = 0.90$ L per minute per L. Values obtained from the mixed

expired gas: $\dot{V}A_2 = 0.89$ L per minute, $\dot{V}A_2/L_2 = 0.21$ L per minute per L, $\dot{V}A_1/L_1 = 1.2$ L per minute per L. Values for the distribution of blood flow were: by platinum electrode, $\dot{Q}_2/Q_T = 69\%$; by saturation method, $\dot{Q}_2/Q_T = 71\%$. The lung volume, the distribution of ventilation, and the distribution of blood flow can be determined from changes of oxygen tension in the arterial blood.

Lysozymuria: An Enzymatic Measure of Renal Tubular Damage. WARREN D. DAVIDSON AND DARWIN J. PROCKOP, Philadelphia, Pa. (introduced by Truman G. Schnabel, Jr.).

Previous clinical studies (New Engl. J. Med. 1964, **270**, 269) suggested that increased urinary excretion of the enzyme lysozyme may be a relatively specific indication of renal tubular damage. When the nephrotic syndrome was produced in rats with renal antibodies or with puromycin aminonucleoside, the massive proteinuria was not accompanied by lysozymuria unless lethal doses were administered. When tubular lesions were produced with 8 mg per kg $HgCl_2$, lysozyme excretion increased 100 times. Kidney tissue lysozyme decreased in the first 24 hours, but returned to normal levels at 48 hours in rats that did not become anuric. Peak levels of lysozymuria appeared before kidney levels of lysozyme returned to normal, suggesting that the urinary lysozyme originated in the kidney. The initial decrease in kidney lysozyme was paralleled by a decrease in kidney alkaline phosphatase activity, and the recovery of lysozyme levels was accompanied by increased protein synthesis as reflected by increased RNA:DNA ratios. These results suggest that kidney lysozyme changes may reflect first the destruction and then the regeneration of renal tubules. When tubular lesions were produced with 0.5 g per kg of maleic acid, lysozyme excretion increased up to 3,000 times. Although the histological changes were as severe as those with $HgCl_2$, the lysozymuria disappeared more rapidly. Earlier recovery of renal tissue was also suggested by the fact that kidney lysozyme and alkaline phosphatase levels were normal at 24 hours, and the lysozyme levels were greater than normal at 48 hours. These observations suggest that lysozyme assays may be useful in establishing the nature, extent, and repair of renal lesions both in experimental animals and in man.

Evidence for Aminoaciduria and Peptiduria in Adult Celiac Disease. O. DHODANAND KOWLESSAR, LORRAINE J. HAEFFNER, AND HOWARD D. BRONSTEIN, Jersey City, N. J. (introduced by Harry J. Robinson).

The concentration of free amino acids was determined quantitatively on 24-hour urine samples of 15 normal subjects, 8 untreated celiac patients, and 11 celiac patients on a gluten-free diet. The amino acids were separated by high voltage electrophoresis (HVE) at pH 2.2 (4% vol/vol formic acid; 0.3% pyridine) at 2,000 v for 70 minutes, followed by chromatography in butanol: acetic acid: water (120:30:50). The chromatograms were dipped in Ninhydrin, and the amino acids were eluted and measured colorimetrically. The results obtained are: *1)* Three patients with celiac disease and osteomalacia had striking increases in the excretion of glutamine, serine, citrulline, glutamic acid, threonine, leucine, and alanine. *2)* Celiac patients, without osteomalacia, had normal amino acid excretion. *3)* Long-term studies on the amino acid excretion of two of the three patients with aminoaciduria showed a reversal to a normal excretion with resolution of their osteomalacia. In the patients with aminoaciduria, a yellow Ninhydrin spot was observed with an R_f similar to alanine in the system used, and is a hydroxyproline peptide. Total hydroxyproline was 236.2, 157, and 242 mg per 24 hours in the three patients with osteomalacia, with a fall to normal levels of 50 and 38 mg per 24 hours in two of the patients with resolution of the osteomalacia. The rest of the celiac patients had normal excretion of total hydroxyproline. These studies suggest that celiac patients will have an aminoaciduria when they are vitamin D deficient and have osteomalacia. Further, the increased excretion of bound hydroxyproline is evidence for secondary hyperparathyroidism in these patients.

The Aromatization Reaction: Studies of the in Vivo and in Vitro Conversion of Androgens to Estrogens. MAURICE M. PECHET,* HEINZ KOHLER, AND EVELYN CARROLL, Boston, Mass.

Published results indicate that 19-hydroxytestosterone is an intermediate in the *in vitro* conversion of testosterone to estrogens, especially by placental tissue. Hirsutism and virilism associated with menstrual disorders have been attributed to a failure in conversion of testosterone to estrogens involving an enzymatic defect in 19-hydroxylation of testosterone. It has been postulated that this defect in the aromatization reaction induces an accumulation of testosterone with its resultant androgenic effects. 19-Hydroxytestosterone was prepared and its conversion to estrogens in human subjects investigated. Urinary estradiol, estrone, and estriol were determined by the Brown chromatographic technique. With the administration of a dose of 100 mg daily for 6 days the conversion to estrogens was 0.002%; with 80 mg daily for 6 days the conversion was 0.058%; with 70 mg daily for 6 days, 0.002%; and with 30 mg daily for 6 days, 0.001%. Other parameters indicative of an estrogenic effect were studied. At all doses there were no significant changes in Ca or P balances or in serum P. Thus it is evident that 19-hydroxytestosterone is not a significant precursor of estrogens in man. 19-Hydroxytestosterone was incubated with rat liver homogenate. The isolation and rigid chemical characterization of dihydro-19-hydroxytestosterone and tetrahydro-19-hydroxytestosterones indicate that reduction by enzyme reductases occurs without elimination of the 19-oxygen function. Evidence for the presence of enzymes involved in the conversion of 19-hydroxytestosterone, dihydro-19-hydroxytestosterone, or tetrahydro-19-hydroxytestosterones to estrogens was not found. The studies in man indicate that 19-hydroxytestosterone is not an obligatory

intermediate in the conversion of androgens to estrogens and is not a significant precursor of estrogens in man.

Cerebroside Synthesis in Peripheral Nerves: A Defect in Diabetes. SVEN G. ELIASSON* AND GEORGE CROWLEY, St. Louis, Mo., and Dallas, Texas.

Decreased conduction velocity in the peripheral nerves of rats is noted after induction of diabetes by alloxanization or subtotal pancreatectomy. Measurements on the internodal segments of isolated single nerve fibers from diabetic rats indicate that the defect is due to a change in the transverse resistance of the myelin sheath. The slowing of nerve conduction follows the same time course as a decrease in the incorporation of radioactive precursors into cholesterol and fatty acids isolated from nerve fragments. To elucidate the effect of this decrease on the myelin sheath composition, fragments of nerve fibers were prepared, and the incorporation of C^{14}-labeled acetate and acyl-CoA into myelin lipids was studied. The decrease in cerebroside synthesis in peripheral nerves from diabetic animals was more marked than expected. There was also a change in the type of cerebrosides synthesized with most of the label recovered in unsaturated cerebrosides in the diabetic animals. The radioactivity was found in the saturated side chain of the cerebrosides from normal nerves. Partially purified enzyme systems from nerves of normal and diabetic animals were used to determine that whereas psychosine was preferentially incorporated into the cerebrosides in both cases, the enzyme system from the diabetic animal was much more inactive towards saturated acids than the system isolated from the normal nerves.

Exercise Diffusing Capacity (D_{LCO}) in Normal Men with Acute Pulmonary Congestion. WALTER J. DALY, SAMUEL T. GIAMMONA, AND JOSEPH C. ROSS, Indianapolis, Ind. (introduced by John B. Hickam).

Greater increases in D_{LCO} are found during heavy exercise than can be produced by other means. Such increases in D_{LCO} imply enlargement of the effective pulmonary capillary bed. Procedures acutely increasing pulmonary capillary transmural pressure may also enlarge the pulmonary capillary bed and produce smaller increases in D_{LCO}. In 11 subjects, breathholding D_{LCO} was measured during both rest and exercise (1,100 ml O_2 per minute) in seated, supine, and 60° head-down positions. D_{LCO} increased from 32.8 ± 6.4 (sitting) to 36.1 ± 7.2 (supine) to 43.7 ± 9.4 ml per minute per mm Hg (60° head-down), $p = 0.001$. During exercise at 60°, D_{LCO} increased further to 46.8 ml per minute per mm Hg, $p = 0.025$. During this same exercise, sitting, D_{LCO} was 42.2 ± 8.1 ml per minute per mm Hg. In four subjects, right atrial pressure and D_{LCO} were determined in supine and 15°, 30°, and 60° head-down positions. Despite progressive increase in right atrial pressure from 8 to 20 mm Hg, very little increase in D_{LCO} was produced by tilting beyond 15°. (Supine, 37.6; 15°, 43.2; 30°, 43.4; 60°, 45.0 ml per minute per mm Hg). D_{LCO} and vascular pressure increases so obtained are maintained for at least 5 minutes while similar changes during g-suit inflation decrease after 30 seconds. These data show: *1)* The head-down position is a convenient way to produce relatively stable acute pulmonary congestion and increased D_{LCO} in man. *2)* In the presence of pulmonary congestion, exercise further increases D_{LCO}. It is known that increased blood flow, increased ventilation, and decreased venous P_{O_2} or pH do not increase breathholding D_{LCO}, and that impaired autonomic reactivity does not completely block D_{LCO} increases with exercise. The present study shows that D_{LCO} is further increased by exercise in subjects whose pulmonary capillary bed has already been enlarged by pressure. Exercise may produce a humoral substance that facilitates enlargement of the effective pulmonary capillary bed at a given level of applied pressure.

Studies of Bovine Thrombin Enhancement of Acid Hemolysin Reaction of Paroxysmal Nocturnal Hemoglobinuria (PNH). DAVID E. JENKINS, JR., NORMAN L. LYDE, AND ROBERT C. HARTMANN,* Nashville, Tenn.

The mechanism by which crude commercial bovine thrombin preparations enhance the *in vitro* acid hemolysin reaction of PNH has been disputed. Originally thought due to thrombin clotting activity, subsequent work suggested that this enhancement is due to contaminating heterophile antibodies which agglutinate normal as well as PNH erythrocytes. However, absorption with normal red cells to remove heterophile antibodies decreased but did not abolish the ability of crude bovine thrombin to enhance PNH hemolysis. Studies from this laboratory have confirmed the presence of agglutinating antibodies in crude thrombin. In addition it has been shown that crude thrombin can fix complement to normal and PNH erythrocytes. This suggested a more definite mechanism for enhancement of PNH hemolysis by thrombin. Exhaustive absorption of crude thrombin with normal cells, while removing all agglutinating activity, did not remove all complement-fixing and PNH hemolytic-enhancing activities, indicating the presence of a second substance in crude thrombin which enhances PNH hemolysis. This second substance, heat stable and thereby distinct from thrombin, appears also to be an antibody. To determine the effect of thrombin clotting activity on PNH hemolysis, highly purified thrombin was prepared by gel filtration and cation-exchange chromatography. This highly purified thrombin, at a concentration of 20 NIH units had no effect on PNH hemolysis in comparison to the marked enhancement observed with crude thrombin preparations of equal clotting activity. These findings have been observed with erythrocytes from six patients with PNH. Thus the ability of commercial bovine thrombin preparations to enhance PNH hemolysis appears to be unrelated to thrombin clotting activity but rather due to the presence of contaminating complement-fixing antibodies in these preparations.

Abnormal Triglyceride Dynamics in the Coronary Prone and Addisonian Subject. RAY H. ROSENMAN,* MEYER FRIEDMAN, AND SANFORD O. BYERS, San Francisco, Calif.

Recently we found the serum triglyceride to rise and remain abnormally high after ingestion of a test fat meal by well men exhibiting a behavior pattern (type A) associated with markedly increased incidence of coronary artery disease. To determine whether adrenal function played a part in the disturbed postprandial lipid dynamics of subjects with pattern A, a series of eight apparently well men exhibiting behavior pattern A were given the test meal after a course of *1*) metopirone, *2*) cortisone and ACTH, *3*) cortisone alone, and *4*) ACTH alone. It was found that their control average fasting, 4-hour, and 8-hour postprandial serum triglyceride values of 115, 250, and 167 mg per 100 ml, respectively, remained unchanged after administration of metopirone or cortisone. However, after cortisone and ACTH, or ACTH alone, the average triglyceride values dramatically normalized, becoming 65, 123, and 105 mg per 100 ml, respectively. These data suggested that the abnormal triglycerides found in our well subjects with behavior pattern A were due to some inadequacies in adrenal hormone supply or utilization. The pre- and postprandial triglyceride values of four Addisonian patients (adequately maintained on hydrocortisone and fluorohydrocortisone) were determined after they were given the test meal. It was found that, like our eight type A subjects, Addisonian pre- and postprandial triglyceride values also were abnormal, being 125, 272, and 159 mg per 100 ml, respectively. Unlike what occurred in our eight subjects with pattern A, these values understandably remained unchanged in Addisonian patients after ACTH administration. These data suggest that the abnormal triglyceride dynamics observed in both our subjects with behavior pattern A (and by inference in the coronary patient) and in Addisonian patients stem from an inadequate supply or utilization of some adrenal hormone(s) other than hydrocortisone and fluorohydrocortisone.

Measurements of Lipid Esterification by Internal Mucosa of Patients with Diabetes. MALCOLM P. TYOR AND ROBERT S. BRICE, JR., Durham, N. C. (introduced by Julian M. Ruffin).

In an attempt to evaluate more directly the possibility of a defect in lipid esterification by intestinal mucosa of diabetic patients, homogenates of mucosal biopsies from distal duodenum of five diabetic patients, four students, and one patient with nonspecific diarrhea have been incubated with labeled fatty acid. Four diabetics showed modest steatorrhea; none had a morphologic intestinal abnormality. Insulin was withheld on the morning of the study; blood sugars ranged between 260 to 470 mg per 100 ml. Homogenates were incubated with 1-C^{14}-palmitic acid, 100 mμM, 260,000 cpm, and buffered to pH 7.4 at 30° C. Duplicate homogenates were incubated with ATP, 10^{-3} M. Upon completion of each incubation, the lipid soluble material was extracted and fractionated into triglycerides (TG) and phospholipids (PL) by thin-layer chromatography. Average values for incorporation of labeled fatty acid into TG (mμmoles per 10 mg protein) by duodenal mucosa of diabetics (12 determinations) after 45 minutes of incubation were slightly higher than those of nondiabetic controls (6 determinations); without ATP: 8.24 ± 3.44, 5.58 ± 0.89; with ATP: 57.01 ± 18.38, 38.29 ± 17.11. Similar differences between the two groups were evident in the PL fractions; without ATP: 6.56 ± 1.71, 6.19 ± 2.12; with ATP: 33.32 ± 13.45, 25.54 ± 6.98. ATP uniformly enhanced incorporation of C^{14}-palmitate into both TG and PL of intestinal homogenates of all subjects. However, the increase into TG was consistently greater. When incorporation of labeled palmitate into TG of intestinal homogenates incubated with ATP was compared in four diabetics and five controls after 10-, 20-, 30-, and 45-minute incubations, significantly greater values were obtained from diabetic mucosa: at 10 minutes, 15.32 ± 2.76, 9.52 ± 2.99, $p = <0.05$; at 20 minutes, 36.69 ± 4.40, 17.72 ± 3.79, $p = <0.02$. These experiments demonstrate that intestinal mucosa from hyperglycemic diabetics with steatorrhea has a normal capacity to incorporate fatty acid into TG and PL, and that this incorporation may be enhanced in the presence of excess ATP.

The Influence of Cholesterol on the Shape of Erythrocytes. JOHN R. MURPHY,* Cleveland, Ohio.

This study was designed to determine if the shape of the erythrocyte is related to differences in the lipid composition of the membrane in different areas of the membrane. The cholesterol in the membrane of erythrocytes has been known to be in a dynamic state, rapidly exchanging with free cholesterol in the plasma. Radioautographs were made of erythrocytes containing cholesterol-7α-H^3 after incubation in serum containing cholesterol-7α-H^3. The radioautographs showed that the cholesterol in the membrane of normal erythrocytes was concentrated around the periphery of the cell with less cholesterol in the central biconcave area. In contrast, the cholesterol in the ovalocyte membrane was found to be concentrated toward the ends of the abnormal cells. Cholesterol also influenced the surface area of the erythrocyte membrane. An increase in erythrocyte cholesterol was associated with an increase in surface area, whereas a decrease in erythrocyte cholesterol was associated with a decrease in surface area of the membrane. It is proposed that the shape of erythrocytes is related in part to relative differences in the interfacial tension in different areas of the membrane-plasma interface. The localization of cholesterol, a hydrophobic substance, around the periphery of the cell is associated with a high interfacial tension in that area. The central biconcave area with less cholesterol has a low interfacial tension and is more wettable. The differences in interfacial tension result in the biconcave shape. The relationship of cholesterol and surface area suggests that a change in the amount of cholesterol in

the membrane altered intermolecular forces in the membrane, resulting in changes in surface area.

Natriuresis in Pregnancy during the Infusion of Physiologic Amounts of Antidiuretic Hormone. VICTOR E. POLLAK, CESAR TORRES, AND LIONEL J. SCHEWITZ, Chicago, Ill. (introduced by Robert M. Kark).

Clinically, pre-eclampsia has many features in common with water intoxication; histologically, intracellular edema is found. Recent evidence points to decreased body sodium or increased body water, or both, in women with pre-eclampsia when compared with healthy pregnant controls. Increased antidiuretic hormone (ADH) activity has been reported in serum and urine of patients with pre-eclampsia. The present experiments were therefore designed to investigate the effects of ADH on sodium excretion during pregnancy. Four healthy nonpregnant young women, four healthy second trimester, and four healthy third trimester women were studied under strict control in left decubitus on normal sodium diets. Steady levels of hydration were maintained throughout. When hydrated, 8-arginine vasopressin was infused at a steady rate (dose range: 0.3 to 0.6 mU per minute) in each of two 90-minute periods. Each infusion was preceded and followed by a 60-minute control period. Three timed urine collections were made in each of the two experimental and three control periods. During ADH infusion urine flow and free water clearance decreased markedly in all studies. Creatinine clearance was unchanged in nine studies and decreased slightly in three. Sodium excretion, unchanged in nonpregnant subjects, increased 80% in the second trimester, 21% in the third. This was due to tubular effects of ADH as the percentage of filtered sodium excreted during ADH infusion was 218% of control in the second trimester. Osmolal and chloride excretions were concordant. Potassium excretion decreased (average, 27%) in all subjects. ADH infusion resulted in a 37% increase in the percentage of filtered urate excreted in the second trimester. Urate excretion rate was related to sodium excretion rate. If ADH secretion is indeed increased in pre-eclampsia, these observations suggest a mechanism that may result in both water retention and sodium depletion in pregnancy.

Anaerobic Sodium Transport by the Isolated Turtle Bladder: Dissociation of Glycolysis from Transport. SAULO KLAHR AND NEAL S. BRICKER,* St. Louis, Mo.

Epithelial cells that transport sodium transcellularly facilitate the investigation of the dependency of active transport upon metabolic energy. Recent studies have shown that the turtle bladder epithelia transport sodium not only aerobically but anaerobically. That anaerobic sodium transport is related to lactate production is suggested by the fact that elimination of transport by substituting choline for sodium in the bathing media was associated with decreased lactate evolution. In the present studies, an attempt has been made to gain insight into the characteristics of the energy transfer mechanisms. Dinitrophenol (DNP) is a known uncoupler of oxidative phosphorylation. Although the precise molecular basis of this uncoupling action is not yet clearly defined, current evidence suggests that DNP may interfere with the formation of a high energy intermediate in the mitochondrial-linked energy transfer pathway. In these studies, DNP (1×10^{-5} M) has been found to inhibit anaerobic as well as aerobic transcellular sodium transport. With DNP, anaerobic glycolysis as measured by lactate production increased greatly. ATP levels decreased only modestly. With antimycin in oxygenated Ringer's solution, sodium transport persisted while lactate production increased appreciably. This response to an inhibitor of mitochondrial electron transport simulated the results obtained with anaerobiosis. The turtle bladder epithelial cells appear capable of providing free energy for sustained active transcellular sodium transport by anaerobic glycolysis. The inhibition of anaerobic sodium transport by DNP occurring in the presence of adequate tissue ATP levels (and markedly increased rates of glycolysis) raises the possibility that DNP may interfere with the formation of a high energy intermediate similar to that arising in mitochondrial energy transfer. The possibility also exists that such an intermediate, rather than ATP, may represent the energy source for transport in this system. An interaction of DNP with the carrier itself is not excluded.

Preservation of Human Platelet Concentrates by Controlled Slow Freezing in a Glycerol Medium. PHIN COHEN, E. JOHN PARKER-WILLIAMS, PATRICIA A. WATROUSE, AND FRANK H. GARDNER,* Boston, Mass.

Human volunteers were bled into one of three anticoagulants: *1*) 440 milliosmolar, pH 3.8 EDTA; *2*) 780 milliosmolar, pH 4.9 ACD, NIH Formula A; *3*) 440 milliosmolar, pH 4.6 modified ACD. Citrate buffer, 1,100 milliosmolar, pH 5.8 when added in 1:10 ratio to platelet rich plasma (PRP) prepared from ACD blood, permitted resuspension of platelet concentrates (PC) and salvaged the red cells for standard blood banking. PRP from all anticoagulants was centrifuged at $200 \times g$ for 5 minutes to sediment most of the red cells. PC were prepared by centrifugation of PRP for 20 minutes at 1,000 $\times g$ and were labeled with 300 μc of chromium^{51}. Glycerol then was added in equal volume as a 16 to 32% (vol/vol) solution in 200 to 240 milliosmolar potassium phosphate buffer. Final glycerol concentration was 8 to 16% (vol/vol). PC were frozen by monitoring the refrigerant bath to achieve a 1° C per minute decrement to −30° C and 5° C decrement to −75° C. Frozen PC were thawed in cold tap water. An equal volume of 1,050 milliosmolar sodium citrate was added, and PC again were prepared by centrifugation. Supernatant citrate-glycerol solution was discarded, and PC were resuspended in 10 ml autologous platelet-poor plasma. PC were given through a filter in 5 minutes. No adverse reactions were seen in 266 transfusions. Thirty per cent of injected radioactivity was harvested from peripheral blood of autologous recipients whose PC were prepared

from modified ACD and frozen in 10 to 12% glycerol (vol/vol). A 45% harvest was achieved from glycerol-treated, unfrozen PC, and a 70% harvest from nonglycerol-treated, unfrozen PC. The platelet lesion of collection in ACD is incompletely reversed by adding citrate buffer to PRP. The maximal harvest from nonglycerol-treated, unfrozen ACD PC was 50 to 60%. The harvest from frozen, glycerol-treated EDTA PC was poorer than with either standard or modified ACD.

Control of Thyroxine Metabolism with 2-Tertiary Butyl 4-Methoxyphenol. JAMES WYNN,* Durham, N. C.

Thyroxine is displaced from microsomal degradation reactions *in vitro* by 2-tertiary butyl 4-methoxyphenol (J. biol. Chem. 1963, **230**, 3490). The possibility that this compound may displace thyroxine from *in vivo* degradation reactions has been investigated. A male volunteer was treated for 31 days with 0.2 mg L-thyroxine to stop function of his thyroid gland. On day 18 of such therapy, 50 μc of L-thyroxine (3',5'I^{131}) was administered orally. On days 23 through 25, 100 mg of 2-tertiary butyl 4-methoxyphenol was administered orally, daily in gelatin capsules. Serum obtained from day 19 through the termination of the experiment was assayed for radioactive content, radioactive constituents, and for PBI. After administration of 2-tertiary butyl 4-methoxyphenol, the rate of disappearance of thyroxine decreased, and the level of PBI increased. These changes were reversed by stopping the drug. At a 1×10^{-5} M concentration, 2-tertiary butyl 4-methoxyphenol has no influence on specific, serum building of thyroxine. These studies suggest that this compound may interfere with thyroxine degradation *in vivo* as it does *in vitro*. This type of ortho-para hindered phenol may provide a means for studying the relationship of thyroxine function to peripheral thyroxine metabolism.

Role of Catecholamines in the Mobilization of Free Fatty Acids during Fasting. GEORGE A. BRAY, Boston, Mass. (introduced by E. B. Astwood).

The thyroidectomized-adrenodemedullated rat is uniquely sensitive to reserpine and shows progressive hypothermia and hypometabolism leading to death. This sequence does not occur in normal or thyroidectomized rats or in animals treated with thyroid hormone or exposed to a warm environment. To elucidate the mechanism for this phenomenon the oxygen consumption of isolated tissues from animals pretreated with reserpine was measured *in vitro,* and liver glycogen, circulating FFA, and glucose were determined. The rats were housed at 30° C for 5 days and given 25 μg per 100 g body weight of reserpine on days 1 and 3 and 50 μg per 100 g on day 5 and bled from the abdominal aorta after an 18-hour fast on day 6. Pretreatment with reserpine raised the Q_{O_2} of heart slices from 244 ± 44 μg per g per hour (mean $\pm$ SE) to 435 ± 75 and of liver slices from 481 ± 48 to 651 ± 42, showing that reserpine does not directly depress tissue metabolism. The increased liver glycogen after reserpine was abolished by adrenalectomy or hypophysectomy, indicating that this effect depends on stimulation of the pituitary-adrenal axis. The rise in circulating FFA in response to fasting was greatly reduced in adrenodemedullated rats. After reserpine the FFA of adrenodemedullated rats was 385 ± 32 μEq per L and of thyroidectomized-adrenodemedullated rats 438 ± 30 compared to 640 ± 54 in normal rats, 649 ± 72 in hypophysectomized rats, and 639 ± 28 in thyroidectomized rats. Blood glucose was also increased by reserpine, but this did not correlate with the effect on FFA, since in thyroidectomized rats blood glucose was 160 mg per 100 ml when there was no effect on FFA, while in thyroidectomized-adrenodemedullated rats with reduced FFA the blood glucose was 114 mg per 100 ml. These findings support the concept that catecholamines play a central role in the mobilization of FFA during fasting.

Correlation of RBC Glutathione Reductase Inhibition by the Chromate Ion and in Vivo Erythrocyte Survival in the Human and Dog. FRANKLIN G. EBAUGH, JR.,* WILLIAM N. VALENTINE,† AND O. ROSS MCINTYRE, Hanover, N. H., and Los Angeles, Calif.

Previous work found that of 17 RBC enzymes only glutathione reductase (GSSG-R) was specifically inhibited by $CrO_4^=$. Fifteen to 25 μg of $CrO_4^=$ (expressed as elemental chromium) per ml of ACD blood lowered the GSSG-R to 31 ± 8% of normal in ten human subjects (normal $= 2.134 \pm 0.660$ μmoles per minute per 10^{10} RBC). The Cr^{51} RBC survival half-time was 83% (24.5 ± 2.4 days; normal $= 29.5 \pm 3$ days), and 20% Cr^{51} RBC survival time was 71% of normal (48 ± 7.7 days; normal $= 67 \pm 8$ days) in the same ten subjects. Sixty to 80 μg of $CrO_4^=$ per ml lowered the GSSG-R to 12% and the Cr^{51} half-time to 66% of normal, and 100 to 220 μg of $CrO_4^=$ per ml reduced RBC GSSG-R to 6.5% and Cr^{51} RBC survival half-time to 54% of normal. Thirty mg of oral primaquine for 14 days did not further shorten *in vivo* Cr^{51} RBC survival in eight subjects, nor were Heinz bodies formed *in vitro* on exposure to 614 mg per 100 ml of primaquine diphosphate. In contrast, increased Heinz body formation is caused by *in vitro* exposure of $CrO_4^=$ RBC to 100 mg per 100 ml acetylphenylhydrazine. The inhibition of GSSG-R by $CrO_4^=$ was not reversed in erythrocytes stored for 22 days in ACD at 5° C. Dog erythrocytes whose normal GSSG-R content is one-third that of humans are more sensitive to the effects of $CrO_4^=$ since 10-minute *in vivo* survivals of 33% and 50% for red cells labeled with 70 and 50 μg of chromate per ml, respectively, were observed. The decreased Cr^{51} RBC survival described above for the human and dog would not appear to be due to increased Cr^{51} elution, since red cell survival curves determined simultaneously by Fe^{59} and Cr^{51} of the same population of cells in the dog showed good agreement after correction for reutilization of Fe^{59}.

Contribution of Glucose, Palmitate, and Amino Acids to Respiration in Heart Muscle: Importance of Endogenous Lipids. J. C. SHIPP, O. E. MATOS, J. M. THOMAS, AND L. E. CREVASSE, Gainesville, Fla. (introduced by G. T. Harrell).

Previous studies in this laboratory have shown directly that endogenous heart lipids are oxidized to CO_2. The purpose of this study was to quantitate the amount of metabolic CO_2 formed from endogenous lipids in hearts perfused with bicarbonate buffer with, and without, exogenous substrates. Hearts from fed rats depleted of glycogen were perfused for 30 minutes in a closed recirculated system; total CO_2 and $C^{14}O_2$ formation were determined. Similar amounts of metabolic CO_2 were formed by hearts perfused with buffer alone, or buffer containing 0.6 mmoles palmitate-1-C^{14}; $C^{14}O_2$ accounted for 40% of metabolic CO_2. With 5 and 10 mmoles glucose-U-C^{14}, metabolic CO_2 was increased by 25%, of which $C^{14}O_2$ accounted for half; this increase in CO_2 was explained by the higher R.Q. for glucose. When 0.6 mmoles palmitate was present with 10 mmoles glucose-U-C^{14}, total CO_2 was identical to that observed with hearts perfused with buffer alone, and with buffer containing palmitate; $C^{14}O_2$ formation was completely suppressed. With 0.25 to 5 mmoles C^{14}-labeled glutamic acid and leucine metabolic CO_2 formation was similar to that with palmitate; $C^{14}O_2$ accounted for less than 10% of total CO_2. C^{14} alanine in similar concentrations was oxidized to a less extent than glutamic acid and consistently suppressed metabolic CO_2 formation; the mechanism of the latter effect was not explained. Thus, exogenous glucose and palmitate, but not amino acids, contributed significantly to total respiration of heart muscle. The results emphasize the important contribution of endogenous lipids, primarily phospholipids and triglycerides, to respiration; this was true even with exogenous substrates that were optimally oxidized.

Failure to Escape: A Mechanism in Idiopathic Edema. NORMAN J. MARIEB AND PATRICK J. MULROW,* New Haven, Conn.

Previous reports have pointed to an increased aldosterone secretion as a cause of fluid retention in patients with idiopathic edema. However, when normal subjects receive large doses of aldosterone, they gain only a few pounds before escaping from the sodium-retaining effects. In the present study four subjects with a history of cyclical weight gain and edema had normal aldosterone excretion rates. One of these patients, an 18-year-old girl with marked edema, was studied extensively. There was no evidence of cardiac, renal, or liver disease, orthostatic hypotension, or hypoalbuminemia. Her edema often fluctuated spontaneously but was improved by salt restriction and worsened by high salt intake. During periods of spontaneous, marked, edema formation, aldosterone excretion and secretion rates were in the low normal range. Plasma renin and angiotensin levels were normal. During two separate periods of normal hospital activity, administration of desoxycorticosterone acetate (DOCA) for 7 days led to progressive, generalized edema, with a weight gain up to 17 lbs. Plasma volume increased, hematocrit decreased, and inulin clearance remained within the normal range. The normal escape from the sodium-retaining effect of DOCA failed to occur. Administration of DOCA while the patient was at bed-rest did not stimulate fluid retention. It is concluded that the mechanism of edema formation in this patient is not increased aldosterone secretion but a failure of the physiological escape mechanism to limit the expansion of extracellular fluid volume. This failure to escape is related to the upright position. Although the presence of aldosterone may be necessary for fluid retention, it is postulated that in many patients with idiopathic edema a major factor is the failure of the escape mechanism.

Oxygen Equilibrium of the Subunits of Hemoglobin. ROBIN W. BRIEHL AND HELEN M. RANNEY,* Bronx, N. Y.

The structural basis of the physiologically advantageous functional properties of human hemoglobin A ($\alpha_2^A \beta_2^A$), i.e., oxygen affinity, heme-heme interaction, and Bohr effect, is poorly understood. Hemoglobin H (β_4^A), composed solely of β polypeptide chains, has a high oxygen affinity and lacks heme-heme interaction and a Bohr effect (Benesch and co-workers). We have recently isolated from human hemoglobin exposed to experimental conditions of hybridization a basic component which resembles the α^A polypeptide chains described by Huehns and associates. This component designated α^A is largely monomeric (mol wt = 22,000) and recombines with hemoglobin H to yield a tetramer, "reconstituted A." The following measurements were made on hemoglobin α^A, H, and reconstituted A: P $\frac{1}{2}$ (oxygen tension required for half saturation) for α^A, 0.09 mm Hg; for hemoglobin H, 0.05; for reconstituted A, 4. Like hemoglobin H, the α^A component lacks heme-heme interaction and Bohr effect. Therefore, the decreased oxygen affinity of hemoglobin A as well as heme-heme interaction and Bohr effect appears to depend upon linkages between α and β chains and tetramerization. Relationships between oxygenation and aggregation of hemoglobin have been demonstrated in lamprey and in human hemoglobin. Oxygenated hemoglobin deaggregates more readily in salt (Benesch and co-workers) and hybridizes more readily in acid (Ranney and co-workers) than does the deoxygenated pigment. Current data obtained by sedimentation equilibria confirm these findings and provide evidence for the existence of monomers in salt and in acid. In lamprey hemoglobin (Briehl), only deoxyhemoglobin aggregates, and this aggregation appears to be the basis of low oxygen affinity. Therefore, in human hemoglobin the low oxygen affinity of hemoglobin A as compared to its subunits may be based on a tendency of the aggregated deoxygenated hemoglobin to deaggregate with oxygenation.

Absorption and Plasma Concentrations of Octanoic Acid in Cirrhosis. WILLEM G. LINSCHEER, WILLIAM P. CASTELLI, EDWARD W. MOORE, JAMES F. PATTERSON, AND THOMAS C. CHALMERS,† Boston, Mass.

Middle-chain fatty acids (MCFA) have been shown to be transported via portal vein rather than lymphatics and cleared by the liver. Parenchymal liver disease or portal systemic shunts, or both, might thus result in elevated plasma MCFA levels. Absorption rates and plasma levels of octanoic acid have, therefore, been studied in three groups of patients: Group A, six noncirrhotics; group B, six cirrhotics; and group C, nine cirrhotics with portacaval shunts. A micellar solution of octanoic acid (1%) and 3% Na taurocholate in 0.85% saline was infused at a constant rate for 60 minutes through a proximal opening of a 4-lumen, 2-balloon tube in an isolated segment of upper small bowel, with continuous distal aspiration. Polyethylene glycol served as nonabsorbable marker. Mean absorption rates were similar in the three groups: 30.9 ± 4.2 (1 SE) mg per minute (group A), 24.5 ± 3.1 mg per minute (group B), and 25.3 ± 2.9 mg per minute (group C). In the same groups, 24-hour fecal fat excretions (100 g normal fat intake) were 3.1 ± 0.5 (1 SE), 5.4 ± 1.6, and 6.4 ± 1.8 g, respectively. Plasma unesterified, octanoic acid levels, determined by gas chromatography as peak heights in millimeters, rose from negligible fasting levels to 12.3 ± 2.4 (1 SE), 28.7 ± 2.7, and 89.2 ± 19.6 mm at 60 minutes in groups A, B, and C, respectively. A return to fasting levels was observed in groups A and B by 120 minutes, while in the shunted patients, the mean level dropped to 2.6 ± 0.9. In one patient (group C) with a colon bypass, colonic absorption rate of octanoic acid was 9.6 mg per minute as compared with 34.8 mg per minute in the upper small bowel. Corresponding peak plasma levels were 68 and 155 mm, respectively. MCFA absorption rates were normal in cirrhosis. Plasma MCFA levels, however, were significantly higher in cirrhotics than in normals, with a further significant increase in patients with surgical shunts. The method may thus provide a diagnostic tool for demonstration of intra- and extrahepatic shunts. Absorption of octanoic acid from the colon has been demonstrated in man.

Studies on the Pathogenesis of Thrombosis: An Experimental Hypercoagulable State Induced by the Intravenous Injection of Ellagic Acid. ROBERT E. BOTTI AND OSCAR D. RATNOFF,† Cleveland, Ohio.

One factor long believed to be important in the pathogenesis of thrombosis is an increase in the inherent propensity of blood to clot, the so-called hypercoagulable state. This report describes a technique to induce a protracted hypercoagulable state in dogs by the iv injection of ellagic acid (4,4′,5,5′,6,6′-hexahydroxy-diphenic 2,6,2′,6′-dilactone). Only when the hypercoagulable state was combined with vascular stasis did thrombosis occur. The iv injection of 30 to 200 ml of 2×10^{-4} M ellagic acid was followed immediately by a striking decrease in the clotting time of whole blood in glass and silicone-coated tubes. For example, the clotting time was shortened precipitously from 18 minutes in glass and 96 minutes in silicone-coated tubes to 8 minutes in both types of tube. Abolition of the difference in clotting time between that measured in glass and in silicone-coated tubes was consistent with the known effect of ellagic acid, the activation of Hageman factor. In agreement with this observation, prothrombin consumption and thromboplastin generation were increased in blood drawn after the injection of ellagic acid. The effect on the clotting time could be maintained for at least 3 hours by repeated infusions of ellagic acid. Despite acceleration of clotting as measured *in vitro,* neither gross nor histologic evidence of intravascular thrombosis could be found, and the concentration of fibrinogen and other clotting factors was maintained. Intravascular clotting occurred only when, in addition, stasis was deliberately induced by isolation of a venous segment with Wessler's technique. The experiments described provide the opportunity to study the effects of protracted changes in the coagulability of the blood on the development of intravascular clots. Whether these observations are applicable to clinical states associated with intravascular thrombosis is yet to be determined.

Renal Ammonia Metabolism in Potassium-depleted Dogs. PHILIP W. HALL III, ROBERT BARENBERG, ROBERT HOPKINS, AND GEORGE J. GABUZDA,* Cleveland, Ohio.

The effects of potassium deficiency on renal ammonia metabolism were studied in six dogs depleted of this cation by hemodialysis. On day 4 after dialysis, serial measurements of arterial, renal venous, and urine ammonia concentrations, urine flow, renal blood flow (electromagnetic flow meter), and blood and urine electrolytes were made before, during, and after giving potassium chloride intravenously (50 mEq in 1 hour). Venous output accounted for 65% of the total ammonia output by the normal and potassium-deficient kidney. Output of ammonia via renal vein and urine in potassium-depleted dogs was increased 3 times above the levels recorded for six normal dogs. This was not accompanied by significant alterations in blood pH or bicarbonate level, urine pH, urine flow, or renal blood flow. The administration of potassium to the depleted animals caused decreases in total renal output of ammonia and increases in urine flow without significant changes in renal blood flow, blood pH, or bicarbonate concentration, or in urine pH. The decrease in total ammonia output was accounted for entirely by decrease in renal venous output. Potassium administration was without effect on renal ammonia output in normal dogs or the increased output that occurred in animals made acidotic with ammonium chloride. We conclude that potassium depletion causes increases in total renal output of ammonia without altering extracellular bicarbonate concentration or pH, or urine pH. This effect is reversed by iv potassium chloride. In potassium-deficient dogs production of ammonia or its availability, or both, to renal venous outflow and urinary excretion are increased.

The Role of Immune Mechanisms in Mediating the Lethal Action of Bacterial Endotoxin. P. J. PORTER AND A. R. SPIEVACK, Boston, Mass. (introduced by E. H. Kass).

The mechanism of action of bacterial endotoxin has been debated. There is evidence to suggest that immune mechanisms may play a mediating role in the pathogenesis of the nonspecific effects of endotoxin. The following series of experiments was designed to test this hypothesis. Thymectomy in the newborn rat did not alter the sensitivity of rats to endotoxic lethality or other nonspecific effects, although the thymectomized animals were immunologically incompetent when tested with respect to antibody response and acceptance of a skin homograft. However, the titers of bactericidal antibody to *E. coli* 0127 were similar in thymectomized and in controls sham-operated on. It therefore remained to be seen whether small amounts of antibody were necessary for the lethal action of endotoxin. Newborn rats were found to be deficient in antibody. Antibody levels increased with age until adult levels were reached at 4 to 6 weeks of age. At the same time the newborn rats exhibited a marked sensitivity to the lethal action of endotoxin. Sensitivity to endotoxin declined so that adult sensitivity was reached at about 14 days of age. These studies indicate that bactericidal antibody is not necessary for the lethal action of homologous endotoxin in the rat. The possibility of some protective effect of bactericidal antibody arose. Studies were undertaken to compare litter mates who had been given hyperimmune *E. coli* 0127 serum and a saline control. Lethality to homologous endotoxin was similar at 7 days of age when bactericidal antibody levels were elevated in the immunized group. This suggests that maturational factors are important in this decline of sensitivity to endotoxin and that specific bactericidal antibody has little effect in the rat.

Inhibition of Protein Synthesis: A Mechanism for the Production of Impaired Fat Absorption. SEYMOUR M. SABESIN, GLADYS D. DRUMMEY, DOROTHY M. BUDZ, AND KURT J. ISSELBACHER,* Boston, Mass.

During intestinal fat absorption, triglycerides are formed in the mucosa and converted to chylomicrons by interacting with lipoproteins. Although lipoproteins are synthesized in the mucosa, their function in the control of fat absorption remains unclear. Their importance in lipid transport, however, is suggested by the occurrence of steatorrhea, mucosal fat accumulation, and low serum lipids in congenital beta-lipoprotein deficiency (acanthocytosis). In the present investigation protein and lipoprotein synthesis was inhibited in the rat with puromycin, and the effects on intestinal lipid transport were studied biochemically and with the electron microscope. After the oral administration of corn oil, animals treated with puromycin (15 mg) showed a striking accumulation of fat in jejunal epithelial cells with relatively little fat in lymphatics. In contrast, in the untreated animal, fat was readily absorbed into the lymphatics, and 6 hours after corn oil serum triglyceride levels were 200 to 530 mg per 100 ml but only 25 to 60 mg per 100 ml in the puromycin group. The inhibition of lipid transport by puromycin did not appear to be secondary to impaired triglyceride synthesis, since *in vivo* and *in vitro* studies with C^{14}-palmitate showed only minimal depression of fatty acid esterification. Puromycin did not interfere with glucose absorption, indicating that under the experimental conditions employed there was no generalized depression of intestinal transport. Furthermore, the defect in mucosal lipid transport was not associated with any obvious derangement in the ultrastructure of the epithelial cells. Similar results have been obtained with acetoxycyclohexamide, another inhibitor of protein synthesis. These studies indicate that by the experimental inhibition of protein synthesis a morphological and biochemical picture may be produced in the intestine which is strikingly similar to that observed in human beta-lipoprotein deficiency and supports the concept that intestinal lipid transport is intimately related to lipoprotein synthesis.

Localization and Pyrazinamide Inhibition of Tubular Secretion of Uric Acid-C^{14} with a Modified Stop-Flow Technique. BERNARD B. DAVIS, JAMES B. FIELD,* GERALD P. RODNAN, AND LAURENCE H. KEDES, Pittsburgh, Pa.

The exact site of renal secretion of urate in the mammalian kidney has not been localized. This study, utilizing stop-flow analysis and injection of uric acid-C^{14}, was undertaken to define this site and the mechanism of drug-induced hyperuricemia. Mongrel dogs were prepared in the usual manner for stop-flow analysis, with additional exposure of the left renal artery. Diuresis was induced with mannitol, and after unilateral urine flow was stabilized at a minimum of 7 ml per minute, the flow was stopped. Three minutes later, uric acid-C^{14} (6 dogs) or inulin-C^{14} (2 dogs) was injected directly into the renal artery. Ten to fifteen seconds after injection, free flow was restored, and small serial samples were collected for 3 minutes. 0.1-ml samples of urine were counted for radioactivity. Three dogs were given pyrazinamide, 0.5 g orally, twice a day, for 3 days before study. They were also given an iv priming dose of 1 g and an infusion of 10 mg per minute for 2 hours before uric acid-C^{14} injection. In six dogs a peak of radioactivity was observed in samples representing a distal portion of the nephron as indicated by maximal creatinine concentration. After the peak, the counts returned to background and then in later samples there was another peak. Only this second peak was present in both dogs given inulin-C^{14}, indicating that it represents new glomerular filtrate and that the first peak is indicative of tubular secretion. The radioactive peak indicative of distal nephron secretion was abolished in all three dogs treated with pyrazinamide. These studies demonstrate that uric acid is secreted by a distal portion of the nephron and that hyperuricemia induced by pyrazinamide is mediated at least in part by inhibition of the secretory process.

Blood Cholesterol and Steroid Hormone Production. JAMES C. MELBY,* THOMAS E. WILSON, AND SIDNEY L. DALE, Boston, Mass.

Experimental evidence suggests that plasma cholesterol is the principal precursor of corticosteroid hormones. Administration of an inhibitor of cholesterol synthesis, the dimethylaminomethoxy derivative of dehydroepiandrosterone, to two patients with Cushing's syndrome due to adrenal neoplasm for 6 days resulted in a mean reduction of 50% of plasma cholesterol, 75% of plasma cortisol, and 64% in cortisol secretory rate determined by the tracer method. Experiments were performed to explore this phenomenon. Ten adult mongrel dogs were fed and received injections of H^3-cholesterol for a time sufficient to achieve uniform labeling of the cholesterol pool. Half received the inhibitor for 1 week. Permanent cannulas were placed in lumboadrenal veins. Comparison of specific activities of plasma cholesterol and adrenal venous cortisol was made between control and test animals before and after ACTH. The specific activity of adrenal venous cortisol in both control and test animals was 80% of the specific activity of circulating cholesterol and 95% after injection of ACTH. Most adrenal venous cortisol was derived from plasma cholesterol. A group of experiments was undertaken in which rats were fed inhibitor for 21 days. Another group was given inhibitor plus 1% cholesterol by weight in the diet. In rats receiving inhibitor alone, there was a sharp reduction in plasma cholesterol and corticosterone. Incubation of sectioned adrenals from these rats demonstrated marked inability to synthesize steroids *in vitro*. Rats receiving both inhibitor and cholesterol in their diet demonstrated no reduction in plasma corticosterone nor in steroidogenesis *in vitro*. In contrast to the effect of the inhibitor on adrenocortical steroid production, experiments were performed in which no alteration in testicular testosterone production could be demonstrated. Preliminary studies suggest that testicular testosterone may be derived from another cholesterol pool.

Iron Absorption Kinetic Studies in the Normal Dog. EDWARD W. MOORE, WILLEM G. LINSCHEER, AND WILLIS R. KEENE, Boston, Mass. (introduced by Joseph M. Hayman, Jr.).

Determination of iron absorption kinetics in man or intact animals is difficult because *1*) intraluminal factors may alter absorbability of available iron and *2*) absorption is necessarily expressed in percentage, rather than more meaningful rate terminology. A decrease in percentage of absorption may actually reflect increased absorption rate. Studies were therefore performed in dogs with isolated duodenojejunal loops in which luminal iron concentration was varied systematically, with direct determination of absorption rates and kinetic equations. Absorption of $Fe^{59}Cl_3 - FeCl_3$, Fe^{59} citrate $- FeSO_4$, or $Fe^{59}SO_4 - FeSO_4$ was studied in nine dogs given 270 infusions. Infusate iron concentration ranged from 0.125 μg per ml to 100 μg per ml, with duplicate 50-ml infusions administered at 2.5 ml per minute and at pH 3.0. On a given day, each animal received 10 to 16 such infusions with progressively increasing iron concentrations; duplicate infusions were given in reverse order. Both ferric and ferrous absorption rates increased curvilinearly with increasing infusate concentrations up to about 25 μg per ml, with linear increase at higher concentrations. On a log-log plot, absorption rate and infusate concentration were linearly related in all studies. Both total absorption (A) and the linear (diffusion) component (B) were greater for ferric than for ferrous iron, but the differences were significant only at concentrations below 25 μg per ml. No significant differences between Fe^{59} citrate and $Fe^{59}SO_4$ were noted. In each study, subtraction of (B) from (A) yielded another curve (C) compatible with an enzymatic or carrier process, in accordance with Michaelis-Menton kinetics. Thus, both ferric and ferrous absorption appeared to be mediated by two processes operating simultaneously: *1*) a first-order process in which absorption rate was linearly related to luminal iron concentration and *2*) a process that appeared to fit the kinetics of an enzymatic or carrier mechanism. At high luminal iron concentrations, diffusion would constitute the major mechanism of absorption, whereas the active or carrier process would primarily contribute to absorption at low concentrations.

What Happens to Patients with Sarcoidosis? SAMUEL F. BOUSHY, BENJAMIN M. LEWIS,* AND RAYMOND J. KURTZMAN, Detroit, Mich.

Of 31 patients with sarcoidosis, 5 died, 4 moved from the city, and 5 were lost to follow-up. The other 17 were re-evaluated clinically, by X ray and by pulmonary function study after a period which averaged 3 years. Combined clinical, radiologic, and function improvement was unusual, occurring in only two patients. Changes in the X ray could not be correlated with pulmonary function tests. Specifically, diffusing capacity decreased in two of four cases with unchanged hilar adenopathy and two of three cases in which diffuse nodular densities resolved, but it improved in one of two in which linear strands (fibrosis) replaced linear densities and one of three with unchanged nodular densities. Nor did all pulmonary function tests change in the same direction. The structural changes that govern lung compliance in this disease are usually separable from those that govern gas transfer (diffusing capacity). Compliance changed in eight, but diffusing capacity changed in parallel fashion in three and in the opposite direction in three. Compliance and lung volume changes, however, were well correlated, but diffusing capacity and lung volume changes were not.

Cation and H_2O Translocation in Mitochondria and in Model Systems. WILLIAM S. LYNN* AND ROSE BROWN, Durham, N. C.

Dehydration of mitochondria requires *1*) energy (ATP or electron-transport), *2*) Mg^{++}, *3*) acidic phospholipids (APL—these act competitively with fatty acids on dehy-

dration), and *4*) maintenance of semipermeable properties of mitochondrial membranes (Ca^{++} and fatty acids destroy this osmotic property). It is also known *1*) that mitochondrial membranes are made of interwoven networks of basic protein and phospholipids bound together by charge and hydrophobic bonds, *2*) that divalent cations cause dehydration of APL micelles by displacement of H^+, and *3*) that energy is required for translocation of cations into mitochondria. Using data obtained from a study of binding constants, solubility, and dehydration of model mitochondrial systems (composed of cytochrome C, APL, divalent cations, fatty acids, ATP, and mitochondrial ATPase) and from mitochondria, we have proposed the following scheme for mitochondrial H_2O and cation translocation: *1*) Hydrated Mg^{++} + intramitochondrial ATP $\overset{\text{Translocase}}{\rightleftarrows}$ intramitochondrial anhydrous Mg-ATP + H_2O; *2*) a. Anhydrous Mg-ATP $\overset{\text{ATP'ase}}{\longrightarrow}$ $Mg_3(PO_4)_2$ + ADP; b. Anhydrous Mg-ATP + Pi $\rightleftarrows$ $Mg_2(PO_4)_2$ + ATP; c. Anhydrous Mg-ATP + hydrated H^+-APL $\rightleftarrows$ insoluble Mg-APL + H_2O + H^+ + ATP; d. Anhydrous Mg-ATP + fatty acids $\rightleftarrows$ insoluble Mg-FA + ATP. The only energetic requirement for this scheme is the generation of intramitochondrial ATP. Contraction should be competitive with Pi concentration. Data on measurement of intramitochondrial Mg-ATP, Mg-APL (as well as state of hydration of mitochondria and model micellar suspension), on Mg-APL-cytochrome C complex formation, and on the competitive inhibition of Pi on water movement in mitochondria validates the above hypothesis and will be presented. The scheme also accounts for the fact that dehydration of mitochondria requires no hydrolysis of high energy phosphate bonds. Thus, intramitochondrial ATP chelates Mg^{++} within the anhydrous micellar portion of mitochondria and facilitates transfer of Mg^{++} to APL, or Pi, or fatty acids. In the model system, Mg^{++} acts as a bridge between cytochrome C and APL to form an insoluble ternary complex. This is presumed to be the function of Mg^{++} in mitochondria also. The role of ATPase in this scheme is to drive all the transfer reactions to the right.

The Response of the Retinal Circulation to Hyperbaric Oxygenation. H. A. SALTZMAN, LEONARD HART, B. ANDERSON, JR., EDWARD DUFFY, AND H. O. SIEKER,* Durham, N. C.

Hyperbaric oxygenation affects central nervous system function and the cerebral circulation. The optic fundus permits direct visualization of the retinal fraction of the cerebral circulation. For this reason, still and motion picture photography was performed in five normal volunteers breathing air or oxygen at 1 and 3 atmospheres of absolute pressure, during quiet respiration and hyperventilation. Hyperbaric oxygenation resulted in marked attenuation of both arterioles and venules, and the smaller retinal vessels disappeared completely. In addition the normal color difference between veins and arteries was markedly reduced. These changes were apparent during the first minute of oxygen respiration and reached a maximal 46% decrease in size within 5 minutes. The changes produced by hyperbaric oxygenation were largely reversed by 1 minute of air breathing. Hyperventilation, with an associated marked decrease in the arterial P_{CO_2}, was not associated with measurable decreases in vessel caliber. These studies indicate a major vasoconstrictor action of oxygen upon the retinal circulation and are consistent with both a decrease of cerebral blood flow and with ophthalmologic pathology previously noted with hyperoxia. The arterial color of the retinal veins during hyperbaric oxygenation suggests that there is a significant increase in retinal oxygen content despite the associated reduction of cerebral blood flow.

Lipid Mobilization in Lean and Obese Individuals. ROBERT F. KLEIN, WILLIAM G. TROYER, JR., THOMAS H. HOOD, KURT W. BACK, AND MORTON D. BOGDONOFF,* Durham, N. C.

The effect of body size upon the process of lipid mobilization has not been well delineated. The present report concerns the patterns of lipid mobilization observed in small group psychophysiological experiments as they relate to body size. One hundred and sixty-seven volunteer male university students were studied in groups of four subjects at a time. The duration of fasting ranged from 16 to 21 hours with a mean of 17 hours. Forearm venous blood was sampled from indwelling needles and analyzed for FFA level. Samples were taken immediately after insertion of the needle and at intervals throughout a 2-hour period. Subjects rested and completed questionnaires and then performed perceptual judgment tasks under experimentally manipulated social conditions. Height: weight ratios (inch: lb.) of the subjects ranged from 0.320 (obese—72 inches: 220 lbs.) to 0.536 (lean—68 inches: 126 lbs.) and followed a normal distribution curve. There was a correlation coefficient of 0.23 between resting FFA and height: weight ratio ($p = 0.01$). A comparison of the 26 most lean subjects (15% of normal) with the 27 most obese subjects showed significantly ($p = 0.05$) higher FFA levels in the lean group throughout the experiments and a larger ($p = 0.05$) rise in FFA in response to the experimental tasks. These findings indicate that in a healthy young adult male population with a normal distribution of weight, fasting (17-hour) plasma concentrations of FFA bear an inverse relationship to weight, and, furthermore, the results also suggest that less lipid mobilization occurs in obese subjects in response to judgmental tasks.

The Impairment of Carbohydrate Tolerance by Elevated Plasma Free Fatty Acids. DON S. SCHALCH AND DAVID M. KIPNIS,* St. Louis, Mo.

Recent *in vitro* studies indicate that many metabolic abnormalities associated with the diabetic state can be produced by elevated nonesterified fatty acid (NEFA) levels per se. The purpose of this study was to examine the effect of elevated plasma NEFA on carbohydrate tolerance in man. To accomplish this, it was necessary

to devise a technique for elevating plasma NEFA without relying upon the administration of hormones that impair carbohydrate tolerance (e.g., epinephrine, growth hormone). After a 60-g fat meal, plasma NEFA response is variable, in some cases increasing from fasting levels of 400 to 600 μEq per L to 600 to 800 μEq per L over 3 to 4 hours. Throughout this period, plasma glucose and immunoassayable insulin remain constant. Heparin (50 mg, iv), given 3 hours after a 60-g fat meal, produces a 2- to 3-fold rise in plasma NEFA to levels of 1,500 to 2,000 μEq per L without a change in either plasma glucose or insulin. Carbohydrate tolerance was measured by the disappearance rate (K) of 25 g of intravenously administered glucose in eight normal subjects and three mild diabetics under fasting conditions with and without a fatty meal and heparin. K values for eight normal subjects decreased 47% after an induced rise in plasma NEFA, falling from a control value of 3.49 ± 0.60 to 1.84 ± 0.33 under experimental conditions. Control K values for three diabetic subjects (1.24 ± 0.24) were significantly lower than experimental values observed in normal subjects and were only minimally depressed (1.07 ± 0.10) by further elevation of plasma NEFA. The impaired carbohydrate tolerance observed in normal subjects appears to be associated with an increased secretion of insulin. The results of this study suggest that the impaired carbohydrate tolerance associated with growth hormone or adrenoglucocorticoid administration, starvation, obesity, and maturity-onset diabetes may be a consequence, in part, of the elevated NEFA levels characteristic of these conditions.

Colonic Absorption and Urinary Excretion of Tryptophan Metabolites in Man. W. M. SCROGGIE, D. E. POLTER, AND J. S. FORDTRAN, Dallas, Texas (introduced by Elias Strauss).

Patients with malabsorption excrete increased quantities of tryptophan metabolites in the urine. This is usually attributed to altered bacterial activity in the gut, and Horning and Dalgleish have stated that urinary indoles must arise from bacterial action on tryptophan in the small intestine which, unlike the large intestine, absorbs lipid-soluble substances. Accordingly, urinary indole excretion would directly reflect bacterial population in the small bowel. Three studies were done to determine the role of the colon and its normal bacteria in urinary indole excretion. *1*) Absorption of indole and skatole was studied in jejunum, ileum, and colon of six normal subjects by a perfusion-marker technique. Absorption of both was extremely rapid in all three areas. *2*) Urinary indole excretion was studied after perfusion of the ascending colon (through an orally introduced tube) with tryptophan or casein in six subjects. A marked increase in urinary indican and indole-3-acetic acid (IAA) occurred in each subject, but 5-hydroxyindole-acetic acid (5HIAA) did not increase. Glucose in the test solution delayed but did not decrease urinary excretion of indican and IAA. *3*) Serotonin was infused into the colon of four subjects. Urinary 5HIAA did not rise. Therefore, colonic bacterial metabolism and colonic absorption can result in elevated indole excretion if amino acids or proteins reach the colon, as in malabsorption, and urinary indoles are not necessarily of small bowel origin. Elevated urinary 5HIAA in celiac patients cannot be related to colonic bacterial action on unabsorbed tryptophan; even if serotonin were formed in the colon by bacterial action on tryptophan, urinary 5HIAA would not rise.

Homologous Disease, a Model for Autoimmune Disease. P. STASTNY, V. A. STEMBRIDGE, T. L. VISCHER, AND M. ZIFF,† Dallas, Texas.

Disease in man, if due to an autoimmune reaction, may result from abnormalities associated either with the antigens involved or with the response of the host. The animal with homologous disease, since it contains in its reticuloendothlial tissues large numbers of immunologically competent homologous cells, offers an experimental model for investigation of the effect of an abnormal lymphoid cell population on normal host tissues. Inbred Fischer rats were rendered tolerant to an inbred strain of Lewis rats by neonatal injection of lymphoid cells. At maturity, the tolerant recipients were given three injections of 200 to 800 million lymphoid cells from the donor strain. In addition to the pancytopenia and hemolytic anemia usually observed in homologous disease, the following striking changes occurred. Over 50% of recipients developed a migratory polyarthritis associated with a mononuclear type of synovitis. Cultures of 25 affected joints for pleuropneumonia-like organisms were negative in all cases. Almost all animals demonstrated inflammatory lesions in the heart, both valvular and myocardial. Vascular lesions with fibrinoid necrosis were, on occasion, present. A variety of skin lesions was also observed. These are believed to result from an immunologic reaction directed against the host for the following reasons: *1*) Although donor strain homografts were accepted, autografts simultaneously applied were uniformly rejected; *2*) the histologic appearance of the spontaneous lesions closely resembled that of the autografts undergoing rejection; *3*) skin lesions contiguous to donor homografts were limited to host skin, sparing the donor skin. Circulating antibody against autologous lymphoid cells was also demonstrated with an immunofluorescent technique. The multisystem abnormalities described, which resemble those seen in human connective tissue disease, and which are a consequence of the presence of foreign lymphoid cells, provide evidence that alteration of the immunologic responsiveness of lymphoid cells may be responsible for the changes observed in human connective tissue disease.

Persistence of Immunological Competence against Bacterial Antigen in Thymectomized Rats. JACK A. BARNETT, LONA L. AKINS, AND JAY P. SANFORD,* Dallas, Texas.

While neonatal thymectomy results in subsequent quantitative immunological incompetence upon challenge with

various antigens, the effect of varying antigen characteristics on quality and quantity of antibody has not been defined. This experiment was designed to characterize qualitatively and quantitatively the antibody response in doubly immunized Holtzman rats thymectomized within 12 hours after birth. These and controls received 5 mg bovine serum albumin (BSA) in incomplete adjuvant at 5 and 6 weeks, then one iv injection of 0.5-ml heat-killed 18-hour culture of *E. coli* 0-111 at 7 weeks of age. Sera for antibody determination were obtained weekly for 3 weeks following immunization. Antibody against *E. coli* was quantitated by bacterial agglutination in serially diluted sera; anti-BSA was quantitated by the ammonium sulfate precipitation technique of Farr. Sera were fractionated by column chromatography and sucrose density gradient ultracentrifugation and the fractions assessed for antibody against each antigen. Sixteen of 49 thymectomized survivors made no detectable anti-BSA. Thirty-three of the 49 produced modest amounts with mean antigen-binding capacity of 1.6 compared with control of 6.4 ($p < 0.01$). Agglutinins against *E. coli* were equal in thymectomized and control animals. Studies on fractionated sera showed anti-*E. coli* to be distributed principally in the rapidly sedimenting fractions in early response and in the slowly sedimenting fractions in late response. Anti-BSA was found principally in the slowly sedimenting fractions in all bleedings. These data demonstrate that neonatal thymectomy of rats did not alter quantitatively or qualitatively the immune response to *E. coli* while, as reported by Jankovic and others, the response to BSA is markedly reduced. Although thymectomy has been considered to produce broad immunologic incompetence, these data demonstrate that interpretation of thymic influence must be based upon assessment of specific immune responses.

The Renal Excretion of Tritiated Digoxin. E. J. Towbin, J. E. Doherty, and C. B. Ferrell, Little Rock, Ark. (introduced by Richard V. Ebert).

Stop-flow and clearance techniques were used to study renal excretion of tritiated digoxin in anesthetized dogs after the iv injection of 0.5 mg of pharmacologically active glycoside having a specific activity of 61 μc per mg. Urine radioactivity and cold digoxin migrate identically when chromatographed. This observation permits the use of urine and plasma radioactivity as a measure of digoxin content. A brisk solute diuresis was induced by infusion of urea and glucose in Ringer's solution. In four experiments ureteral stasis was applied for 5 minutes before tritiated digoxin and a marker of filtration, $Na_4Fe(CN)_6$, were rapidly and simultaneously injected intravenously. Two minutes later the clamp was removed, and urine samples were secured. Digoxin and $Na_4Fe(CN)_6$ concentration curves were superimposable, indicating that digoxin is freely filtered. In a second experimental protocol, creatinine and tritiated digoxin were infused at a constant rate after priming doses. Subsequent to equilibration, the ureteral catheter was clamped. After 3 to 6 minutes of urinary stasis the clamp was released, and ½-ml serial urine samples were collected. Eighteen such experiments showed that filtered digoxin is reabsorbed by the proximal tubule just distal to the site for maximal glucose reabsorption. There is no evidence for tubular secretion of digoxin. Average clearance per kidney during free flow was 9.5 ml per minute for digoxin and 16 ml per minute for creatinine, clearance ratio being 0.66 ± 0.03. Control and postdigitalization electrolyte stop-flow patterns were observed in 21 experiments. Distal tubular potassium reabsorption was inhibited by digoxin in 15 experiments. The sodium pattern was not changed by digoxin.

The in Vitro Conversion of Radioactive 17α-Hydroxyprogesterone and 17α-Hydroxypregnenolone to Corticoids and Androgens by the Human Adrenal. George L. Cohn,* New Haven, Conn.

Previous studies indicate that the predominant pathway for human adrenal androgen biothesis is pregnenolone → 17α-hydroxypregnolone → dehydroepiandrosterone → androstenedione → 11β-hydroxyandrostenedione. Since 17α-hydroxypregnolone is converted also to 17α-hydroxyprogesterone for cortisol biosynthesis (bypassing progesterone), studies were designed to investigate the importance of these 17-hydroxylated intermediates in androgen and corticoid biosynthesis. Slices of an adrenal adenoma removed at operation from a patient with Cushing's syndrome were incubated with *a*) 1·2 μc of H^3-17α-hydroxypregnenolone, *b*) 0·9 μc of C^{14}-17α-hydroxyprogesterone, and *c*) equimolar amounts of the H^3 and C^{14} intermediates. Isotopic corrections for manipulative losses were determined in experiments *a*) and *b*). Cortisol, 11-deoxycortisol, androstenedione, and 11β-hydroxyandrostenedione were isolated from the incubation media and identified after the addition of carriers. The amounts of radioactivity in testosterone and 17α-hydroxyprogesterone were too small for adequate identification. The relative amounts of the two precursors incorporated into cortisol and androstenedione, expressed as the ratios of percentage of incorporation of H^3-17α-hydroxypregnenolone: C^{14}-17α-hydroxyprogesterone, were 0.41 and 3.6, respectively. The data indicate that hydroxylases and 3β-ol-dehydrogenase enzymatic activities occur more readily than side-chain cleavage in this human adrenal adenomatous tissue.

Apparent Biosynthetic Labeling of Thyrotropin with C^{14} Amino Acids. J. M. McKenzie * and S. H. Solomon, Montreal, Quebec, Canada.

The single *in vitro* rat pituitary was found to release measurable thyrotropin into the medium. C^{14}-labeled mixed amino acids (*Chlorella bacillus* hydrolysate, Volk) were added with fresh medium for five successive hourly periods, and the medium was removed hourly for filtration on Sephadex G-25. Thyrotropin and up to 4% of the C^{14} were found in the material excluded by the gel (i.e., with mol wt >5,000) even when the amino acids were allowed to remain in the medium without change for all 5 hours. When crude aqueous extract of rat hypothalamus was

added to the medium for the third hour of incubation, an increase in the release of thyrotropin was found, and when the radioactive amino acids were included, C^{14} (about 20% of total) was found with the large molecular component. In a repeat experiment with equivalent C^{14} as a single amino acid (L-threonine-U-C^{14}) thyrotropin release was increased, but the C^{14} associated with it was negligible. The G-25 excluded material containing thyrotropin and mixed C^{14} amino acids, together with carrier pituitary extract, was fractionated by trichloroacetic acid precipitation, filtration on Sephadex G-100, or Bates' percolation procedure. Thyrotropin and C^{14} were consistently found in association, although 32.5%, 73%, and 74.9% of the C^{14} were dissociated, respectively, by these procedures. Electrophoresis of the preparation on cellulose acetate at pH 8.6 gave a fraction potent in thyrotropin and containing about one-tenth the applied C^{14}. These data suggest that a preparation of thyrotropin labeled with C^{14} has been obtained. This presumably may be applied to metabolic studies with less risk of artifactual result than currently used radioiodinated preparations.

Antigenic Heterogeneity of Human Plasma High Density Lipoprotein. ROBERT I. LEVY AND DONALD S. FREDRICKSON,* Bethesda, Md.

High density lipoprotein (HDL) isolated in the ultracentrifuge between densities 1.063 and 1.21 and corresponding to α_1 lipoproteins by electrophoresis transports roughly one-third of plasma lipid. Its deficiency is associated with tissue storage of cholesterol (Tangier disease). It is generally accepted that HDL contains a single protein moiety, but conflicting evidence of heterogeneity has complicated purification of the lipoprotein and interpretation of clinical and biochemical experiments related to it. The present studies have established that HDL is present in ultracentrifugal isolates in at least two antigenic forms. These have an important relationship to current methods for isolating and quantifying HDL. Six different antibodies prepared in three animal species have consistently produced two precipitation lines with HDL, which are designated here as α and α_D. Both stain for lipid. Whole serum contains mainly, if not exclusively, α, which is present throughout the entire density range of 1.063 to 1.21. α_D is predominately found in the HDL fraction of density 1.1 to 1.21, with some also sedimenting at 1.21. α_D appears in increasing amounts with repeated freeze-thawing or ultracentrifugation, but is not produced by subjecting plasma to dialysis, high salt concentrations, or prolonged standing at room temperature. Both α and α_D antigens have identical N-terminal amino acids and amino acid compositions. These results are not inconsistent with previous studies suggesting that the biphasic distributions of HDL obtained in the preparative or analytical ultracentrifuge (the HDL_2 and HDL_3 fractions of D 1.06 to 1.1 and 1.1 to 1.2) represent monomer and dimer forms of the same protein. They indicate that only one form may be present in native plasma, the other arising mainly during isolation procedures and being readily detectable because of accompanying changes in antigenicity. The findings have bearing on a number of problems related to polymorphism in lipoproteins.

Evidence for a Mechanism of Transmission of Viral Respiratory Disease Based on Aerosol Studies with Coxsackie A-21. R. B. COUCH, T. R. CATE, W. F. FLEET, P. J. GERONE, W. R. GRIFFITH, AND V. KNIGHT,† Bethesda and Ft. Detrick, Md.

The concept that viral respiratory diseases spread via infectious aerosols has often been proposed, but without knowing the properties of such aerosols required to produce infection in man or whether an ill person is likely to produce viral aerosols that possess these properties. Assay of nasopharyngeal secretions from individuals with natural or induced infection with Coxsackie A-21 revealed 10^3 to 10^6, 50% tissue culture infectious doses ($TCID_{50}$) per ml. Since sneezes are known to contain approximately 1 million particles, over 90% of which are less than 20 μ in diameter, it is estimated that 30 to 30,000 $TCID_{50}$ of virus are expelled in particles known to remain airborne for long periods of time. To determine the infectivity of such aerosols, tissue-culture propagated virus or natural secretions containing Coxsackie A-21 were aerosolized by a Collison atomizer as 1-μ particles or by vibrating reed as 15-μ particles. Each of 71 antibody-free volunteers inhaled 10 L of the aerosolized tissue-culture propagated agent, and the 50% human infectious dose for aerosols of both particle sizes was found to be 33 $TCID_{50}$. Aerosolized natural virus showed similar infectivity. In 40 ill volunteers, the characteristic clinical response was febrile respiratory disease, ranging from the common cold syndrome to virus pneumonia (two cases). These findings suggest that persons with Coxsackie A-21 illness can readily produce small particle aerosols containing the minute doses of virus required to initiate human infection and that such aerosols may be chiefly responsible for the natural spread of this disease. This method of transmission may be equally important in the spread of other respiratory viral diseases.

Marriage to a Spouse with Asthma or Hay Fever as a Factor Predisposing to the Development of These Diseases in the Adult. J. MONTGOMERY SMITH AND LLOYD KNOWLER, Iowa City, Iowa (introduced by Willis M. Fowler).

The epidemiology of asthma and hay fever has been studied in the following populations: *1*) 611 consecutively seen patients with asthma or hay fever; *2*) 508 people with asthma or hay fever in a field interview study of 1,440 Iowa City households (every 4th household); *3*) 603 people with asthma or hay fever in a field interview study of 1,760 Iowa farm households (the entire rural population of 14 townships); *4*) 253 alumni seen with hay fever or asthma 10 to 20 years ago—their roommates and the next on the alumni list of the same age and graduation date have been followed up by mail. This makes a total of 1,975 people with asthma or hay fever in four

separate population groups. In each of these separate studies, the incidence of asthma and hay fever was three times greater in previously normal people without the disease in their family who had spouses with these conditions than in those who had normal spouses. The combined figure for the clinic and interview studies is 9.2% for those with affected spouses and 2.8 for those with normal partners. This difference is highly significant. In each of the separate studies from 20 to 30% of all adults with a negative family history who developed asthma or hay fever as adults did so after marriage to an affected partner. Other observations made in the course of these investigations made it most unlikely that exposure to common antigens could account for this phenomenon. The question is posed as to what factor or factors predisposing to the development of this kind of sensitization could be transmissible between marital partners.

The Pituitary-adrenal Relationship Is Abnormal in Cushing's Disease, Normal in Addison's Disease. MARTIN S. ROGINSKY, WILLIAM D. DRUCKER, AND NICHOLAS P. CHRISTY,* New York, N. Y.

The pituitary-adrenal relationship was studied in three patients with Cushing's syndrome associated with bilateral adrenal cortical hyperplasia who had been bilaterally, subtotally adrenalectomized, and in two patients with Addison's disease who did not require glucocorticoids for maintenance. In both groups, there were detectable quantities of plasma and urinary corticosteroids. The following measurements were made: secretory rate of cortisol by isotope dilution (cortisol-6,7-H^3); response of plasma cortisol to iv ACTH; diurnal variation of plasma cortisol levels; suppression by dexamethasone of urinary 17-ketogenic steroids, chromatographically isolated tetrahydrocortisone (THE) and tetrahydrocortisol (THF), and plasma cortisol. In the patients with partially treated Cushing's disease, secretory rate of cortisol was normal (14 to 20 mg per day); plasma cortisol levels were normal (12 to 18 μg per 100 ml) but did not rise after ACTH; there was no diurnal variation of plasma cortisol values; urinary 17-ketogenic steroids (8 to 11 mg per day) did not fall after dexamethasone, 2 mg per day for 2 days (7 to 13 mg per day); isolated urinary THE and THF were not suppressed; and plasma cortisol also was not normally diminished. In Addison's disease, cortisol secretory rate was low, and low normal values of plasma cortisol did not rise after ACTH, but there was normal diurnal variation of plasma cortisol, and urinary 17-ketogenic steroids (6 to 11 mg per day) were normally reduced (2 to 4 mg per day) after dexamethasone, as were urinary THE and THF and plasma corticosteroid values. As previously observed (Williams and associates, J. clin. Endocr. 1961, 21, 427), plasma ACTH levels are higher in patients adrenalectomized for Cushing's disease than in primary adrenal cortical insufficiency. The present data further support the concept of abnormal pituitary regulation of ACTH release in Cushing's disease. Even with long-standing absence of adequate adrenal cortical secretion, the pituitary responds normally in Addison's disease. In contrast, the abnormal pituitary-adrenal regulation persists even after subtotal adrenalectomy in Cushing's syndrome.

A New Inherited Metabolic Abnormality of Human Erythrocytes Characterized by Elevated Levels of Adenosine Triphosphate (ATP). GEORGE J. BREWER, Ann Arbor, Mich. (introduced by Fred M. Davenport).

During a study of ATP in erythrocytes of Negroes a male glucose-6-phosphate dehydrogenase (G6PD)-deficient individual was observed who exhibited twice the normal level of ATP in his erythrocytes. His values were 7 SD above the normal mean. Inheritance of high erythrocytic ATP was established by detection of elevated levels in four biological relatives of the propositus, none of whom was G6PD-deficient. In two of the affected relatives, the values were similar to that of the propositus (twice the normal mean), whereas in the other two the values were significantly, but not so markedly, elevated. The data are consistent with simple monomeric autosomal inheritance of high erythrocytic ATP, with all affected individuals in this pedigree heterozygous for the gene. Erythrocytes of affected individuals probably have an abnormality of an enzyme involved in utilization, regeneration, or synthesis of ATP, but we have not discovered this postulated primary disturbance. Possession of this abnormality of ATP metabolism has not been found to be harmful; affected individuals are clinically and hematologically normal. On the contrary, the trait may in some ways be beneficial. In one test system there is good evidence of superior performance of high ATP erythrocytes. After 6 weeks of storage in ACD solution under blood bank conditions, 74% of Cr^{51}-labeled autotransfused erythrocytes of the propositus survived 24 hours in contrast to 28 and 29% survival for similarly treated erythrocytes from control individuals. In the erythrocyte this genetically determined metabolic abnormality should prove useful in testing the role of ATP in various cellular functions; whether or not it will be of similar usefulness in other tissues of the body will depend upon the tissue distribution of the abnormality.

Hemodynamic Consequences of Changes in Blood Volume. JAMES CONWAY, Ann Arbor, Mich. (introduced by S. W. Hoobler).

To determine whether factors regulating blood volume may induce secondary alterations in cardiovascular function, repeated measurements have been made of cardiac output (dye-dilution) and blood pressure in unanesthetized dogs over periods of 4 to 5 hours after blood volume had been expanded or contracted by approximately 15% of the initial value. Six experiments were performed for each procedure. Blood volume was expanded by dextran infusions and contracted by bleeding. The hematocrit was held within 4% of the initial value by the

additional infusion of a suspension of homologous red cells. Hemorrhage produced variable changes in CO and TPR within the first 3 hours. Thereafter all animals showed a decline in TPR (from 31.9 ± 5.6 control to 24.4 ± 3.3 U), although CO (3.6 ± 0.5 U) was unchanged (4.1 ± 0.7 U). Expansion in blood volume initially caused a small increase in CO (2.4 ± 0.9 control to 3.1 ± 1.04 L per minute), but after 2 hours in spite of continued elevation in central venous pressure it returned to control levels (2.5 ± 0.54 L per minute). The TPR (38.3 ± 5.3 U control) changed little as the cardiac output increased, but after 2 hours exceeded the control value (49.1 ± 7.5 U). Blood volume expansion under ganglionic blockade produced the same pattern of response with an increase in TPR from a control value of 33 ± 9.5 U to 41.5 ± 8.5 U after 2 hours. Changes in peripheral resistance can be produced, therefore, by changes in blood volume, and the mechanism may be similar to the one involved in autoregulation. The differences between the immediate and delayed responses demonstrated here may explain the inconsistencies formerly noted in the hemodynamic effects of changes in blood volume.

Stereoscopic Fluoroscopy and Cineangiocardiography in Man. GEORGE G. ROWE,* WILLIAM C. ZARNSTORFF, AND CHARLES W. CRUMPTON,* Madison, Wis.

It is natural for man to view objects in three dimensions. Since it has not been practicable cineangiographically in the past, considerable skill has been developed in utilizing various projections. The results of these studies never reveal the true shape, and interrelationships may be inferred, but are never really seen. The present method permits recording of either 30 or 60 stereographic pairs per second, permitting observation of the moving heart in three dimensions. Two X-ray sources beneath the radiographic table emit ultrashort bursts alternately from a right and left source. These rays pass through the patient to an image intensifier and are recorded as alternate frames on a standard movie camera. A partially silvered mirror reflects a portion of the light for simultaneous fluoroscopic viewing. A rotating mirror is synchronized with the X-ray source, so that light is alternately reflected and permitted to pass to a second mirror which is mounted behind. The planes of the rotating and of the fixed mirror are sufficiently different that each eye sees only its proper image. Since each eye receives either 30 or 60 images per second, fusion is achieved and the picture is seen in three dimensions. Viewing is accomplished by projecting two frames at once through a suitable prism arrangement which permits superimposition of appropriately polarized right and left eye images. When viewed with properly polarized glasses the image is seen in three dimensions. The method has been in clinical use for approximately 7 months. It has proven of great teaching value and has added a new dimension to the diagnosis of heart disease. It is particularly useful in coronary arteriography.

Esophageal Pressure Studies in Achalasia: Evidence of an Abnormally Elevated Esophagogastric Sphincter Pressure with Relaxation on Swallowing. BERNARD R. COHEN, New York, N. Y. (introduced by Henry D. Janowitz).

The prevailing concept that in achalasia the resting pressure in the esophagogastric sphincteric area is normal but fails to fall on swallowing is based upon the conventional technique of measuring intraluminal pressures on dry swallows with intermittent catheter flushing to maintain patency. If pressure determinations are done during continuous fluid perfusion of the catheters (approximately 5 ml per minute), the results differ from those cited. This was demonstrated in twelve patients in whom all of the other features were consistent with conventional achalasia. A mecholyl test performed in ten of these patients was positive. Fundic, sphincteric, and body of esophagus pressures were determined in these patients and normal subjects by the method described. Resting absolute esophagogastric sphincteric pressure was higher (mean = 38 mm Hg) in patients with achalasia than in normals (mean = 19 mm Hg). A fall of resting sphincteric pressure occurred in 92% of voluntary swallows (mean fall = 19 mm Hg) in the achalasia group. The duration of this fall in pressure on swallowing was shorter in achalasia (mean = 4 seconds) than in normals (mean = 9 seconds). This shorter duration was caused by either a delayed onset of the pressure fall or its earlier termination. Sphincteric pressure during swallowing remained higher than fundic pressure. The minimal sphincteric pressure during a swallow could be correlated with the degree of dysphagia and of sphincteric narrowing seen on cine and conventional radiographs. These results indicate that under the above conditions in achalasia there is an abnormally elevated pressure within the sphincter that can partially relax on swallowing. This interpretation is more consistent with a number of clinical, radiological, endoscopic, and neuropharmacological aspects of the disease.

The Effects of Angiotensin, ACTH, and Potassium upon Steroid Biosynthesis in Vitro. NORMAN M. KAPLAN, Dallas, Texas (introduced by Gladys J. Fashena).

Previous studies with beef adrenal slices have demonstrated a selective stimulation of the synthesis of cortisol with small amounts of ACTH and of the synthesis of aldosterone with small amounts of angiotensin or increased concentrations of potassium. Additional studies were done to investigate the interrelationships between these agents. Steroids were measured by a double isotope derivative assay. When only aldosterone and corticosterone were studied, outer slices of beef adrenals were used; when cortisol was also studied, the next inner two slices were also used. Angiotensin stimulated the synthesis of corticosterone and, in larger doses, the synthesis of cortisol in 30 minutes. In 2 hours angiotensin stimulated the synthesis of aldosterone, sometimes in the absence of an effect upon corticosterone. The activity of

adrenal phosphorylase was not affected by angiotensin. ACTH, in doses insufficient to stimulate the synthesis of aldosterone, did potentiate the stimulatory effect of angiotensin upon aldosterone. ACTH stimulated the synthesis of cortisol in the absence of either calcium or potassium in the media. Potassium had profound effects upon the synthesis of aldosterone and corticosterone but no effect upon the synthesis of cortisol. As the potassium concentration in the media was increased from 1.5 to 11.0 mEq per L, corticosterone synthesis increased from 2.7 to 13.0 μg per g tissue per hour and aldosterone synthesis increased from 2.5 to 8.0 μg per g tissue per hour. At all concentrations of potassium angiotensin stimulated the synthesis of both steroids. The effects of potassium were maintained in the presence of the stimulation of steroid synthesis by angiotensin. These results support the initiative role of angiotensin and the supportive role of ACTH in the biosynthesis of aldosterone and suggest that angiotensin acts relatively early in the biosynthetic pathway and independently of the effects of potassium.

Mechanisms of Hemodynamic Stimulation of 17-Hydroxycorticosteroid Secretion. DONALD S. GANN AND RICHARD H. EGDAHL,* Richmond, Va.

Hemorrhage is a potent stimulus to adrenal secretion of 17-hydroxycorticosteroids (17OHCS), but the mechanisms of stimulation are unknown. The present studies were undertaken to define these mechanisms. Experiments were performed on anesthetized dogs with chronic adrenal venous cannulae; 17OHCS were determined in adrenal venous blood by the method of Peterson. All dogs had low resting secretion in 17OHCS, and all were responsive to ACTH. Hemorrhage, accompanied by hypotension, is a maximal stimulus to 17OHCS secretion. Administration of trimethaphan camphor sulfonate (Arfonad) to produce hypotension without hypovolemia is also a maximal stimulus. Maintenance of arterial pressure with *l*-norepinephrine during Arfonad infusion prevents the increased 17OHCS secretion, whereas maintenance of arterial pressure during hemorrhage does not prevent maximal response of 17OHCS secretion. Thus hypotension and hypovolemia are effective and separate stimuli to 17OHCS secretion. Regional hypotension and denervation of receptor areas have been used to study the mechanisms involved in the adrenal cortical response to hypotension. These experiments have demonstrated the importance of vagal pathways, carotid receptors, and the kidneys in the mediation of responses to regional hypotension. Neither combined vagal and carotid denervation nor bilateral nephrectomy alone prevents the response of 17OHCS secretion to systemic hypotension. In contrast, preliminary results suggest that the combination of vagal and carotid denervation and nephrectomy prevents the response to systemic hypotension. The data suggest that vagal, carotid, and renal receptors all participate in the adrenal cortical activation following hypotension.

The Role of Phosphofructokinase in the Accelerated Glycolysis of Neoplastic Tissue. DANIEL W. FOSTER AND JOSIAH B. TAYLOR, Dallas, Texas (introduced by Marvin D. Siperstein).

A fundamental characteristic of neoplastic tissue is the capacity to maintain a high rate of glycolysis in the presence of oxygen. Recent evidence indicates that control of glycolysis is mediated through the enzyme, phosphofructokinase, in a wide variety of tissues. A study of phosphofructokinase activity in mouse liver and the mouse hepatoma, *BW 7756,* was carried out to determine whether an abnormality of this enzyme might be associated with the accelerated glycolysis of tumor tissue. Phosphofructokinase activities in high speed extracts of the hepatoma were 5 to 10 times greater than those in the liver. Furthermore, kinetic studies with crude and partially purified preparations suggested that the phosphofructokinase of hepatoma is a different enzyme from that in liver. V_{max} with respect to the substrate fructose-6-PO_4 was 2.7×10^{-4} M for the liver and 4.0×10^{-3} M for the tumor. Although differences in activities of enzymes in tumors have been reported, this appears to be the first report of distinct enzymes catalyzing the same reaction in a tumor and its tissue of origin. Since activity of the tumor enzyme is greater than that of liver at any given concentration of substrate or activator, the increased rate of aerobic glycolysis in neoplastic tissue may be due to synthesis of an altered phosphofructokinase by the hepatoma.

The Hyperosmotic Effects of Ethanol and Sucrose on the Left Ventricle. TIMOTHY J. REGAN,* ALAN B. WEISSE, HENRY A. OLDEWURTEL, AND HARPER K. HELLEMS,* Jersey City, N. J.

The role of non-nutritional factors in the cardiac response to alcohol has not been defined. An increase of plasma osmolality has been found to occur in ten intact anesthetized dogs receiving 15% ethanol intravenously for 2 hours at a rate of 0.1 ml per kg per minute. At a plasma ethanol level of 95 mg per 100 ml and an osmolality rise of 30 mOsm per L the plasma volume (serial RISA measurements) was increased maximally by 30 minutes to 18% ± 6 above control with a corresponding decrease in plasma protein. Simultaneously, left ventricular end-diastolic pressure began to rise, and stroke work progressively declined to 70% of control without significant heart rate or arterial pressure change. Sampling of arterial-coronary sinus (A-CS) blood at 15-minute intervals revealed a net loss of K^+ and PO_4 ions beginning by 1 hour. The mean maximal PO_4(A-CS) was −0.7 mg per 100 ml ($p < 0.001$) and that of K^+ was −0.33 mEq per L ($p < 0.01$). Release of enzyme from the heart began at about 2 hours, with a mean maximal SGOT (A-CS) of −84 U ($p < 0.001$), that was not attributable to a decline in coronary blood flow (hydrogen gas method). To determine if these left ventricular alterations are related to plasma hyperosmolality induced by alcohol, hypertonic sucrose was infused intravenously into 8 animals for 2 hours to achieve a comparable plasma

osmolality rise. Subsequent to the maximal rise in plasma volume and decrease in plasma proteins at 30 minutes, there was a net loss of ions, beginning at 1 hour. The mean maximal PO_4(A-CS) was −0.86 mg per 100 ml ($p < 0.001$) and that of K^+ was −0.29 mEq per L ($p < 0.02$). Enzyme release from the heart appeared at about 2 hours with a maximal SGOT (A-CS) of −45 U ($p < 0.01$). These findings were not related to a coronary flow decrease. A decline in ventricular contractility was observed with end-diastolic pressure above control while stroke work was diminished. Thus the decline in left ventricular function and loss of myocardial ions and enzyme seen at moderate levels of ethanol appear to be largely attributable to its osmolar properties.

Characteristics of an Immune System Common to Certain External Secretions. THOMAS B. TOMASI, JR., Burlington, Vt. (introduced by K. J. Thomson).

Certain human secretions such as tears, saliva, and colostrum have been found to contain significant concentrations of γ-globulin by quantitative immunologic analysis. The γ-globulins present in these fluids are largely of the γ_{1A} type, and the γ_2:γ_{1A} concentration ratios are less than one (compared with ratios in normal serum in the 5 to 8 range). Several possible explanations for the selective occurrence of γ_{1A} in these fluids have been considered. These include selective transport of γ_{1A} from serum to fluid and the local production of γ_{1A} by the corresponding gland. In an attempt to distinguish between these possibilities the γ_{1A} in two of these secretions (colostrum and parotid saliva) and in serum was isolated in homogenous form (as determined by ultracentrifugation, electrophoresis, and immunologically), and their physical and chemical properties were compared. The properties studied include sedimentation, electrophoresis, optical rotatory dispersion, hexose sugar and sialic acid content, and immunological properties such as group specificity and genetic (Gm, Inv) characteristics. Sedimentation was studied over a range of pH's, in 8 M urea, and after treatment with disulfide reagents and proteolytic enzymes (papain and pepsin). Salivary and colostral γ_{1A} were similar if not identical, but they differed significantly in certain of their properties from serum γ_{1A}. These results suggest the possibility of an immune system common to certain external secretions, which is quite different at least in the nature of its antibody from that produced by the systemic system.

Left Ventricular Performance in Aortic Regurgitation in Man. GILBERT E. LEVINSON, GABRIEL KOROXENIDIS, AND MARTIN J. FRANK, Jersey City, N. J. (introduced by Harper K. Hellems).

Previously we have shown that regurgitant flow ($\dot{Q}_R$) in aortic regurgitation (AR) is measured accurately from ventricular (LV) and arterial dilution curves during continuous indicator infusion into the aortic root. This technic, in conjunction with measurements of end-diastolic and end-systolic volumes (EDV and ESV) by indicator-dilution, permitted study of LV performance in 16 patients with AR. Twelve subjects with normal ventricles served as controls. With $\dot{Q}_R$ ranging from 0.12 to 7.05 L per minute per m^2, the LV maintained normal forward flow ($\dot{Q}_F$) in pure AR (mean = 3.0 L per minute per m^2), and flows commensurate with the stenotic lesion with concomitant aortic or mitral stenosis (means = 2.66 and 1.94 L per minute per m^2, respectively), by pumping total flows ($\dot{Q}_T$) up to 10.42 L per minute per m^2. Total work was increased in all patients and rose with increasingly severe AR to 219 g-m per beat per m^2 (normal = 56 ± 4). EDV (normal = 91 ± 12 ml per m^2) rose with increasingly severe AR, up to 148 ml per m^2 in compensated patients and 276 ml per m^2 with LV failure. ESV was normal (49 ± 9 ml per m^2) or diminished, except with decompensation, where it rose to twice normal. Thus, the LV dilated in compensated AR only in proportion to the augmented stroke volume. LVED pressure was elevated in most patients and correlated poorly with EDV. Mean fiber-shortening rates (MFSR), calculated assuming a spherical LV, were elevated in all compensated patients, up to 66 cm per second (normal = 13 ± 2). With decompensation, MFSR approached normal. The LV maintains a normal effective flow and work in AR by augmenting total flow and work. It accomplishes this by dilating only enough to accommodate the augmented stroke and by enhanced emptying through rapid fiber-shortening. Decompensation is not reliably indicated by ED pressure, but is identified by disproportionate dilatation, less complete emptying, and inappropriately small fiber-shortening rates.

Transport of Cystine and the Dibasic Amino Acids by Leukocytes from Normal Subjects and Patients with Cystinuria. LEON E. ROSENBERG AND SYLVIA J. DOWNING, Bethesda, Md. (introduced by N. I. Berlin).

Recent *in vitro* studies using jejunal mucosa and kidney cortex slices have demonstrated the following abnormalities in patients with cystinuria: *1)* defective transport of the dibasic amino acids, lysine and arginine in gut and kidney; *2)* impaired cystine transport in gut but not in kidney. The present studies were undertaken to determine whether amino acid transport is also modified in leukocytes from cystinuric patients and to compare the transport system(s) for cystine and the dibasic amino acids with that in other human tissues. Leukocytes were isolated from as little as 100 ml of whole blood by dextran sedimentation, and contaminating erythrocytes were removed by hypotonic lysis. Leukocytes prepared in this way appeared normal by light microscopy, resisted trypan blue staining, oxidized glucose to carbon dioxide, and incorporated amino acids into protein, indicating the metabolic competency of this tissue preparation. Leukocyte suspensions, incubated with C^{14}-labeled lysine and S^{35} cystine in modified saline-bicarbonate buffer, were noted to accumulate these amino acids rapidly against chemical concentration gradients, achieving steady state conditions within 10 to 15 minutes. Studies carried out over a wide range of substrate concentrations indicated

the presence of saturable sites for these amino acids. Lysine and arginine were again noted to be mutally inhibitory and competed for a common site, which was not shared by cystine. In contrast to results in gut and kidney, no defect in lysine or arginine transport was found in leukocytes from patients with cystinuria, but preliminary results in five cystinuric subjects and seven normals suggest that cystine uptake may be defective in this disease. These results present further evidence of the complexity of the transport dysfunction(s) in cystinuria and indicate that leukocytes provide a useful tool for studies of transport mechanisms and abnormalities in man.

Hyperreactivity to Endotoxin Induced by Etiocholanolone. SHELDON M. WOLFF, MELVIN RUBENSTEIN, AND JOHN H. MULHOLLAND, Bethesda, Md. (introduced by James H. Baxter).

Some infectious diseases are capable of inducing pyrogenic tolerance to endotoxin. To assess the effect of fever per se on endotoxin reactivity, six normal volunteers were given 5 or 7 im injections of 10 or 20 mg of etiocholanolone during an 8- to 10-day period. Identical doses of endotoxin (Lipexal, the lipopolysaccharide of *Salmonella abortus equi*) were given intravenously before and after the series of etiocholanolone injections. Febrile response (°C) was plotted for 7 hours and the area under the curve measured in cm^2 as the fever index. Each injection of etiocholanolone produced a febrile response. The administration of endotoxin after the series of etiocholanolone injections was accompanied by a significant increase in fever index (100% in three subjects and 33% in one) as compared to the pyrogenic response to endotoxin before etiocholanolone administration. In addition, marked subjective reactions (chills, headache, nausea) were noted in all subjects. The remaining two subjects had chills and rapid rise in temperature so pronounced as to necessitate interruption of fever by aspirin. A return to base-line endotoxin response was noted in three of four individuals retested within 2 weeks. In two subjects the capacity of the reticuloendothelial system to clear I^{131}-aggregated albumin was accelerated after the etiocholanolone injections. Despite the etiocholanolone-accelerated clearance of aggregated albumin endotoxin tolerance was not induced; on the contrary, hyperreactivity to endotoxin was observed.

Immunoglobulins: Clarification of Their Significance in Renal Disease and Demonstration of Response to Immunosuppressive Therapy. A. F. MICHAEL, K. N. DRUMMOND, R. A. GOOD,* AND R. L. VERNIER,* Minneapolis, Minn.

An investigation of the immunologic mechanisms in renal disease was undertaken in 81 patients. Immunofluorescent and morphologic studies on kidney biopsy tissue were correlated with the clinical course. Antibody to purified 7 S gamma globulin, B_{1c}, albumin, fibrin, and fibrinogen was used. In nine children with poststreptococcal acute glomerulonephritis B_{1c} was localized in all cases in discrete nodular masses along the glomerular basement membrane. By electron microscopy, these masses were seen on the basement membrane and within the epithelial cells. Gamma globulin was present in a similar distribution in two of nine patients. The lesions decrease in intensity or disappear with recovery. The fluorescent and electron microscopic appearance of the deposits is identical to that described in antigen-antibody complex nephritis in rabbits. In 18 children with idiopathic nephrotic syndrome showing no or minimal glomerular alterations by light microscopy, neither gamma globulin nor B_{1c} was present. Sixteen had a complete remission and cessation of proteinuria after corticosteroid therapy. Five patients with nephrotic syndrome and definite glomular pathology showed gamma globulin and B_{1c} on the glomerular basement membranes and were resistant to steroid therapy. These findings suggest that most cases of the nephrotic syndrome in childhood are not due to immunological mechanisms and that the presence of immunoglobulins is usually associated with steroid resistance. Three patients with severe renal disease (disseminated lupus, chronic glomerulonephritis, and subacute nephritis) showing gamma globulin and B_{1c} deposits on the glomerular basement membrane were treated with high doses of cortisone and Imuran (4 to 10 mg per kg). No immunoglobulins were detected in glomerulonephritis after 1 month of therapy; a striking reduction in fluorescent staining was seen in lupus nephritis. These changes were accompanied by marked clinical improvement, reduction of blood urea nitrogen to normal, and increased renal function and serum complement.

Pathogenesis of Immunologic Deficiency Disease. RAYMOND D. A. PETERSON AND ROBERT A. GOOD,* Minneapolis, Minn.

Studies of patients with immunologic deficiencies provided direction for experimental studies that have clarified many aspects of the normal immune system. This communication will synthesize the clinical and experimental data and begin a classification of immunologic deficiency diseases based upon the developmental scheme of the immune system. The anlage of the immune system is an epithelial thymus derived from foregut endoderm. The epithelial thymic cells are induced by mesenchyme to become lymphoid. These lymphoid cells differentiate and directly or indirectly populate the peripheral lymphoid tissue. The peripheral lymphoid tissue is the anlage for the plasma cells that are the principal producers of immunoglobulins. A deficiency of immunoglobulins would result therefore from a defect located anywhere from the gut anlage to the plasma cell. Morphologic study of cellular aspects of the immune system, particularly study of the thymic structure of patients, now permits a pathogenetic classification of several of the clinical types of immunologic deficiency diseases. Patients with lymphopenic agammaglobulinemia and with reticular dysgenesis have failed to develop a normal epithelial thymus. Children with ataxia-telangiec-

tasia have a thymus that appears incompletely developed. Eight of these patients have died with lymphoreticular malignancies. The congenital sex-linked type of agammaglobulinemia is probably due to a block beyond the central lymphoid tissue, as the thymus appears normal but the peripheral lymphoid tissue is undeveloped. Three of these patients have died of lymphatic leukemia. The primary acquired agammaglobulinemia of adults is also probably a failure of peripheral lymphoid tissue, 10% incidence of an associated thymic tumor being secondary to a defective feedback mechanism. A consideration of the pathology of a defective immune system directs further study of these patients and may clarify the observed association of these syndromes and lymphoreticular malignancies.

Production of Autoantibodies to Insulin in Man and Rabbits. GEROLD M. GRODSKY, San Francisco, Calif. (introduced by Richard J. Havel).

Antibodies to crystalline insulin are demonstrable in man and animals after insulin treatment. Since immunized animals maintain normal glucose levels, it was presumed that these antibodies are produced only by foreign sites on the exogenous crystalline insulin and do not affect endogenous hormone. Techniques were devised to measure directly serum antibody-bound endogenous hormone in the presence of residual exogenous insulin. The antibody fraction of serum from immunized rabbits and an uncontrolled maturity-onset resistant diabetic, all treated only with bovine insulin, was isolated by ultracentrifugation or preferential salt precipitation, or both, and total insulin was extracted from antibody with acid alcohol. Yields of insulin in these procedures were determined in separate experiments by the recovery of chromatographically intact insulin-I^{131}. Bovine exogenous and leporine or human endogenous insulin was immunoassayed (Grodsky and Forsham, 1960) differentially, with species-specific antibodies as reagents. Serum antibody titers were determined chromatographically with insulin-I^{131}. Extractable insulin in normal serum was 0 to 50 μU per ml, none being associated with any antibody fraction. In serum containing elevated antibodies, circulating insulin was always bound (> 95%). In rabbits with normal blood sugar, 3 weeks after the last immunizing dose of bovine insulin, exogenous insulin was undetectable but bound leporine insulin proved elevated (1.5 to 3 mU per ml). In the diabetic, bound exogenous insulin, high 17 hours after insulin administration (4 to 6 mU per ml), declined at 3 days (2 mU per ml) and was undetectable at 11 days. In contrast, high levels of human endogenous insulin were consistently associated with antibody (1 to 2 mU per ml) for 5 weeks, decreasing slowly with decreasing antibody levels. Blood sugar remained elevated. Thus in man and rabbits exogenous insulin, a pure, low molecular weight protein, can produce circulating autoantibodies that bind and inactivate large amounts of endogenous hormone. Furthermore, the presence of circulating endogenous insulin demonstrated in the diabetic subject described emphasizes that some resistant diabetics have a continuing capacity to secrete insulin.

Competition between Several Antithyroid Compounds and Iodide for a Common Oxidizing Enzyme System in Thyroid Tissue. F. MALOOF* AND M. SOODAK, Waltham and Boston, Mass.

A cytoplasmic particulate fraction of thyroid tissue has been isolated that oxidizes the thiocarbamide, thiourea, the pseudohalide, thiocyanate, and the halide, iodide, *in vitro,* similar to their oxidation *in vivo.* The system has been solubilized by sonication of an acetone powder of the particles. A common enzyme system for these oxidations is suggested by the following observations. *1*) All three reactions are carried out by the same particulate fraction and the same soluble enzyme system. *2*) The latter is protected from inactivation during solubilization by thiourea, thiocyanate, or iodide (10^{-4} to 10^{-3} M), all substrates for this system. *3*) These reactions are inhibited by the thyroid supernatant fluid, by anaerobiosis, azide, thiosulfate, various thiols, aromatic antithyroid compounds, and by the nucleophilic anions, bisulfite and cyanide. *4*) Preincubation studies with these nucleophilic anions which cleave disulfide (—S—S—) bonds reveal that an intact —S—S— bond is a requisite for activity in all three reactions. *5*) There is competitive inhibition between the nucleophilic anions and thiourea and between thiourea and thiocyanate, suggesting competition for a common site on the enzyme. There is evidence that this thyroidal enzyme system may be a peroxidase. This is based on the fact that these reactions are also inhibited by catalase and by the heme-binding protein, globin. The latter inhibition is overcome by hematin. Furthermore, spectrophotometric analysis reveals that this enzyme system contains a unique hemoprotein. Oxygen and hydrogen peroxide are requirements for activity. The lack of activity with hydrogen peroxide under nitrogen suggests that oxygen does not merely produce hydrogen peroxide and that hydrogen peroxide is not the primary oxidant. These data indicate that there is a common oxidizing enzyme system for the thiocarbamides, thiocyanate and iodide. Competition for oxidation may explain the inhibitory action of these antithyroid compounds on iodination.

The Source of Energy for Active Sodium Transport in the Toad Urinary Bladder. ROBERT P. DAVIS, MITZY CANESSA-FISCHER, CHESTER M. EDELMANN, JR., AND LEE HOFFMAN, New York, N. Y. (introduced by Howard A. Eder).

Mitochondrial ATP synthesis is coupled to cell respiration through a series of high energy intermediates. These intermediates include, sequentially, a bound form of reduced pyridine nucleotide, a nonphosphorylated compound and a phosphorylated intermediate that reacts with ADP to form ATP. We have previously shown that energy for active sodium transport in the toad bladder is derived directly from oxidative phosphorylation. The effect of ADP on metabolism and sodium transport

indicated that ATP was not this direct source of energy for ion transport. To identify which intermediate of oxidative phosphorylation is involved, studies were performed with inhibitors and uncouplers of oxidative phosphorylation which act on specific intermediates of mitochondrial energy reactions. In mitochondria, amytal administration and anaerobiosis cause accumulation of bound reduced pyridine nucleotide by preventing its oxidation. By spectrofluorometric studies we have shown this same specific metabolic effect to occur in the intact toad bladder. Yet, under these conditions, sodium transport is inhibited. These and other metabolic studies show no correlation between levels of bound pyridine nucleotide and sodium transport. Carbonyl-cyanide-chlorphenylhydrazone and guanidine compounds inhibit oxidative phosphorylation by preventing formation of the nonphosphorylated compound. Secondarily, levels of the phosphorylated intermediate are reduced. In the toad bladder, these agents decrease sodium transport while polarographic studies of respiration demonstrated characteristic inhibition of oxidative phosphorylation. To separate the roles of the two intermediates, studies were performed with oligomycin, which specifically inhibits formation of the phosphorylated intermediate by mitochondria without inhibiting the nonphosphorylated compound. Oligomycin similarly inhibits sodium transport by the toad bladder. Whenever levels of intermediates have been varied, transport of sodium by the toad bladder parallels levels of the phosphorylated intermediate. We propose that this phosphorylated compound is the specific intermediate of oxidative phosphorylation which directly donates energy for active sodium transport in the toad bladder.

The Quantitative Assessment of Pulmonary Edema. O. Robert Levine, Robert B. Mellins, Mounir E. Nassar, and Alfred P. Fishman,* New York, N. Y.

In the intact animal or man, the degree of pulmonary edema is generally assessed by clinical and radiographic criteria. In this study, the degree of pulmonary edema was quantified by determining the interstitial water space of the lung (ISF) and the mechanics of breathing in dogs with graded degrees of pulmonary venous congestion. Pulmonary venous return was obstructed by inflating a balloon in the left atrium of anesthetized dogs followed by saline hemodilution. The ISF was determined from simultaneous T-1824 and tritiated water dilution curves; the results were compared with the water content of the lungs at autopsy. The forces responsible for formation of pulmonary edema were estimated from direct measurements of pulmonary arterial, left atrial, and oncotic pressures. In 33 dogs, control measurements of ISF ranged from 1.73 to 4.76 ml per kg (mean, 3.48 ml per kg; SD $\pm$ 0.22 ml per kg), and the pulmonary arterial-aortic arch blood volume (PBV) ranged from 11.5 to 17.3 ml per kg (mean, 15.2 ml per kg; SD $\pm$ 2.8 ml per kg). Balloon inflation and hemodilution resulted in pulmonary congestion in all 33 dogs; in 22 of these, there was also an increase in the ISF of 1 to 9 ml per kg. The ISF correlated significantly with lung weight and the moisture content of the lungs at autopsy. Pulmonary compliance and air flow resistance were also determined in eight dogs with pulmonary congestion. In those with normal ISF, compliance fell by 20 to 30%. In those with an expanded ISF (by 2.7 to 3.2 ml per kg) pulmonary compliance fell by 60 to 80%. Air flow resistance remained within normal limits in both groups. These data indicate that *1*) measurement of ISF distinguishes reliably between pulmonary congestion and edema and *2*) the effect of pulmonary congestion and edema on pulmonary compliance is quantitatively different.

Quantitation of the Effects of Penicillamine Therapy in Cystinuria. Myron Lotz and John T. Potts, Jr., Bethesda, Md. (introduced by Robert W. Berliner).

The urinary amino acid excretion of five patients with cystinuria and of normal subjects has been studied during oral administration of 1 to 6 g of D-penicillamine daily. Modifications of the conventional methods of amino acid determination with a Spinco automatic analyzer (lowering effluent buffer pH and increasing temperature) permitted separation and quantitation of urinary amino acids including cystine, cysteine-penicillamine mixed disulfide, and penicillamine disulfide. In cystinuria, stepwise increase in penicillamine dosage results in a progressive decrease in cystine excretion with a proportional rise in the excretion of mixed disulfide. With 2 to 3 g of penicillamine the reduction in cystine excretion was 80 to 100%. On penicillamine, normal subjects exhibit a 10- to 20-fold increase in the total excretion of half-cystine (principally as mixed disulfide), whereas cystinuric patients exhibit a 35 to 50% reduction in excretion of half-cystine (cystine + mixed disulfide). This suggests that in cystinuria the response to penicillamine is more complex than a simple disulfide interchange in the kidney tubule between unreabsorbed cystine and filtered penicillamine. Penicillamine disulfide and penicillamine-cysteine mixed disulfide are 50 to 500 times as soluble as cystine. During 10 months of therapy with penicillamine in doses sufficient to suppress urinary cystine to less than 50 mg per day, crystalluria has ceased and no stones have formed. Toxicity has been minimal with transient fever and skin rash occurring in two patients early in the course of therapy. No abnormalities have been observed in copper, iron, or ceruloplasmin metabolism. Guarded optimism for the effectiveness of D-penicillamine therapy in cystinuria appears warranted.

Studies on Renal Acidification in Hyperglobulinemic, Nonmyelomatous States. Curtis Morris, Linares Johnson, and H. H. Fudenberg,* San Francisco, Calif.

The syndrome, renal tubular acidosis, in association with the Fanconi syndrome has been described in a number of patients with multiple myeloma. In those affected, the most striking and commonly the only significant pathological change of the renal tubule is limited to the proximal convoluted tubule where cellular degeneration and

inclusion bodies having the staining characteristics of protein have been described. To test whether hyperglobulinemic states other than that of multiple myeloma might be associated with defects of renal acidification, this function has been investigated in nonazotemic, hyperglobulinemic patients without demonstrable multiple myeloma. In three female patients, ages 32, 16, and 56 with serum globulins of 5.6, 5.9, and 7.9 per 100 ml, frank hyperchloremic renal tubular acidosis was demonstrated. The predominant globulin in each case was a broad base 7 Sγ and occurred in association with idiopathic hyperglobulinemia, lupoid hepatitis, and Sjogren's syndrome, respectively. Two of these patients had nephrocalcinosis. In a fourth nonacidotic female patient with Hodgkin's disease and a serum globulin of 4.6 g per 100 ml, predominately of 7 Sγ spike, a markedly reduced urinary excretion rate of titratable acidity and ammonium and a subnormal reduction of urinary pH was demonstrated after loading with ammonium chloride. Maximal attainable urine osmolarity after water restriction for 12 hours and im Pitressin was 349, 394, 453, and 427 mOsm per L in the respective patients. Tubular reabsorption of solute-free water, T_{H_2O}, in response to infusion of 20% mannitol and a maintained state of antidiuresis was 1.35, 0.64, 1.14, and 1.46 ml per minute, respectively. These data indicate a concentrating defect. Neither acidosis nor hypokalemia existed at the time of these concentration studies. Neither glycosuria nor Bence-Jones proteinuria was demonstrated in these patients. One patient (with lupoid hepatitis) had aminoaciduria. We conclude that hyperglobulinemic states other than that of multiple myeloma may be significantly associated with defects of renal acidification and concentration.

The Character of the Aldosterone Response to Changes in Potassium Balance and to Angiotensin: Dependence of Both Effects upon Sodium Balance. PAUL J. CANNON, RICHARD P. AMES, AND JOHN H. LARAGH,* New York, N. Y.

A renal-adrenal interaction involving angiotensin release and stimulation of aldosterone may play a major role in regulation of sodium balance. Fifteen angiotensin infusions in normal man indicated that the adrenal response, as well as the pressor response, is a graded one, related to the state of sodium balance. Angiotensin produced greater increases in aldosterone secretion in subjects on a high sodium diet; this augmenting effect declined with sodium depletion. Because a number of situations occur in which there is hypersecretion of aldosterone without clinical evidence of angiotensinemia, the contribution of changes in potassium balance to the regulation of aldosterone secretion also was evaluated by metabolic balance technics. Potassium administration did not significantly increase aldosterone secretion in ten normal subjects receiving normal constant amounts of dietary sodium. Similar amounts of potassium produced significant to marked increases of aldosterone in these subjects after dietary sodium deprivation. Dietary potassium deprivation blocked the hypersecretion of aldosterone of sodium deprivation. Peculiarly, much greater potassium loss and hypokalemia induced by thiazides or ethacrynic acid were associated with enormous hypersecretion. Despite normal sodium intake, potassium administration strikingly increased aldosterone secretion in thirteen studies of the following diseases: unilateral or bilateral renal hypertension, malignant hypertension, renal tubular acidosis, and sodium-losing nephropathy. In the latter two, after salt depletion potassium boosted aldosterone secretion to 2,618 and to 6,532 μg per day. In these renal diseases dietary potassium intake is therefore a major factor in causing hyperaldosteronism. This investigation demonstrates that potassium administration can exert stimulating effects upon the secretion rate of aldosterone at least as great as, or greater than, those produced by angiotensin. In normal subjects, the spectrum of adrenal cortical responsiveness to potassium is the reverse of that for exogenous angiotensin, with the stimulating effect of both agents being determined by the state of sodium balance.

Alterations in Cortisol Kinetics by Estrogens, ACTH, and Obesity. EDWIN M. BRADLEY, Rochester, N. Y. (introduced by Christine Waterhouse).

Available information on the effects of protein binding on cortisol metabolism and distribution *in vivo* is largely indirect. Data from disappearance curves of labeled cortisol have been limited to the late exponential phase. With the early phase as well, a two-compartment system can be analyzed which allows calculation of rates of distribution and catabolism. Plasma cortisol disappearance curves were obtained by drawing arterial blood specimens at 2, 4, 8, 16, 30, 45, 60, and 75 minutes after injection of trace amounts of cortisol-4-C^{14}. Cortisol was isolated and the radioactivity determined. Analysis of disappearance curves from an Addisonian subject receiving constant infusions of high and low levels of exogenous cortisol showed the calculated rate of catabolism to be equal to the rate of infusion. The initial distribution of the tracer was confined to the vascular space. Cortisol disappearance curves were obtained in normal subjects under basal and ACTH conditions and were repeated after protein binding had been increased by exogenous estrogens. The effects of obesity were studied under basal and ACTH conditions. ACTH expands both the intravascular and extravascular compartments proportionately. Transfer in all directions is greatly increased, but the probabilities for catabolism and distribution of any given cortisol molecule are not significantly changed. Increased protein binding causes disproportionate expansion of the intravascular pool and decreases the probability of metabolism of a given cortisol molecule. These latter relationships are maintained under ACTH stimulation. In obesity there is expansion of the extravascular pool with increased probability for a cortisol particle to leave the blood. Although increased protein binding radically changes the distribution and probability of cortisol catabolism, absolute production and degradation rates are not greatly changed. Increased amounts of free cortisol as

seen with ACTH speed up all processes nearly proportionately. An expanded extravascular pool as seen with obesity is associated with an increased tendency for cortisol catabolism.

The Response of the Rectum of Patients with Celiac Sprue to Wheat Enemas. WILLIAM O. DOBBINS III AND CYRUS E. RUBIN,* Seattle, Wash.

Twelve of 22 celiac sprue patients showed some histologic abnormality in multiple rectal biopsies (81). It therefore seemed desirable to study the possible relationship between gluten exposure and rectal mucosal damage. One hundred forty-two biopsies were taken in 13 experiments. The following enemas were given to normal controls: wheat, 2; gliadin, 1; gluten, 1; isotonic saline, 1; blood, 1. Wheat enemas were given to four celiac sprue patients whose diets had been gluten free for 3 to 24 months; several months later, gliadin, oats, and corn enemas were administered to one of each of the same patients. Rectal biopsies were obtained before the enemas and at 4, 8, and 24 hours thereafter. The various enemas given to normal controls produced no symptoms and only the minimal polymorphonuclear infiltrate that may be seen after any enema. In contrast there was a characteristic histologic response to wheat enemas in four celiac sprue patients and in the single patient given gliadin; it consisted of obvious polymorphonuclear infiltration of the surface epithelium, lamina propria, and submucosa, maximally apparent at 8 hours. Three of these five patients developed symptoms. Oats produced no symptoms in a celiac sprue patient; the histological response was delayed to 24 hours but was otherwise identical to that after wheat. A gluten-free corn enema was given to a celiac sprue patient who had reacted characteristically to wheat; it produced only the minimal nonspecific changes observed in our controls. The rectal reaction to wheat enemas in celiac sprue was similar in timing but far milder histologically than that seen in previous experiments where wheat was instilled into the ileum. If the absorptive cell is the primary site of abnormal gene action in this disease, then the milder rectal than intestinal reaction to wheat is best explained by the presence of fewer rectal absorptive cells.

Epithelial Hyperplasia after Massive Small Bowel Resection in Man. RICHARD L. PORUS, Seattle, Wash. (introduced by Wade Volwiler).

Hypertrophy and hyperplasia of residual small intestine after extensive resection have been reported in animals. Although similar changes might be expected in man, these were not obvious histologically in peroral duodenojejunal biopsies, and therefore quantitative analysis was undertaken. A male teenager with 75% resection (70-cm duodenojejunal remnant) and two adult women with 50% resection (ileectomy) were studied 2, 3, and 10 years, respectively, after operation. All had malabsorption but maintained normal nutrition. Eleven peroral biopsies were taken at the duodenojejunal junction from the three patients and 22 biopsies from 11 age- and sex-matched normal controls. The length of epithelial border was determined with a map measurer in four different flat fields from two well-oriented, 4-μ serially sectioned biopsies in each patient and control (160 $\times$ magnification; field diameter, 21.5 cm). The number of cells per unit length of epithelial border was counted in the middle third of 90 different, well-oriented villi at 430 $\times$ magnification. There was no significant difference in length of epithelial border or in number of cells per unit length between the two 50% resections and their normal controls. Furthermore, there was no difference between these controls and younger controls of the patient with 75% resection, a finding consistent with extensive earlier work from this laboratory showing no significant variation in the length of epithelial border with age or sex. In the patient with 75% resection the number of cells per unit length of epithelial border was increased 22% over normal controls ($F_{1,4}$ test significant at 1% level). This patient's epithelial border length was in the upper normal range. Thus hyperplasia of intestinal epithelial cells following massive small bowel resection has now been clearly demonstrated in a human, but no evidence has been found for accompanying hypertrophy of villi. No changes were observed following two 50% resections.

Evidence for a Common Carrier in the Renal Reabsorption of All Alkali Cations. MACKENZIE WALSER * AND W. JOSEPH RAHILL, Baltimore, Md.

Recent micropuncture data are consistent with the possibility that, in the absence of K^+ deficiency or agents which induce net K^+ secretion (such as sulfate, bicarbonate, urea, and acetazolamide), excreted K^+ represents chiefly unreabsorbed K^+ and that reabsorption of K^+ may be less complete at each point in the nephron than Na^+. If both cations share the same carrier, the amounts reabsorbed of each, in relation to the local tubular fluid concentrations, should be proportionate at any point in the tubule. If this proportionality is maintained constant throughout the tubule, excreted/filtered K^+ should be a constant power of excreted/filtered Na^+. In 122 observations in dogs in normal conditions, salt-depleted, or undergoing saline or mannitol diuresis, or both (log E/F K^+) $\div$ (log E/F Na^+) was 0.38 ± 0.10 (SD), independent of flow, over a 3,000-fold range of E/F Na^+. The remaining variability could reflect a small but inconstant contribution of $K^+ - Na^+$ exchange. To test this possibility, Cs^{134} was given by priming injection and constant infusion. Net secretion of Cs^+ did not occur, despite loading with CsCl, KCl, $KHCO_3$, $NaHCO_3$, or Na_2SO_4. Log E/F Cs^+) $\div$ (log E/F $Na + K^+$) was 0.34 ± 0.05 ($n = 100$) and was uninfluenced by K^+ loading, or by flow. Clearance of Rb^{86}, however, equaled that of K^+ (ratio, 1.00 ± 0.14). Recalculation of published data indicates that E/F Li^+ is also a constant power (0.25 ± 0.05) of E/F Na^+. Thus only K^+ and Rb^+ appear to be secreted, but all five cations are reabsorbed in an interdependent manner. The form of this interdependence suggests that a common carrier is involved, with preferential binding

reflecting naked ion radius in the sequence: Li < Na > K = Rb > Cs.

Characteristics of Cell Proliferation in Untreated Acute Leukemia. ALVIN M. MAUER AND VIRGINIA FISHER, Cincinnati, Ohio (introduced by Richard W. Vilter).

To obtain information about the proliferative capacity of leukemic cells, tritiated thymidine was given intravenously to eight children with untreated acute leukemia. Six children were given a single dose of 200 μc per kg, and two were given serial injections at 10-hour intervals. Multiple blood and bone marrow samples were obtained after a single injection, and samples were obtained before and 1 hour after the serial injections. Radioautographs were prepared, and the percentage of cells labeled and the number of grains over each labeled cell were determined. The percentage of leukemic cells labeled initially in blood was less than in marrow for each patient. The subsequent time course of labeled cells in the two compartments was likewise dissimilar. In two patients adequately studied before blood transfusions were needed, a mitotic division of labeled marrow cells was evident within 10 hours and again 18 to 26 hours after the injection. With this generation time for labeled cells of 15 to 20 hours and the percentage of initially labeled cells (5.5 and 7.5), it could be calculated that less than one-third of the marrow leukemic cells was actively proliferating. A nonuniform population of leukemic cells was also found in the patients given serial injections of label. Relabeling of marrow leukemic cells was found at the 20-hour injection in that there was an increase in grain concentration over labeled cells without increase in the percentage of labeled cells. Although almost all mitotic figures were labeled and at least one generation time encompassed, the final proportion of labeled marrow cells was less than one-third. Thus these morphologically similar leukemic cells were comprised of both proliferative and nonproliferative elements. In these experiments considerable reutilization of label also was found.

The Miscible Serotonin Pool and Daily Serotonin Production. SIDLEE W. LEEPER, HAROLD BROWN,† VIRGINIA E. DAVIS, CLARENCE P. ALFREY, MICHEL E. KAHIL, AND ENOS L. SIMONS, Houston, Texas.

Serial measurements of the specific activity of 5-hydroxyindoleacetic acid (5-HIAA) excreted in the urine after the administration of C^{14}-labeled serotonin (5-HT) approximate a semilog curve when plotted against time. The slope of this curve and its extrapolation to zero time afford a means of calculating a readily miscible serotonin pool. Eighty-eight per cent of the injected radioactivity was recovered in the urine in the 24 hours following the administration of the isotope (range, 52 to 104%). Sixty-eight per cent of the administered serotonin was converted to 5-HIAA (range, 40 to 96%). The daily serotonin production was calculated by dividing the 24-hour 5-HIAA excretion by the fraction of radioactive 5-HT converted to radioactive 5-HIAA. In nine subjects, without carcinoid tumors, the readily miscible serotonin pool ranged from 0.28 to 1.9 mg, and the daily serotonin production ranged from 4.0 to 10.3 mg. In two subjects with extensive nonfunctioning carcinoid tumors, the serotonin pool was 0.58 and 0.64 mg, and the daily serotonin production was 5.0 and 5.9 mg, respectively. In seven subjects with functioning carcinoid tumors, the serotonin miscible pool was 6.8 to 78.0 mg, and the daily serotonin production was 27.9 to 615 mg. The serotonin pool of the patients with symptoms of the carcinoid syndrome was larger than that of the asymptomatic individuals (20.9 to 78 mg vs. 6.8 to 11.2 mg). The administration of reserpine, with the resultant striking reduction in the blood platelet serotonin level, did not affect the size of the serotonin pool, or the daily serotonin production. These studies suggest that the serotonin miscible pool and the daily serotonin production are independent of the circulating platelet serotonin level and that the symptomatology of the carcinoid syndrome in patients with carcinoid tumors is related to the size of the serotonin pool.

Effects of a Unilateral Reduction in GFR on Sodium Reabsorption during Osmotic Diuresis. RICHARD M. STEIN, RUTH G. ABRAMSON, D. DANNY BERCOVITCH, AND MARVIN F. LEVITT,* New York, N. Y.

Hypertonic mannitol was administered at increasing rates to anesthetized hydropenic dogs. Constriction of one renal artery performed early in the diuresis produced unilateral reductions in GFR up to 60%. Thereafter, GFR remained stable in each kidney and as the rate of infusion was increased C_{osm} rose bilaterally (i.e., 3 to 20 ml per minute on the control side compared to 3 to 10 ml per minute on the constricted side). However, in successive collection periods obtained during the diuresis and at every level of C_{osm}, the urinary concentration of sodium per level of nonreabsorbed solute (U_{Na}/U_{osm}), $U_{Na}V$ and $U_{Na}V$/filtered Na remained distinctly lower on the constricted side. Thus, the enhanced gradient for sodium reabsorption provoked by a reduction in GFR could be maintained despite progressive increments in solute excretion and urine flow. Although sodium reabsorption was enhanced on the constricted side, U_KV per level of $U_{Na}V$ was comparable on both sides. Immediately after a decrease in GFR, $T^c_{H_2O}$ fell on the constricted side. As the diuresis proceeded, $T^c_{H_2O}$ in both kidneys increased toward maximal levels but thereafter often fell toward C_{H_2O}. However, in each collection period obtained during the diuresis $T^c_{H_2O}$ per level of GFR was distinctly different in the two kidneys. Early in the diuresis, as $T^c_{H_2O}$/GFR rose in both kidneys, levels on the constricted side were consistently lower than those noted on the control side. Later in the diuresis, when $T^c_{H_2O}$/GFR was falling on the control side, this parameter continued to rise on the constricted side to levels higher than those on the control side. These data suggest that an acute reduction in GFR does not, per se, decrease distal tubular permeability to water. Instead, this stimulus appears to enhance the gradient against which sodium can be reabsorbed in part within the proximal tubule.

Possible Identification of the Chromosome Bearing the Haptoglobin Locus. PARK S. GERALD,* SUSAN WARNER, JACK D. SINGER, PATRICIA A. CORCORAN, AND IRVING UMANSKY, Boston, Mass.

This investigation concerns a child with congenital malformations whose chromosome complement includes 46 chromosomes, one of which is a ring chromosome derived from a member of the "D" group. Since ring chromosomes are believed to be formed by loss of chromosomal material from the two ends of a chromosome (followed by union of the new ends of the chromosome to form a ring), the patient presumably has lost some of the genes present on one of his "D" chromosomes. Exhaustive investigation of blood groups of the patient and both parents has been carried out. No unexpected blood grouping results were found except in the haptoglobin (alpha chain) typing, for which an anomalous inheritance pattern was encountered. The haptoglobin results were: patient, Hp 1-1; mother, Hp 2-1; father, Hp 2-2. Haptoglobin subtyping was performed by an independent laboratory, and the patient was found to have only the fast type of haptoglobin alpha chains (mother, Hp 2-1^F). The failure of the child to inherit an Hp^2 gene from his apparently homozygous father could have resulted from the loss of this haptoglobin gene during formation of the ring chromosome. Nonpaternity could also explain the anomalous inheritance pattern but was not otherwise demonstrable. The blood groups of the child are such that only 0.6% of unrelated Caucasian males could have been her father. Anomalous inheritance of haptoglobins due to a silent gene has been described in two families, but correspondence with other investigators indicates that no further instances have been found and hence its frequency must be much less than 1%. We tentatively propose that the haptoglobin (alpha chain) locus is on the end of the long arm of a "D" chromosome.

The Effect of Vasopressin and of Theophylline on the Concentration of Adenosine 3',5'-Monophosphate in the Intact Urinary Bladder of the Toad. J. S. HANDLER, R. W. BUTCHER, E. W. SUTHERLAND, AND J. ORLOFF,* Bethesda, Md., and Nashville, Tenn.

It has been proposed that vasopressin increases the permeability of the toad bladder, and by analogy the renal tubule, by stimulating the production of an intermediate, adenosine, 3',5'monophosphate (cyclic 3',5'-AMP), which in turn is responsible for the permeability changes. Direct evidence in support of this thesis was obtained by measuring the effect of vasopressin on the concentration of cyclic 3',5'-AMP in the intact urinary bladder of the toad. In each of 15 paired experiments, arginine vasopressin (100 mU per ml) caused an increase (mean increase = 106%) in the concentration of cyclic 3',5'-AMP in the bladder. The response was evident as early as 3 minutes after addition of the hormone. This response to antidiuretic hormone is specific in that insulin, angiotensin, and alkali-inactivated vasopressin had no effect on the concentration of cyclic 3',5'-AMP in the tissue. Theophylline, an inhibitor of cyclic nucleotide diesterase, the enzyme that catalyzes the inactivation of cyclic 3',5'-AMP, also produced an increase in the concentration of cyclic 3',5'-AMP in the bladder. As would be expected, theophylline and vasopressin have a synergistic effect on the concentration of cyclic 3',5'-AMP. Since the physiological effects of cyclic 3',5'-AMP and of theophylline on the bladder resemble those of vasopressin (increased permeability to water and increased sodium transport), we conclude that vasopressin acts by stimulating the production of cyclic 3',5'-AMP in this tissue.

Treatment of Thyrotoxicosis with a Single Daily Dose of Propylthiouracil. MONTE A. GREER,* WALTER MEIHOFF, AND HUGO STUDER, Portland, Ore.

Antithyroid drugs have been an important agent in the treatment of thyrotoxicosis since their introduction by Astwood 20 years ago. However, one of the chief disadvantages to their use in long-term control of this disease is the widespread belief that the drug must be given at frequent intervals (usually every 8 hours) to maintain satisfactory control. Although such frequent dosage is rationalized by a presumed rapid inactivation of the drug, no adequate documentation of the necessity for frequent dosage has ever been made. Over the past 4 years we have routinely treated thyrotoxic patients selected for chronic therapy (1 year) with propylthiouracil, using several plans comparing the efficiency of a single daily dose of 300 mg with the same daily dose divided into equal 8-hour fractions. Eighteen patients achieved a satisfactory clinical remission taking a single daily dose, the majority in 3 months or less. In six patients initial control was achieved with divided daily doses, and control was maintained when they were given the same quantity in a single daily dose. Two patients were not controlled by a single daily dose after 3 to 4 months; these patients also did not respond to the same total daily dose given every 8 hours. In two cases treatment was more satisfactory with divided than with single daily doses. These results indicate that the administration of propylthiouracil in a single daily dose of 300 mg is adequate to produce a remission in the majority of thyrotoxic patients.

The Role of Connective Tissue in Pulmonary Mechanics. GERARD M. TURINO,* RUY V. LOURENÇO, AND GEORGE H. MCCRACKEN, New York, N. Y.

Normal pulmonary function is determined by the mechanical characteristics of pulmonary tissue. The contribution of connective tissue to these characteristics remains unknown. In this study the effects of altering elastin and collagen were determined in both the isolated trachea and in whole lung *in vivo*. Pressure-volume measurements of rabbit trachea ranging from collapse to maximal distension were made before and after exposure for 1 hour to .5% pancreatic elastase or .15% clostridial collagenase. Altering elastin markedly increases tracheal distensibility and collapsibility at transmural pressures of normal breathing but did not affect distensibility at high distending pressures; altering collagen increased distensibility only at high distending pressures but produced minor

effects at normal transmural pressures. Histology confirmed specific enzymatic effects. The effects of altering pulmonary elastin were studied in 800- to 1,200-g rabbits injected daily for 3 months with 5 mg pancreatic elastase. Controls received isotonic saline. Animals were studied under light anesthesia. Tidal volume and tracheal airflow were measured by pneumotachograph and intrapleural pressure by an intrathoracic needle. In 10 animals treated with elastase, lung compliance was increased and averaged 20 ml per cm water (SD ± 12) while five controls averaged 4 ml per cm water (SD 1.1). Nonelastic lung resistance was unaffected. Elastase-treated animals breathed with higher tidal volumes (average 24 ml, SD ± 9.0) and lower frequency (average 53, SD ± 21) than did controls, averages of 11, SD ± 13, and 82, SD ± 17, respectively. These results indicate that *1*) enzymatic degradation of connective tissue elicits characteristic changes in pulmonary mechanics *in vitro* and *in vivo*, *2*) the mechanics of the trachea over the range of physiologic pressures are determined by elastin rather than collagen, and *3*) altering pulmonary elastin *in vivo* increases pulmonary distensibility and alters the pattern of spontaneous breathing.

Membrane Surface Sulfhydryl Groups, Red Cell Survival, and Glucose Transfer. ROBERT I. WEED AND JOHN VAN STEVENINCK, Rochester, N. Y. (introduced by Scott N. Swisher).

The reactive sulfhydryl (SH^-) groups on the outer surface of the membrane of intact human red cells number 1 to 2×10^{-18} M per cell. This is approximately 5% of those in the entire membrane or 0.25 to 0.5% of the total cell SH^- content. This investigation was designed to define the importance of the outer surface SH^- groups for *in vivo* red cell survival and to relate their inactivation to membrane parameters measurable *in vitro*. *P*-hydroxymercuribenzoate (PMB) and chlormerodrin both permeate the intact red cell membrane slowly and are capable of producing generalized cation permeability at high concentrations. At lower concentrations they will produce increased K^+ efflux, inhibition of transport ATPase, and inhibition of transmembrane glucose transfer. Jacob and Jandl have demonstrated decreased survival with splenic sequestration associated with doses of PMB that also cause some increase in K^+ permeability. The membrane is almost impermeable to *p*-mercuribenzenesulfonic acid (PMBS), however, because of its marked water solubility, and thus PMBS reacts with only outer surface SH^- groups. PMBS does not affect ATPase in intact cells. Yet *in vitro* reaction of PMBS with the outer surface SH^- groups can produce up to an 80 to 90% inhibition of carrier mediated glucose transfer and produce marked shortening of red cell life span ($t_{\frac{1}{2}}$ of 9 days after exposure to 0.2 μmole per ml red cells to $t_{\frac{1}{2}}$ of 2.5 hours after exposure to 2 μmoles per ml red cells) with no evidence of splenic sequestration. These effects of PMBS indicate the critical nature of the 1 to 2×10^{-18} M per cell of surface SH^- groups necessary for glucose transfer and for normal red cell survival.

A Candidacidal Substance in Plasma. DONALD B. LOURIA * AND ROBERT G. BRAYTON, New York, N. Y.

A substance lethal for *Candida albicans* has been found in human plasma and serum. One million *Candida* cells are added to 1 ml of plasma and specimens rotated at 37° C. At 0, 6, and 24 hours, fungus populations are enumerated by pour plate techniques. A candidacidal effect of normal plasma, defined as a 10- to 100-fold reduction in populations, was found in 18 of 21 neonates, 7 of 9 infants under 1 year, 17 of 17 children, ages 1 to 9, 38 of 38 adults under age 50, and 27 of 30 over 50. No reduction in activity occurred in 35 patients with lymphoma, leukemia, or myeloma, or in 19 older diabetics. In contrast only 19 of 24 with carcinoma had the factor, as did 25 of 35 with liver disease, 9 of 17 diabetics under age 50, and 11 of 24 with nondiabetic azotemia. The most striking reduction was noted in patients with mucocutaneous or systemic candidiasis (5 of 30 had activity) and diabetes with azotemia (1 of 9 had the factor). The candidacidal substance is inactive against other *Candida* species and other yeasts. It is partially destroyed by heating to 60° C and is dialyzable. It is destroyed by trypsin and alcohol and does not appear in ultrafiltrates, suggesting a molecular size of 10,000 to 20,000. It travels with alpha and beta globulins on starch block electrophoresis. It is not related to agglutinating antibody, mycelial transforming factor, lysozyme, or transferrin, and is not influenced *in vitro* by glucose concentrations or the addition of antibodies or glucocorticoids. Twenty of 42 sera without candidacidal activity destroyed the candidacidal activity of normal serum. The interfering substance is heat labile and nondialyzable and migrates primarily with beta and gamma globulins. In one family, three siblings with congenital hypoparathyroidism-hypoadrenalism and superficial candidiasis had no candidacidal activity nor did one of the two apparently healthy, endocrinologically normal parents. We suggest that the presence or absence of this factor may determine susceptibility to *Candida* infections.

Erythrocyte Membrane Alteration Associated with Marrow Stress. ROBERT S. HILLMAN AND ELOISE R. GIBLETT, Seattle, Wash. (introduced by Clement A. Finch).

Erythroid marrow output is the product of the marrow capacity and the intensity of stimulation. A decrease in capacity or an increase in stimulus, or both, create a situation of marrow stress. In studies of conditions manifesting marrow stress, structural alterations in the red cell, as reflected by the interaction between the red cell membrane and the antibody anti-i, were observed. This increase in i-activity is normally limited to infant cells. The phenomenon was further examined in a patient whose erythropoietic rate was increased to two, four, and six times normal by repeated phlebotomy. The i-activity of the newly formed cells rose parallel with erythropoiesis. This study and observations made on blood of patients with thalassemia major, pernicious anemia, chronic blood loss, hypoplastic anemia, and hemolytic anemias indicate

that the membrane change persists throughout the lifespan of the cell and that it is not necessarily associated with ineffective erythropoiesis. Correlations with the erythrokinetics, erythropoietin, and fetal hemoglobin levels will be discussed. We postulate that red cell precursors, in situations of stress as defined above, differentiate and mature more rapidly than normal, and, in so doing, retain characteristics, such as i-activity, which are ordinarily lost with full maturation.

Decreased Plasma Clearance and Hepatic Extraction of Aldosterone in Patients with Heart Failure. CARLOS A. CAMARGO, ERNEST W. HANCOCK, ANNE J. DOWDY, AND JOHN A. LUETSCHER,† Palo Alto, Calif.

The plasma level of a hormone is regulated not only by its daily production but also by the rate at which it is degraded and removed from the circulation. The secretion rate, hepatic extraction, and metabolic clearance rate (MCR) of aldosterone were studied by infusion of 1,2-H^3-*d*-aldosterone in ten patients undergoing cardiac catheterization. A significant association between cardiac output at rest and MCR was found ($p < 0.03$). Four patients with severe heart failure and cardiac output between 1.7 and 2.2 L per minute per m^2 had subnormal plasma clearance of aldosterone (0.28 to 0.30 L per minute per m^2) associated with decreased hepatic extraction (50 to 75%). In three patients with normal cardiac output, the hepatic extraction of aldosterone was above 95% in one passage of blood through the liver. Low hepatic extraction was associated with a decreased oxygen saturation of the hepatic vein blood (28 to 58%). The secretion rate of aldosterone was within normal limits in eight of the ten patients studied and did not appear to be related to the severity of the heart failure. The estimated mean plasma level of aldosterone calculated as secretion rate/MCR was elevated (17 to 52 mμg per 100 ml) in half of the cardiac patients studied. The data suggest that decreased cardiac output, probably acting through a decrease in hepatic blood flow and ensuing hepatic anoxemia, results in a decreased hepatic extraction and plasma clearance of aldosterone. This may contribute toward maintenance of an elevated plasma level of the hormone even in the presence of a normal secretion rate.

A Role for Nasal Secretions in Allergy and Specific Host Immunity. JACK S. REMINGTON, KENNETH L. VOSTI, AND ARTHUR LEITZE, Palo Alto, Calif. (introduced by Lowell A. Rantz).

These studies revealed the presence of immune globulins in nasal secretions of normal and allergic individuals. Nasal secretions obtained from 67 human volunteers were dialyzed against distilled water and lyophilized. Immunoelectrophoretic studies of concentrated nasal secretions revealed $gamma_{1A}$ globulins and albumin in all. A more sensitive analysis using the Ouchterlony technique revealed the additional presence of $gamma_2$ globulins. Four peaks corresponding to $S_{20,w}$ values of 12.3, 6.2, 4.3, and 2.4 were present on Schlieren patterns obtained by ultracentrifugal analyses of pools of nasal secretions. A similar study of gamma globulins separated from nasal secretions revealed 2 peaks with $S_{20,w}$ values of 5.4 and 0.9. The concentrations of the immune globulins in samples of nasal secretions were compared to their concentrations in corresponding sera. $Gamma_{1A}$ globulins ranged from 2 to 10 times their concentrations in the corresponding serum and $gamma_2$ globulins from 0.5 to the same. Antibody activities of the gamma globulins of nasal secretions were studied by measurements of hemagglutinins for streptococcal M protein and for tetanus and diphtheria toxoids. When hemagglutinating antibody was present in nasal secretions, high levels were always present in the corresponding sera. Specificity of the serologic reactions was demonstrated by hemagglutination inhibition techniques. Nasal secretions and sera were collected from asymptomatic patients with known allergy and skin hypersensitivity to grass pollens. Reagenic antibody was demonstrated in their nasal secretions and sera by the passive transfer technique, but not in those obtained from nonallergic individuals. The presence of immune globulins with antibacterial activity in nasal secretions of normal and with reagenic activity in those of allergic persons suggests they may play important roles in local immunity and nasal allergy.

Studies of Hepatic and Plasma Triglyceride Turnover in Man. J. W. FARQUHAR, G. M. REAVEN, R. M. WAGNER, AND R. C. GROSS, Palo Alto, Calif. (introduced by Halsted R. Holman).

Hepatic-plasma triglyceride turnover was previously defined as a 2-pool, liver (precursor) to plasma (product) nonrecycling catenary system. In study of carbohydrate-induced lipemia (CIL), triglycerides of plasma lipoproteins (density <1.006) (VLD-TG) rose exponentially when influx from liver was >1.25 g per hour. Maximal removal rates before lipemia were similar, and the primary variable in cause of lipemia was therefore hepatic overproduction. Unique to man was the finding that rate constants of precursor exceeded those of newly synthesized plasma triglyceride (VLD-TG). Incorporation of glycerol-2-H^3 and of palmitate-1-C^{14} into liver triglyceride at t_{max} of plasma triglycerides was then studied (five experiments). Liver triglyceride pool size averaged 11 g, plasma 1.8 g; 83% of the hepatic pool turned over as a single precursor of VLD-TG. Therefore, from these data, the system is incompletely coupled, and only 6% of newly synthesized hepatic triglyceride (H-TG) was secreted into plasma. Caloric equivalents of triglyceride turnover within hepatic and plasma pools averaged 2,550 and 144 daily. In six individuals with CIL, VLD-TG turnover produced no higher than 600 potential calories, and hepatic turnover remained >80% of total. From palmitate studies, >75% of plasma FFA incorporates (at rest) into the hepatic pool, indicating that calculated intrahepatic triglyceride turnover is of proper magnitude. Fatty acids and glycerol released within liver from H-TG probably furnish the principal energy source for liver and may inhibit intrahepatic

carbohydrate oxidation, thus sparing glucose for export. Whereas 80% of H-TG glycerol was oxidized during one turnover, at least 50% of H-TG fatty acid recycles in this pool. These studies provide information concerning synthesis, hydrolysis, oxidation, and secretion rates of a large, rapidly turning over intrahepatic triglyceride pool. The proportion of H-TG secreted into plasma is also defined. These interrelationships influence hepatic and peripheral energy metabolism, lipoprotein secretion, and the genesis of fatty liver.

The L-Chain Types of Erythrocyte Autoantibodies. JOHN P. LEDDY AND RICHARD F. BAKEMEIER, Rochester, N. Y. (introduced by John H. Vaughan).

Human 7 S gamma globulin is composed of heavy (H) and light (L) polypeptide chains. The latter are of two distinct antigenic types, termed L_I and L_{II}. Normal individuals make gamma globulins having both type I (60%) and type II (30%) L chains. Antibodies of a given specificity isolated from normal persons have generally been reported to contain both L-chain types in varying ratios. Myeloma globulins and Bence Jones proteins (BJP) from a given patient possess L chains of entirely one type, perhaps reflecting their origin from a very homogeneous cell population. In the present study, the L chains of the 7 S gamma globulins coating the RBC of 12 patients with autoimmune hemolytic disease (AHD) were typed to determine whether they more closely resembled normal antibodies or myeloma proteins in this respect. Concentrated eluates from autosensitized RBC were used to sensitize normal RBC for antiglobulin reactions with rabbit antisera specific for type I or II BJP. Eight of these eluates produced sensitized RBC which reacted only with anti-BJP_I. Two eluates sensitized RBC to anti-BJP_{II} alone. The remaining two eluates sensitized RBC to both antisera. Incomplete anti-D antibodies reacted with both antisera. The four eluates available in sufficient concentration to be studied by agar gel diffusion gave patterns consistent with the hemagglutination data. When RBC were sensitized less strongly, as judged by reactions with antisera to whole 7 S gamma globulin, they sometimes failed to react either with anti-BJP_{II} or with both anti-BJP sera. Therefore, with apparent type I gamma globulin sensitization alone, very small amounts of type II antibody might have escaped detection. With such quantitative reservations, the data suggest that some autoantibodies to human RBC resemble paraproteins in L-chain structure; other autoantibodies may be more like normal antibodies in L-chain content. Autoantibodies apparently possessing only one type of L chain were found both in idiopathic and in symptomatic cases of AHD.

The Metabolism of Iodine during Normal Human Pregnancy. JOHN T. NICOLOFF, J. THOMAS DOWLING,* AND SHEROLD FISHMAN, Seattle, Wash.

The causes of goiter and thyroidal hyperfunction during human pregnancy are only partly understood. The hazards of fetal radiation have precluded the use of radioactive iodine to investigate them. However, the recent availability of the short half-life isotope, I^{132}, and low-level counting devices has allowed the following studies of 12 normal women, pregnant from 2.5 to 8.5 months. For 250 minutes after iv injection of 5.0 μc of I^{132}, thyroidal, urinary, and total body radioactivity was monitored. Sera were obtained for estimates of clearance values and serum iodide concentrations. Thyroxine (T_4-turnover was next determined by forearm counting twice daily for 7 days after administration of 0.2 μc of I^{131}-T_4. By these methods radiation exposure was 0.5% of that required for conventional studies. Total body iodide clearance (81 ± 10 ml per minute, mean and standard error) was greatly increased over values of nonpregnant controls (39 ± 2 ml per minute). Increased renal (46 ± 3 ml per minute) and thyroidal (20 ± 4 ml per minute) clearances accounted for this greater turnover. The increased renal clearance of iodide paralleled the augmented creatinine clearance of these women (131 ± 8 ml per minute). Calculated plasma iodide concentrations were reduced (0.22 ± 0.08 μg per 100 ml; control, 0.32 μg per 100 ml). Despite their increased PBI (7.1 ± 0.6 μg per 100 ml), a decreased fractional rate of T_4-turnover (10.1 ± 0.8% per day) led to a normal hormonal disposal (57 ± 5 μg I per day; control 51 μg I per day), thus confirming in normally pregnant women earlier observations made in the macaque and during pathologic pregnancies. The apparent thyroidal hyperfunction of pregnancy must result, therefore, from the profound hemodynamic alterations of the state which lead to increased renal iodide clearance and compensatingly increased thyroid function. Relative iodide deficiency may contribute to the goiter of pregnancy. If there is physiological significance to the increased PBI of pregnancy, it relates to altered distribution of hormone in the body rather than to increased homonogenesis and total body turnover.

The Relationship of Urinary Tract Infection to Urologic Surgery. ROGER P. KENNEDY, JAMES J. PLORDE, AND ROBERT G. PETERSDORF,* Seattle, Wash.

Of 69 patients undergoing prostatic resection (52 transurethral, 17 open), 39 had bacteriuria preoperatively while the urine was sterile in 35. Despite multiple blood cultures during and after operation, bacteremia was detected only three times, in every instance due to a break in operative technique. Two of these three patients were not infected preoperatively. Postoperative bacteriuria occurred twice as frequently in patients with carcinoma, concomitant debilitating diseases, and mechanical obstruction of the catheter as in those in whom these complications were absent. To evaluate the role of antimicrobials in preventing postoperative bacteriuria, 21 patients were treated with a drug giving high concentration in urine but not in tissue, 21 received an agent capable of achieving high levels in both tissue and urine, and 27 served as controls. In the group receiving only urinary antisepsis 10 had bacteriuria and 6 fever, whereas among patients receiving a drug achieving high

tissue levels only 4 had infection and 4 had fever. Among 27 controls, 17 had bacteriuria and 10 fever. Hospital stay was not reduced by prophylaxis. In a smaller number of patients, bacteriuria occurring at least 1 month after operation was the same in all groups. In 29 instances infections were clearly acquired in the hospital. To determine the origin of infecting strains, simultaneous cultures were obtained from urine and stool, and when found, *E. coli* were typed serologically. The stool flora of hospitalized patients consisted predominately of *E. coli* belonging in O groups 4, 6, 75, and 117, along with *Klebsiella-aerobacter, Proteus sp.* and *Pseudomonas.* Only 2 hospital-acquired infections were caused by other serotypes of *E. coli.* In 15 instances of nosocomial urinary infection, the organism was present in the stool before or at the time of infection, whereas in 14 the infection emanated from exogenous sources.

Clinical Expression of Coxsackie A-9 Infections by Age. ALVIN NOVACK, DOUGLAS W. VOTH, AND HARRY A. FELDMAN,† Syracuse, N. Y.

Extensive surveillance has revealed an iceberg-like picture of a Coxsackie A-9 epidemic which occurred in this community in the summer of 1963. A population of 33 families under continuous observation for respiratory disease was studied most intensively. Others studied were patients with aseptic meningitis admitted to City Hospital, children attending well baby clinics, and elderly residents of the county home. The first indication of infection in the families was a rash disease that began on July 2, 1963. This was limited to children under 12 years of age. In those less than 2 years of age, 79% had rashes. Coxsackie A-9 virus was isolated from one or more members of 28 families; 32 families had at least one person with virus or rash, or both. During the summer, 20 patients (6 months to 38 years) were admitted with aseptic meningitis. The spinal fluids of four and the throat of another were positive for Coxsackie A-9 virus. Of these 20 cases, 10 had A-9 infections by either virus isolation, antibody change, or both. Two of these cases (9 months and 24 years) had rashes. No significant antibody rises to A-9 were found in the sample of elderly persons (78+ years). Among 480 well baby clinic children (under age 7), 58% had antibodies for A-9. No one under age 7 in the family group had antibodies for A-9 in sera obtained before the epidemic. Coxsackie A-9 virus, which apparently had been absent from the community for about 7 years, caused significant morbidity in children and young adults. Rash was seen almost exclusively in young children.

The Augmentation of Proteinuria by an Acute Sodium Depletion That Stimulates the Secretion of Renin. LOUIS TOBIAN * AND POLLY NASON, Minneapolis, Minn.

Proteinuria increases temporarily in certain humans during orthostasis, physical exertion, recumbent pooling of blood in the legs, and congestive heart failure. The proteinuria of orthostatsis or pooling is frequently associated with some structural renal pathology. These four situations all augment the secretion of renin. Injections of renin greatly increase proteinuria in the rat even without raising arterial pressure. Thus, these four situations may produce temporary proteinuria by stimulating renin secretion. If this hypothesis were true, acute sodium depletion should enhance proteinuria since it stimulates renin secretion. This principle was tested in the rat, which has a slight proteinuria normally. Sodium depletion was accomplished by injecting a 10% glucose solution intraperitoneally (15 ml per 100 g body weight) and removing it 1 hour later. The injected solution always had concentrations of K, Mg, Ca, and HCO_3 similar to extracellular fluid to prevent depletion of these ions. The rats were fed a low salt diet after the dialyses. Ninety-three separate depletion dialyses were performed on 32 rats. The amount of proteinuria on the day following the depletion dialyses averaged 13.1 times as much as the proteinuria on the day preceding the dialyses. In these same 32 rats, nondepleting control dialyses were also done by injecting a fluid identical in every way except that the NaCl had been added up to 145 mEq per L. The amount of proteinuria on the day following 72 nondepleting dialyses averaged only 1.5 times that of the day preceding these dialyses. Thus, acute sodium depletion augmented proteinuria about ninefold over control dialyses ($p < 0.00001$). These results are compatible with the concept that situations which temporarily increase the secretion of renin tend to magnify proteinuria. In a kidney possessing the structural abnormalities of some human orthostatic proteinurias, lesser degrees of renin stimulation may be sufficient to enhance proteinuria.

Uptake and Metabolism of Glucose by the Ischemic Myocardium. NORMAN BRACHFELD AND JAMES SCHEUER, New York, N. Y. (introduced by Robert F. Watson).

An experiment was designed to evaluate the effects of myocardial ischemia on the uptake and metabolism of glucose. The left coronary artery in the open chest dog was cannulated and perfused from a reservoir of normoglycemic, fully oxygenated, arterial blood at normal pH. A hemodynamic steady state was established at normal coronary flow rates (mean = 82 ml per minute). Coronary arterial and coronary sinus (CS) samples were analyzed for oxygen, glucose, pH, lactate, and pyruvate. After a control period, perfusion was gradually reduced until an ischemic steady state was established (mean = 30 ml per minute) as evidenced by decreased oxygen consumption [control (C) = 10.4, ischemia (I) = 5.8 ml per 100 g per minute], atrial hypertension (C = 6.9, I = 25.0 mm Hg), and the appearance of a significant increase in CS (excess) lactate (% change = +116%, $p = 0.001$) despite fixed arterial lactate and pyruvate concentrations. Each animal served as its own control. When coronary flow was reduced, oxygen extracted rapidly became maximal and oxygen consumption and tension time index fell. These changes were disproportionate so that calculated myocardial efficiency was increased during ischemia. The coefficient of glucose extraction ($a\text{-}v/a \times 100$) increased 404% (C = 5.4%, I = 27.2%, $p < 0.001$). Since this in-

crease was proportionately greater than the reduction in flow, absolute glucose consumption also showed an increase (92% over controls). In the ischemic myocardium compensatory anaerobiasis acquired greater significance as an energy source and may be partially responsible for the apparent increase in calculated efficiency. The increased dependence on glycolytic pathways stimulated a marked acceleration of glucose uptake and utilization. This data is consistent with descriptions of increased glucose uptake by the isolated perfused rat heart and with quantitative conversion of extracted glucose to lactate in anoxic *in vitro* preparations.

A New Screening Procedure for the Detection of Galactosemia. ERNEST BEUTLER,* MARYELLEN C. BALUDA, AND GEORGE DONNELL, Duarte and Los Angeles, Calif.

The early diagnosis of galactosemia is of particular importance because the serious irreversible manifestations of this disease may be prevented by sufficiently early institution of a galactose-free diet. Galactosemia is associated with virtual absence of the enzyme PGal-uridyl transferase from body tissues, including the red blood cells. Definitive diagnosis of the disorder can be established by assay of this enzyme in the red cells, but such assay is an exceedingly expensive and tedious procedure. A simple visual screening method for the presence of this enzyme has been devised, based on the reduction of methylene blue. UDPG and Gal-1-P are provided as substrate. If PGal-uridyl transferase is present, glucose-1-phosphate is formed. This is changed to glucose-6-phosphate by phosphoglucomutase and serves as substrate for glucose-6-phosphate dehydrogenase and TPN. Methylene blue acts as a receptor dye. The reaction mixture contains Gal-1-P, 1.9 mM; UDPG, 0.39 mM; Tris buffer, pH 8.0, 110 mM; TPN, 0.042 mM, 1/7 saturated digitonin; and methylene blue, 0.57 mM. The reagents may be premixed and are relatively stable. One-tenth ml of blood is added to 0.7 ml of reaction mixture, the system is gassed for 2 minutes with CO, capped to exclude air, and incubated at 37° C approximately 10 cm from a 100-watt bulb in a gooseneck lamp (approximately 900 foot candles of light). All but very occasional samples of blood from presumably normal adults, children, newborns, and glucose-6-phosphate dehydrogenase-deficient Negro males decolorized dye in less than 30 minutes; galactosemic samples failed to decolorize dye even after several hours. Known heterozygotes tend to decolorize dye at a slower than normal time, but clear distinction from normal may not always be possible. Rare samples from a random population decolorized dye in considerably more than 30 minutes and could represent heterozygotes.

CSF—Blood Acid-Base Relationships in Respiratory Insufficiency. JEROME B. POSNER AND FRED PLUM,* New York, N. Y.

Paired arterial and CSF pH, P_{CO_2} and HCO_3^- levels were determined in 35 controls and seven patients with CO_2 retention. In all patients and some controls lactic acid concentrations were also measured. Five patients were studied serially as CO_2 retention was treated. Control determinations were similar to other published data: CSF pH was 0.1 U less than blood (7.311 ± 0.026 to 7.414 ± 0.023); CSF P_{CO_2} was 9.4 ± 4.5 mm Hg greater than blood (47.9 ± 5.7 to 38.3 ± 1.3); CSF HCO_3^- was 0.7 ± 1.5 mEq per L lower than blood (22.9 ± 2.3 to 23.4 ± 2.4). In six patients, the initial CSF-arterial P_{CO_2} difference was less than the mean difference of controls (less than 1 mm Hg in two patients). In the same six patients mean CSF HCO_3^- was 6.6 mEq per L less than blood, each value being more than 2 SD from the mean control difference. Despite the narrowed P_{CO_2} differences, the initial CSF pH in three patients was more abnormal than blood. CSF-arterial P_{CO_2} differences varied during treatment but tended to remain narrow. CSF HCO_3^- remained several milliequivalents per liter below blood until normal blood acid-base balance was restored. Correcting anoxia did not affect the CSF-arterial HCO_3^- difference. CSF lactate was not elevated. CO_2 retention is unique among the chronic metabolic acid-base disturbances described by ourselves and others in that pH alterations in CSF are often greater than those in blood. The present data suggest that CSF pH is partially protected by elevation of cerebral blood flow which narrows the CSF-arterial P_{CO_2} difference. This protective effect is counteracted by the absence of a CSF HCO_3^- rise comparable to that in blood. The failure of CSF HCO_3^- buffering is not explained by anoxia or by CSF lactic acidosis.

Effect of Ethanol on Human Polymorphonuclear Leukocyte Phagocytosis. PETER E. STOKES AND BETTY J. LASLEY, New York, N. Y. (introduced by Sidney Rothbard).

Human polymorphonuclear (PMN) leukocyte phagocytosis using PMN leukocytes obtained before and after administration of ethanol has been measured in 20 individual tests in normal volunteers or hospitalized alcoholics. Infusions of ethanol in saline were given, resulting in peak blood levels of 150 to 250 mg per 100 ml. Comparison of the phagocytic activity of harvested human PMN leukocytes was made between those leukocytes obtained before, at termination of, and after cessation of the ethanol infusion. Phagocytic activity was assessed utilizing bacterial death as an indirect measure of phagocytosis (Hirsch and others). Comparisons were made of colony counts observed on 24-hour incubation pour plates of the pre-ethanol and postethanol suspensions of harvested PMN leukocytes and added bacteria. The microorganism used (RIA) was known to be rapidly killed within PMN leukocytes. Duplicate tubes of each bacterial PMN leukocyte suspension, one rotated and the other stationary before plating, acted as suitable controls and served to rule out extracellular bacterial death. Additional observations were made coincidentally on stained smears using *Staphylococci* and *Monilia*. With both techniques used some diminution in human PMN leukocytic phagocytosis was observed after ethanol admin-

istration. The alterations were not significant according to the criteria published by Hirsch and others. *In vitro* exposure of human PMN leukocytes to ethanol in concentrations of 100 to 300 mg per 100 ml revealed no significant reduction in phagocytosis of RIA *Staphylococci* or *Monilia*. These findings suggest a minimal direct effect of ethanol on PMN leukocyte phagocytic activity in normal volunteers and alcoholics. These results are consonant with data presented previously from this laboratory in collaboration with Brayton and others, in that alcohol markedly impedes diapedesis of PMN leukocytes.

In Vitro Synthesis of Hemoglobins A and S in Heterozygotes: A Model of Gene Action. SAMUEL H. BOYER,* MICHAEL D. GARRICK, AND PETER HATHAWAY, Baltimore, Md.

Blood from sickle-trait individuals contains more hemoglobin A than S. Similar inequalities exist in other hemoglobin heterozygotes. Such inequalities may result from differences in *1*) quantity of messenger (m)-RNA, *2*) beta-chain synthesis, *3*) assembly of polypeptide chains into complete units, or *4*) survival of whole molecules. We have obtained evidence supporting possibility *2*, differential beta chain synthesis, as the responsible factor and also suggesting contribution by *1*. Washed cells from blood of sickle-trait subjects were incubated with H^3-leucine for 5, 10, 20, and 40 minutes and with C^{14}-leucine for 5 to 6 hours. After incubation samples of a single C^{14}-hemoglobin preparation were added to each H^3 preparation. These mixtures were purified by passage through Sephadex G-25 and Amberlite IRC-50. Further purification and separation of hemoglobins A and S were obtained by successive starch gel-starch granule electrophoresis. Disintegrations of H^3- and C^{14}-hemoglobin were assayed and results expressed as relative specific activity, i.e., H^3/C^{14}, where C^{14} provides an index of recovery. The ratio of relative specific activities (A*/S*) is always greater than unity but decreases over a 40-minute period. It is difficult to explain such results by possibilities *3* and *4*, since these mechanisms allow an alteration in the A*/S* ratio from unity but not the observed decrease of this ratio in short-term incubations. Possibility *2* is probable, since it permits decrease in A*/S* ratios with time. Possibility *1* is not excluded, since the specific activity (counts per minute per milligrams) of A always exceeds S. Such excess tends to become greater in reticulocytes aged *in vitro*. These results suggest that m-RNA for S is less stable than that for A. Additional types of experiments testing possibilities *1* and *2* will be presented.

Alterations in Phagocytic Function of Rabbit Exudate Leukocytes. PHYLLIS BODEL AND J. W. HOLLINGSWORTH,* New Haven, Conn.

Although the leukocyte's environmental factors clearly modify the efficiency of phagocytosis, little is known about changes in activity that may occur in the leukocyte itself during inflammation. Studies comparing the phagocytic abilities of circulating and exudate rabbit polymorphonuclear leukocytes (PMN's) were undertaken after the observation that exudate PMN's were markedly less phagocytic for *Listeria monocytogenes* than were blood leukocytes. Rabbit leukocytes were obtained from saline or glycogen-induced peritoneal inflammations and from heart blood. Suspensions of leukocytes were prepared by dextran-sedimentation of red cells, hemolysis of remaining RBC by brief exposure to hypotonicity, and washings with Krebs-Ringer phosphate buffer. Cells from all sources were treated identically. Samples containing equal numbers of PMN's were suspended in buffer containing 0.05% bovine serum albumin and incubated in a Warburg respirometer. Bacteria were added from the sidearm, and 1 hour later samples were removed, homogenized, and plated to determine total, supernatant, and cell-associated live bacteria. Circulating rabbit PMN's easily phagocyte and kill *Listeria* under these conditions. When exudate PMN's are employed, however, more than 100 times as many live bacteria are present after incubation. This difference results from both decreased phagocytosis and decreased intracellular inactivation of *Listeria* by exudate cells. A similar but smaller difference in phagocytic activity occurs when *Staphylococcus albus* is employed. Incubation of exudate cells in fresh serum before the washing procedure does not improve their phagocytosis of *Listeria*, nor does the addition of glucose or peritoneal exudate fluid to the incubation medium. Four-hour and 18-hour exudate cells have the same activity. These observations suggest that the leukocyte undergoes important alterations in function during transition from the circulation to an inflammatory exudate.

Demonstration of the Enzyme Defect in Xanthinuria. KARL ENGELMAN, R. W. E. WATTS, JAMES KLINENBERG, ALBERT SJOERDSMA,* AND J. EDWIN SEEGMILLER,* Bethesda, Md.

The discovery of xanthinuria in a patient who also had a pheochromocytoma with cardiomyopathy, mental deficiency, and multiple developmental somatic defects permitted extensive clinical and biochemical studies that have defined the basic nature of this disorder of purine metabolism. The presence of xanthinuria was suggested by the very low content of uric acid in plasma (0.4 mg per 100 ml) and urine (5 mg per 24 hours) and was confirmed by demonstrating an increased oxypurine content in plasma (0.5 mg per 100 ml) and urine (180 mg per 24 hours) of which 70% was xanthine and 30% was hypoxanthine. Administration of uric acid precursors resulted in formation of the oxypurines xanthine and hypoxanthine instead of uric acid, thus demonstrating a gross defect in uric acid synthesis in this patient. The xanthine pool size and turnover rate were measured by infusion of xanthine-6-C^{14}. Renal clearance studies revealed a normal mechanism for oxypurine clearance. Extremely sensitive radiochemical methods were developed to enable measurement of xanthine oxidase activity in biopsies of jejunal mucosa and of liver. The patient had a xanthine oxidase activity for both hypo-

xanthine and xanthine which was less than 0.1% of that of her normal mother or of control subjects. These studies have demonstrated that the basic defect in xanthinuria is a deficiency of xanthine oxidase activity. The possibility that the high plasma concentrations of xanthine may have potentiated the ability of catecholamines from the pheochromocytoma to produce a cardiomyopathy is suggested by some of the known biochemical effects of these two substances. Catecholamines stimulate the formation of 3',5' cyclic adenylic acid (AMP), which is necessary for the activation of cardiac glycogen phosphorylase, whereas xanthine inhibits the phosphodiesterase that destroys cyclic AMP.

Changes in Myocardial Enzyme Patterns in Heart Disease. SIGMUNDUR GUDBJARNASON AND CHRISTIAN DESCHRYVER, Detroit, Mich. (introduced by Richard J. Bing).

The purpose of this study was to investigate the possibility of altered myocardial metabolism in human heart failure. The activities of intracellular enzymes were determined in human autopsy material from patients with relatively normal heart muscle and patients who had suffered from congestive heart failure (CHF) or arteriosclerotic heart disease (ASHD). The enzymes chosen for this study were selected as representatives of three metabolic pathways: the Embden-Meyerhof pathway (lactate dehydrogenase [LDH], glyceraldehyde phosphate dehydrogenase [GAPDH], and aldolase [Ald.]), the citric acid cycle (malate dehydrogenase [MDH] and isocitrate dehydrogenase [IDH]), and the pentose phosphate shunt (glucose-6-phosphate dehydrogenase [G-6-PDH]). The activity of α-glycerophosphate dehydrogenase (α-GDH) was also measured. The results show a significant reduction in the activity of isocitrate dehydrogenase in cardiac muscle of patients with CHF and ASHD (20% of normal). The activities of LDH and Ald. were also decreased, whereas the activity of GAPDH was increased two to three times. These changes in enzyme activities of cardiac muscle could indicate impairment of oxidative metabolism and energy production in the myocardium and a compensatory increase in anaerobic phosphorylation.

Hemodynamic Effects of Endocardial Pacemaking in Patients with Complete Heart Block. ROBERT J. MARSHALL,* Morgantown, W. Va.

In eight patients, ages 63 to 78 years, with complete heart block a bipolar electrode catheter was inserted into the right ventricle before surgical implantation of a permanent pacemaker. Cardiac output was measured by the dye dilution method at the idioventricular rate (34 to 42 per minute) and at 5 to 7 different rates (48 to 140 per minute) temporarily induced by the pacemaker. Systemic arterial pressure, right atrial pressure, respiration rate, and an electrocardiogram were also recorded. Cardiac output increased from 2.30 L per minute at the idioventricular rate to 3.63 L per minute at rates of 60 to 80 per minute (mean values); at faster rates it tended to fall slightly while the stroke volume decreased sharply. At identical pacemaking rates the output was greater during exercise or infusion of isopropylarterenol than during rest. Changes in blood pressure were of particular interest. In four patients cyclical fluctuations of systolic and pulse pressure by as much as 48 and 40 mm Hg, respectively, occurred, especially at faster heart rates. There was an inverse linear relationship between systolic or pulse pressure and the duration of the preceding PR interval. In a fifth patient systolic pressure was constant, but the systolic ejection period was considerably prolonged in cycles in which the PR interval was optimal (0.1 to 0.2 second). Lesser cyclical variations in both pulse pressure and duration of systole occurred in three patients. Since variations in both parameters reflect variations in effective stroke volume, these studies confirm previous findings in blocked dogs that properly coordinated atrial contraction enhances ventricular function. Therefore a pacemaker activated by and appropriately timed after the P wave would be preferable to those now commercially available.

Insulin Effect on Hepatic Glucokinase: Studies of Mechanism. JOHN W. VESTER, Pittsburgh, Pa. (introduced by I. Arthur Mirsky).

Others have shown that insulin perfusion increases hepatic extraction of glucose *in vitro* and *in vivo*. Absence of a permeability block to entry of glucose into liver cells has also been shown elsewhere. We have shown that insulin is capable of direct action on hepatic glucokinase to produce an increase in the measurable activity of this enzyme. A particle-free supernatant fluid of a homogenate of normal rat liver is incubated 4 minutes with insulin. Then appropriate reagents and diluents are added so that TPNH production as measured by absorption of light at a wave length of 340 mμ is a function of glucokinase content. Preparations treated with insulin show reproducibly greater glucokinase activity than those identically treated with water instead of insulin. Appropriate controls have ruled out a nonspecific protein effect, and supernatant fluids from livers of alloxan diabetic rats do not respond to insulin. At all substrate levels the degree of increase is dependent on the dose of insulin used. Increasing substrate concentrations irregularly increase the degree of maximal stimulation. Results: glucose, 0.0083 M—no insulin, 0.71 $\pm$ 0.07 μmoles TPNH per g liver per minute ($n = 27$), + 300mU insulin per ml, 1.29 $\pm$ 0.18 ($n = 11$), 81% increase; glucose, 0.025 M—no insulin, 1.51 $\pm$ 0.08 ($n = 27$), + insulin, 2.41 $\pm$ 0.12 ($n = 9$), 59% increase; glucose, 0.05 M—no insulin, 1.63 $\pm$ 0.11 ($n = 24$), + insulin, 2.60 $\pm$ 0.15 ($n = 9$), increase 59%; glucose, 0.10 M—no insulin, 1.61 $\pm$ 0.08 ($n = 28$), + insulin, 3.40 $\pm$ 0.3 ($n = 10$), increase 111%. When a double reciprocal plot is constructed, Km is 1.6×10^{-2} M whether insulin is present or not. Slopes: no insulin, 0.0081; + insulin, 0.0042. These data suggest that insulin may increase the measurable activity of hepatic glucokinase by blocking the action of a noncompetitive inhibitor. The fact that 8×10^{-6}

M Versene produces an effect similar to insulin suggests that such an inhibitor could be a metal ion.

The Effect of Dietary Phosphate on the Intermediary Metabolism of Epiphyseal Cartilage from Rachitic Rats. ARTHUR S. KUNIN AND STEPHEN M. KRANE,* Boston, Mass.

Anaerobic glycolysis through formation of phosphopyruvate is required for the calcification of rachitic epiphyseal cartilage *in vitro*. This study was initiated to determine if vitamin D and phosphate, fed to phosphate-deficient rachitic rats, would alter the metabolism of this calcifiable tissue. Epiphyses from tibiae of young rats on low phosphate (0.1%), high calcium (1.2%), vitamin D-free diets were compared to those from normal rats on stock diets. In other animals 1.0% NaH_2PO_4, vitamin D (10 U per g diet) or phosphate plus vitamin D was added to the rachitogenic diet for 7 days. The florid rickets of the deficient animals was partially reversed by dietary phosphate and less readily by vitamin D. Animals were killed by cervical traction, and tibial epiphyses were removed, cleaned, pooled, blotted, and weighed before incubation. DNA per wet weight (mg per 100 mg) was 0.159 in epiphyses from rachitic, 0.334 from normal, 0.165 from vitamin D-fed, and 0.301 from phosphate-fed animals. Incubations were performed at 37° C in Krebs-Ringer bicarbonate buffer for 1 hour with either glucose-1-C^{14}, glucose-6-C^{14}, pyruvate-1-C^{14}, or citrate-6-C^{14} as substrates. Epiphyses from rachitic rats produced over 3 times more lactate-C^{14} from labeled glucose and pyruvate and lower ratios $C^{14}O_2$/lactate-C^{14} than did normal rat epiphyses based on DNA. Prior phosphate but not vitamin D administration to rachitic animals decreased lactate production towards normal. Whereas citrate contents were lower, production of $C^{14}O_2$ from labeled citrate was 7 times higher in rachitic than in normal epiphyseal cartilage. The addition of either vitamin D phosphate, or both, to the diet decreased citrate oxidation by rachitic epiphyses and increased the citrate content of this tissue. Pronounced alterations in intermediary metabolism occur in the calcifying epiphyseal cartilage of phosphate-vitamin D-deficient rats which are reversed by the addition of phosphate to the diet.

Sucrose Absorption in Man: Differential Absorption of Hydrolysis Products. GARY M. GRAY AND FRANZ J. INGELFINGER,† Boston, Mass.

Absorption of sucrose is believed to consist of hydrolysis at or within the brush border of the epithelial cell followed by the intracellular phenomena of glucose and fructose transport. Such monosaccharides as have been found intraluminally during sucrose absorption have been ascribed to intraluminal sucrase activity and regarded as of little significance. Twenty normal subjects were studied on 42 occasions with a double-lumen tube placed at various intestinal levels. Sucrose solution, 73 mM, made isotonic with NaCl, was perfused at 15 ml per minute through proximal lumen, and samples were collected 30 cm distally. Polyethylene glycol was the nonabsorbable marker. Sucrose was determined after invertase incubation from glucose liberated by Tris-buffered glucose oxidase, and total glucose plus fructose by a hexokinase-phosphohexose isomerase system; glucose was subtracted to determine fructose. Sucrose disappearance was accompanied by monosaccharide appearance in amounts that could not be explained by measured intraluminal sucrase activity (means: glucose 4 mmoles per hour; fructose 17 mmoles per hour). Although we have reported that sucrose absorption is more rapid from jejunal than ileal segments, monosaccharide appearance at these two sites was not significantly different. Since no significant sucrose is believed absorbed unsplit, sucrose disappearance was assumed to equal sucrose hydrolysis. Absorption rates of individual monosaccharide products were determined by subtracting glucose or fructose appearance from sucrose hydrolyzed. Sucrose absorption rates, in terms of potential hydrolytic products, were glucose 35 ± 12 mmoles per hour and fructose 21 ± 12 mmoles per hour. These amounts, as shown in 21 paired studies, were comparable to absorption rates from a solution containing 73 mM glucose and 73 mM fructose—glucose, 39 ± 11 mmoles per hour; fructose, 25 ± 7 mmoles per hour. Sucrose hydrolysis does not appear rate-limiting in the absorption process; sucrose absorption seems to be defined by individual absorption rates of its monosaccharide components, accounting for the differential intraluminal accumulation of glucose and fructose.

ADP Platelet Thrombosis. J. F. MUSTARD,* F. LOTZ, E. A. MURPHY, AND H. C. ROWSELL, Guelph and Toronto, Ontario, Canada.

Commonly in death attributed to vascular occlusion no thrombosis is found at autopsy. The observation that adenosine diphosphate (ADP) produces platelet aggregation has provided an important clue. We have found that iv ADP in swine (25 to 1,000 μg per kg body weight) produces widespread platelet thrombosis and enhances thrombosis from flowing blood in our extracorporeal shunt. The shunt thrombi do not reverse, but the vascular thrombi rapidly disintegrate. The platelets return to the circulation and survive normally. We have explored the pertinence of ADP thrombosis to the function of some vital organs. Intravenous ADP in pigs produces transient apnea, convulsions, precipitous fall in blood pressure, dropped beats, ischemic changes in the EKG, and marked thrombocytopenia. Tissues fixed immediately show widespread platelet thrombi in the small vessels in the lungs and heart. Within minutes as the thrombi break up, the platelet count, blood pressure, and usually the EKG return to normal. If beforehand the platelets are depleted, or treated so that they are clumped by ADP, the whole syndrome is prevented. Infused into the left ventricle or a main coronary artery, ADP causes a precipitous blood pressure fall, decreased amplitude, and ischemic changes in the EKG and platelet thrombosis throughout the small coronary vessels. Large iv doses of heparin do not prevent this.

Recovery occurs within minutes as the thrombi break up, but ischemic EKG changes may persist. Ventricular fibrillation and death may develop after all thrombi have disappeared. Infusion into the descending aorta produces only trivial circulatory changes. Adenosine and AMP infusions have little effect. Evidently ADP in amounts comparable to that available from injured tissue produces platelet thrombi, circulatory collapse, and even death and yet leaves little trace at post-mortem. The clinical implications are possibly important.

Lipid Composition of the Duodenal Mucosa in Normal Fasting Man. PETER WAYS AND CHERILL PARMENTIER, Seattle, Wash. (introduced by Robert H. Williams).

As a basis for investigating the intracellular phase of lipid absorption and chylomicron formation in man, tissue obtained from the fasting duodenojejunal mucosa by peroral biopsy was quantitatively extracted and its lipids analyzed. Chemical techniques, thin-layer, silicic acid paper, and gas liquid chromatography were employed. In 13 biopsy runs on ten individuals 1.5 to 11.3 mg of lipid was obtained, representing 3.0 to 4.4% (average, 3.8%) of the biopsy wet weight. The major constituents were phospholipid (average, 46.8%; SD, 7.8%), triglyceride (average, 20.2%; SD 5.8%), and cholesterol (average, 7.9%; SD, 1.3%). Small amounts of FFA, monoglyceride, and diglyceride were present. Of the major phospholipids (seven determinations) lecithin comprised 44%, phosphatidyl ethanolamine and phosphatidyl serine 19% each, and sphingomyelin plus phosphatidyl inositol 16%. The major fatty acids in the combined phospholipids were palmitic (18 to 29%), oleic (14 to 23%), linoleic (19 to 32%), and arachidonic (4 to 13%). In the triglycerides palmitic (22 to 36%), stearic (9 to 18%), oleic (27 to 47%), and linoleic (9 to 28%) acids predominated. In two experiments, tissues from three widely separated duodenojejunal sites were analyzed. Their fatty acid composition was found to be virtually identical, indicating that failure to biopsy the same region in each study could not explain the wide individual variations observed. In three experiments individuals were biopsied after 2 weeks of identical formula diets. Triglyceride fatty acids were then more similar from individual to individual (e.g., triglyceride linoleate was 26, 28, and 23%), but there was no less individual variation in the phospholipid fatty acid composition. These studies demonstrate that *1*) analysis of lipids in tissue obtained by peroral biopsy is feasible; *2*) individual variations in fatty acid composition of mucosal lipids are wide; and *3*) 2 weeks of dietary control partially equalizes triglyceride fatty acid composition but has no effect on variations in phospholipid fatty acid composition.

Repopulating Potential of Blood and Marrow. FRANK E. TROBAUGH, JR., AND JERRY P. LEWIS, Chicago, Ill. (introduced by Theodore B. Schwartz).

During studies of marrow preservation and dynamics of hematopoietic repopulation, we have made interesting observations concerning hematopoietic stem cells. We present evidence that *1*) stem cells exist in murine peripheral blood in concentration 1/100th that of marrow, *2*) stem cells from peripheral blood generate the same hematopoietic tissue as those from marrow, and *3*) stem cells differentiate predominantly along one cell line. After 750 r irradiation, isologous marrow or blood was injected intravenously into CAF_1/J mice. Hematopoietic tissue so injected forms grossly visible colonies on the spleen. Others have shown that each colony is a clone, developing from a single stem cell. We have previously demonstrated that the number of colonies is proportional to the repopulating capacity of injected material, providing an assay of viable stem cells. In the present investigation, statistical analysis confirms the validity of the bioassay and demonstrates that stem cells are 100 times more numerous among marrow cells than in peripheral blood leukocytes. The size, location, and exact cell type of colonies formed from peripheral blood were compared to those formed from marrow. From each of 254 spleens containing 2 to 30 colonies, more than 500 serial sections were cut, and every fifth section was studied. Of the marrow-induced colonies, 47% contained only erythrocytic elements, 22% only developing granulocytes, and 19% only megakaryocytes; 12% grew mixtures of these cell types. Colonies from blood were similar in all respects, including differentiation, to those from marrow. Addition of irradiated blood to injected marrow had no effect on colonization. Surface colonies consisted of either erythrocytic or granulocytic cells, but megakaryocyte colonies were uniformly deep in the splenic pulp. The identity of the hematopoietic stem cell remains enigmatic. These studies suggest that such cells are unipotential, at least from the time of seeding.

Extracellular and Intracellular Acid-Base Relations in Patients with Chronic Anemia. FELICE MANFREDI, Indianapolis, Ind. (introduced by Paul J. Fouts).

Chronic moderate to severe anemia may be associated at rest with findings of respiratory alkalosis in the extracellular fluid compartment (ECF), presumably resulting from protracted alveolar hyperventilation and attendant H_2CO_3 deficit. In this study ten nonedematous male patients between 41 and 74 years of age with chronic anemia of various origins (hematocrit ranging from 10 to 30%) were investigated to elucidate these acid-base disturbances in the ECF and, possibly, to educe similar alterations in the intracellular fluid compartment (ICF). For the ECF, hydrogen ion concentration, bicarbonate concentration, and carbon dioxide tension were determined from routine measurements on arterial blood; for the ICF, they were determined by measuring mean ICF hydrogen ion concentration with the use of the indicator 5,5-dimethyl-2,4-oxazolidinedione (DMO) assuming that ICF P_{CO_2} was equal to ECF P_{CO_2}. Ten nonedematous male subjects of comparable age (42 to 78), with normal measured acid-base parameters and with normal to high hematocrit levels (40 to 50%), served as control. In the anemic patients the ECF (sucrose space) was ex-

panded ($22.9 \pm 3.1\%$ of body weight, $p < 0.001$), and the plasma contained a slightly low H^+ concentration (37 ± 2 mμEq per L, $p < 0.10$), a low HCO_3^- concentration (21.5 ± 2.0 mEq per L, $p < 0.025$), and a low P_{CO_2} (35 ± 3 mm Hg, $p < 0.005$). The ICF (antipyrine space-sucrose space) was contracted ($33.4 \pm 4.0\%$ of body weight, $p < 0.10$) and contained a normal mean H^+ concentration (108 ± 34 mμEq per L) and a normal apparent HCO_3^- concentration (7.8 ± 2.2 mEq per L). These results suggest that in the steady state of chronic anemia, the acid-base relations of the intracellular fluid are maintained within normal range even when findings of respiratory alkalosis are present in the extracellular fluid. The water shift from the former to the latter compartment probably contributes to maintaining a normal osmotic equilibrium in the internal milieu.

The Biosynthesis of Small Polypeptides as Distinguished from Protein Biosynthesis. BERNARD MACH, New York, N. Y. (introduced by Grant W. Liddle).

The specific amino acid sequences of proteins are known to be under direct genetic control, and the successive reactions involved in protein biosynthesis have been well studied. It is not known, however, how smaller polypeptides' molecules are synthesized; these include many biologically active polypeptides such as hormones and antibiotics. Several aspects of protein biosynthesis were studied and compared with the biosynthesis of tyrocidine, a decapeptide antibiotic produced by *B. brevis*. The two processes could be clearly uncoupled, indicating that a distinct series of reactions, different from those involved in protein synthesis, are capable of directing the synthesis of polypeptide chains. Protein synthesis in *B. brevis* requires continuous RNA synthesis; the biosynthesis of tyrocidine, however, was unaffected by several agents that suppressed RNA synthesis. We have previously reported that the amino acid activation processes involved in the two types of synthesis were probably enzymatically distinct and that chloramphenicol and puromycin inhibited protein synthesis but not tyrocidine biosynthesis. Single amino acid substitutions observed in proteins (such as hemoglobin) are the result of alterations in the genetic material itself, and the recognition and incorporation of amino acids in protein biosynthesis are characterized by absolute specificity. Three single amino acid substitutions observed in the decapeptide tyrocidine were studied. It was found that these substitutions occurred as a function of the environmental concentration of the amino acids involved. This new type of amino acid replacement, which involves structurally related amino acids, is not under direct genetic control and implies a low specificity of the enzymes involved in the recognition of certain amino acids. The control of the sequence specificity of these active polypeptides is, therefore, somewhat flexible. One might speculate that the structural specificity (and therefore the functional specificity) of certain biologically active polypeptides depends in part upon environmental factors and is only indirectly under genetic control.

Microangiopathy and Intolerance to Carbohydrate in Idiopathic Edema. ETHAN A. H. SIMS, TAKESHI SHIRAI, AND BRUCE R. MACKAY, Burlington, Vt. (introduced by Paul H. Lavietes).

Many etiologic mechanisms have been suggested for the syndrome of idiopathic edema, the recurrent edema seen predominantly in emotionally labile females. Exaggerated orthostatic reflexes, with increased secretion of aldosterone, have been emphasized by some. Others have suggested a capillary abnormality with increased permeability, but this has not been demonstrated. We and others have noted a family history of diabetes more frequent than expected (five of eight in our series). We have therefore studied carbohydrate tolerance and ultrastructure of capillaries in this condition. Eight women with idiopathic, noncyclic edema, ages 29 through 49, were studied. Four were emotionally labile. Five were overweight. Five had had babies of excessive size. Two had abnormal oral glucose tolerances, and an additional three had abnormal cortisone-glucose tolerance tests (Fajans-Conn). Aldosterone secretory rates were measured under basal conditions to exclude primary hyperaldosteronism and were found within normal limits. Capillaries of the gastrocnemius muscle were studied by electron microscopy after embedding in araldite epoxy resin, and the size of the basement membrane was evaluated by two methods. The actual size of the capillaries taken for measurement was comparable in patients and in control subjects. The total mass of the basement membrane was estimated by calculating the ratio in six to twelve capillaries of the area of the basement membrane to the total area of the pericytes, endothelial cells, and capillary lumen. In four normal subjects this ratio was 0.22, 0.24, 0.21, and 0.28 (mean, $0.23 \pm$ SD 0.04). Six patients out of eight biopsied, all of whom had abnormal glucose tolerance or positive family history of diabetes, had ratios greater than 2 SD beyond the controls (0.36 to 0.82 with a mean of 0.47). A high incidence of thickening of the capillary basement membrane, of familial diabetes, and of decreased carbohydrate tolerance has been noted in six of eight patients with idiopathic edema. Although thickening of the basement membrane cannot be equated with capillary permeability, this alteration suggests that a capillary defect leading to increased translocation of fluid on standing may be a significant factor in idiopathic edema and may explain the secondary hyperaldosteronism that has been reported in this condition. The coexistence of microangiopathy and of impaired tolerance to carbohydrate suggests an etiologic interrelationship.

Reduction of Estimated Hepatic Blood Flow during Mild to Maximal Exercise in Upright Man. LORING B. ROWELL, JOHN R. BLACKMON, AND ROBERT A. BRUCE,† Seattle, Wash.

The supplemental role of the hepatic-splanchnic circulation in man has been assessed during upright exercise requiring from 26 to 97% of the maximal oxygen intake. Initially the disappearance rate of indocyanine green

(ICG), which is extracted exclusively by the liver, was determined repeatedly in ten normal young men during rest and exercise and was found to be inversely proportional to oxygen intake (milliliters per kilograms per minute) ($r = -0.77$), particularly when oxygen intake was expressed as percentage of maximal ($r = -0.89$). At rest, disappearance rates averaged 23.5% per minute ($t_{\frac{1}{2}} = 3$ minutes) and fell to 4.3% per minute ($t_{\frac{1}{2}} = 16$ minutes) during near-maximal exertion. The studies were repeated in five normal young men and two class I cardiac patients. Catheters were passed into the radial artery, axillary vein, and a hepatic vein. Estimated hepatic blood flow (EHBF) was determined by the single injection technique using ICG. Also hepatic a-v oxygen difference was determined. At rest EHBF averaged 1,614 ml per minute; during various intensities of exercise EHBF ranged from 820 to 390 ml per minute. Percentage changes in EHBF estimated from percentage changes in ICG disappearance rate underestimated true alterations by only 8.6%. Hepatic extraction efficiency for ICG increased slightly from rest (0.77) to exercise (0.84). Changes in EHBF were also estimated from changes in a-v oxygen difference and agreed to within 2% of the true percentage alteration. Hepatic oxygen consumption averaged 68 and 69 ml per minute during rest and exercise, respectively. Thus, the hepatic-splanchnic circulation is heavily compromised during exercise providing in excess of 1,000 ml of blood to working muscle when total blood flow becomes inadequate to meet completely oxidative demands of muscle.

Hormonal and Nonhormonal Factors in the Renal Excretion of Calcium, Magnesium, and Phosphorus. C. R. KLEEMAN,* S. LING, D. BERNSTEIN, AND M. H. MAXWELL, Los Angeles, Calif.

Hormonal and nonhormonal factors must be integrated in the regulation of calcium (Ca^{++}), magnesium (Mg^{++}), and phosphorus ($PO_4^{=}$) excretion. Studies were done in normal trained dogs before, immediately after parathyroidectomy, and days to weeks postoperatively. Renal clearance of sodium, and diffusible Ca^{++}, Mg^{++}, and $PO_4^{=}$, were simultaneously measured in all studies. The ratio, diffusible and free Ca^{++} ion: total serum Ca^{++}, was unchanged regardless of the total serum [Ca^{++}]. Ratios for diffusible Mg^{++} and $PO_4^{=}$ were variable and unpredictable. Parathyroidectomy caused 3- to 4-fold increases in Ca^{++} and Mg^{++} clearance in 5 hours despite falling filtered loads and falling sodium excretion. $PO_4^{=}$ clearance decreased to one-fifth of control. While each dog was normal, chronically hypoparathyroid, or receiving parathyroid hormone (PTH), the filtered load of Ca^{++} was varied by calcium chloride infusion. This showed that decreasing endogenous PTH secretion during Ca^{++} infusion contributed significantly to the acute increase in Ca^{++} clearance. Ca^{++} infusion caused a significant decrease in $PO_4^{=}$ clearance, even in hypoparathyroid dogs. At all filtered loads Ca^{++} clearance varied inversely with the level of PTH and, except at very low sodium, or Ca^{++} clearance, the clearance of these ions paralleled each other. During sustained hypercalcemia ($CaCl_2$) infusion, 25 to 40% decrease in GFR by aortic occlusion caused Ca^{++} clearance to approach zero regardless of maintained excretion of sodium or level of circulating PTH. Hormonal and nonhormonal factors are critically integrated in the renal regulation of divalent ion excretion.

Implication of the Kallikrein System in Production of the Carcinoid Flush. KENNETH MELMON, WALTER LOVENBERG, JOHN A. OATES, LOUIS GILLESPIE, JR., AND ALBERT SJOERDSMA,* Bethesda, Md., and Nashville, Tenn.

Previous studies have cast doubt on the role of serotonin in the flushing of carcinoid patients. The findings of a vasoactive peptide in blood during flushing and a peptide-forming enzyme (kallikrein) in hepatic metastases in several patients suggested another flush mediator. Studies were undertaken to identify the peptide formed *in vitro* during incubation with tumor enzyme, to characterize the peptide formed *in vivo,* and to detect the enzyme in arterial blood during epinephrine-induced flushing. Kallikrein was extracted from 0.5 g of tumor, purified 40 times, and incubated (pH 7.2, 37° C, 2 hours) with an excess of substrate (kallidinogen) prepared from human plasma. The product formed, equivalent to about 700 μg bradykinin by assay on isolated estrous rat uterus, was purified by serial chromatography on DEAE-cellulose, carboxymethyl-cellulose, and Sephadex (G-25). A major peak of biologic activity was recovered, and the material isolated had a mobility similar to that of the decapeptide, lysyl-bradykinin (kallidin II), on high voltage electrophoresis and an identical amino acid composition as shown by Stein-Moore analysis. Isolation of the peptide in 30 ml of hepatic venous blood obtained from a patient during flushing indicated chromatographic characteristics and biological activity similar to that of the nonapeptide, bradykinin. This finding is explained by studies showing rapid conversion of lysyl-bradykinin to bradykinin in blood. Since it was suspected that release of kallikrein from the tumors might be the initiating event in flush reactions, the arterial levels of this enzyme were measured during induced flushing. Seven- to 10-fold increases over control levels were observed in three patients. The kallikrein system may function in several other clinical states accompanied by cutaneous flushes, as it apparently does in carcinoid patients.

Use of Radioisotopic Renal Function Studies to Select Patients for Surgery in Renal Arterial Stenosis. MELVIN H. FARMELANT, CHARLES E. SACHS, GERALD J. HINE, AND BELTON A. BURROWS,* Boston, Mass.

Normotension is achieved in about half of reported hypertensive patients with renal arterial stenosis undergoing surgical repair or nephrectomy. The status of renal function, determined preoperatively, might be used to select patients for surgery. The descending slope of renal radioactivity after iv injection of I^{131}-Hippuran or I^{131}-Diodrast with carrier Diodrast was determined by external measurement. The first 3 to 5 minutes of this

slope in hydrated patients approximates an exponential which can be expressed as a half-time of disappearance ($t_{\frac{1}{2}}$). In dogs studied at differing renal blood flows produced by constriction of a renal artery or uninephrectomy, excellent correlation between degree of reduction of C_{PAH} and prolongation of $t_{\frac{1}{2}}$ was observed. Preoperative radioisotopic renal function studies were available in 45 hypertensive patients with proved renal arterial disease who had uninephrectomy (35) or arterial repair (10). Other patients in whom arterial repairs later thrombosed were excluded. Twenty (45%) became normotensive, whereas 25 (55%) showed no or only partial response at follow-up of 1 to 4 years. Half-time of the nonaffected kidney was between 2.6 and 4.2 minutes in 19 of the 20 patients who became normotensive; the other, who was sodium restricted, had a $t_{\frac{1}{2}}$ of 5.0 minutes. The 25 who remained hypertensive had $t_{\frac{1}{2}}$ values of 4.4 to over 20 minutes. Other observations, such as serum creatinine, BUN, 15-minute PSP excretion, patient age, and extent of vascular lesion were comparable in both groups. The duration of hypertension did correlate with surgical response but with considerable overlapping. These findings indicate that blood pressure response to surgery in renal arterial stenosis depends on the renal functional status, which can be determined by analysis of radioisotopic renal function studies to select patients for surgical treatment.

Primary Role of Hepatocellular Damage in the Genesis of Hyperammonemia as Demonstrated in Patients with Schistosomiasis. KENNETH S. WARREN, GILBERTO REBOUÇAS, AND AUGUSTO GENTIL BAPTISTA, Bahia, Brazil (introduced by Dieter Koch-Weser).

The hyperammonemia accompanying liver cirrhosis is believed to result from bypass of the hepatic parenchyma by portal-systemic collateral circulation rather than from inability of the parenchyma to detoxify ammonia. Thus oral ammonia tolerance tests and ammonia determinations during upper gastrointestinal bleeding are considered indicators of portal systemic shunting, not measures of liver function. These opinions are based on data obtained largely from patients with cirrhosis who have both generalized hepatocellular damage and shunting. The present study of ten patients with hepato-splenic schistosomiasis mansoni who had extensive shunting but good liver function has yielded results which conflict with the above-mentioned concepts. These patients were in good physical condition, had no stigmata of liver parenchymal disease, and had normal average serum albumin and bilirubin concentrations. BSP retention averaged 6.5%. All patients had previous hematemeses and had extensive natural portal-systemic shunting as revealed by esophagoscopy and splenoportography. Ammonia studies revealed: *1*) Arterial concentrations during ammonia tolerance tests within normal limits in 70% of the patients; *2*) average venous concentrations within normal limits throughout the day while the patients were receiving 120 g of protein and oral NH_4Cl; *3*) a normal response to a 1-hour iv NH_4Cl infusion. Moreover, six other patients had normal arterial ammonia levels during severe episodes of bleeding from esophageal varices. Clinical signs of hepatic coma and EEG changes were never observed under any of the above conditions. These occurred only after surgical shunting, when liver function deteriorated and hyperammonemia appeared. These studies have revealed the primary role of hepatocellular damage in the pathogenesis of hyperammonemia and hepatic coma in patients with nonsurgical portal-systemic shunting.