PROCEEDINGS OF THE FIFTY-SECOND ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD IN ATLANTIC CITY, N. J., MAY 2, 1960

PRESIDENTIAL ADDRESS

PROBLEMS OF THE SOCIETY, 1960

BY ROBERT W. BERLINER

It is one of the ancient and, to the victim, barbaric customs of this rite of spring for the president to address the members of the Society on some aspect of the current scene in clinical investigation. The message is then recorded in the Journal of the Society where it is available for posterity, or rather for later presidents in search of suitable quotations pertinent to their own addresses. I had thought that the subjects I plan to discuss were of only immediate interest and too topical to have any significance beyond the next year or so. However, a reading of the recently compiled history of the Society indicates that these same subjects have been the center of discussion almost since the Society was organized. These two matters—the form of the meeting and the criteria for the election of new members—again demand the attention of the membership. The considerations that influence our attitudes on these questions today may differ from those which have been debated in the past and from those which will determine the position of the Society in the future. Nevertheless, we must make decisions based on the current situation, recognizing that they will require continuous reconsideration and possible revision in the future.

Since action on each of these matters is, at this time, required of the Council and the membership, it seems appropriate that the presidential address be devoted to an attempt to clarify the issues involved. The time available for the transaction of business at our annual meeting is sufficiently limited to preclude much adherence to the forms of democratic procedure. Nevertheless, the officers of the Society act in the conviction that they understand and represent the will of the majority of the members and, although the formal opportunities for the members to make known their wishes are few, the informal occasions are many. It is in this spirit that I place before the Society my personal convictions on the problems requiring decision.

As you may know, three years ago the president appointed a committee of the Council to meet with representatives of the Federation for Clinical Research to arrange for the conduct of section meetings on various subspecialties of clinical investigation. The committee was instructed to plan the meetings, in cooperation with the Federation if possible but separately if necessary, and to arrange that the section meetings should not conflict with the general scientific program of the Society on Monday. Agreement was reached, and jointly sponsored meetings were set up for Sunday afternoon and, in some subspecialties, Sunday evening. In order to make this possible, the Federation gave up its Sunday afternoon general meeting. It was also agreed that following a reasonable trial of this arrangement there would be a review to determine whether changes were indicated. A possible expansion of the section meetings was to be subject to such a review. It was conceded, without commitment, that the Society might entertain the possibility that such expansion would replace part of its Monday general scientific session. The time for such a review is at hand and I feel it appropriate to indicate to the members of the Society the possible factors upon which our decision should be based.

I believe it safe to say that the section meetings have been highly successful. They have permitted the presentation of a considerable number of papers which, because of the limitations of time, could not have been included in the general programs of the two societies. More important, they have permitted the inclusion of a number of excellent papers which, because they deal with technical matters of a highly specialized nature, would be inappropriate for a general audience, but are received with interest and insight by workers in the same field. Discussion is far less inhibited by the smaller audience and the much less overwhelming size of the meeting halls and is encouraged by the greater community of interest and understanding.

As to the increase in the number of papers presented, those submitted to the Society have fared somewhat better both absolutely and relatively than those submitted to the Federation. The section meetings have permitted the presentation of more than twice as many of the papers submitted to the Society: just under one of every three papers offered finds a place on one or another program. Because of the sacrifice of part of its general session, there is a smaller increase in the number of papers accepted for the Federation program. In addition, because of a continuing rapid increase in the number of abstracts submitted to the Federation, only one in every six or seven is actually presented.

It is always difficult to evaluate, from an abstract, the work which it represents, but it is my feeling and the considered opinion of the chairmen of the various section meetings, both this year and last, that, except for two of the sections, the programs could not be appreciably expanded without reducing their present high level. Since three of the sections now meet only in the afternoon, additional programs could be fitted into the even-

ings by further subdivision of the more prolific special areas, but it must be conceded that the evening sessions are considerably less satisfactory than those in the afternoon.

This, then, is the background upon which a decision concerning the future of the section programs must be based. I, personally, am opposed to their expansion. There is today, as there was when Dr. Meltzer delivered the first presidential address to the Society, cause for concern about the science of clinical medicine. The basis for this concern is, however, entirely different. The establishment of the science of clinical medicine as a branch distinct from its practice has long since been accomplished. Today we find clinical investigation grown so massive that it has become subdivided into many smaller branches, each of which has tended to fuse back with the corresponding basic science, thus leading not only to a widening separation between the science and practice of medicine, but also to an increasing individualization of the specialized investigative areas. The growing complexity of technology as well as the snowballing accretion of information in each field makes it virtually impossible for an individual to be expert in more than one. Nevertheless, few of us can afford ignorance in fields outside our own, and a single day's exposure to a reasonable sampling of the better work in clinical investigation is all too inadequate.

That only a minority of papers submitted finds a place on the program is, perhaps, unfortunate, but so long as this fact does not discourage submission of excellent material for the program it should not be an important consideration. There is, these days, no lack of opportunity for communication. The ease and speed of travel and the availability of funds to support it has led to such a massive proliferation of meetings, conferences and symposia that one wonders when the participants will find the time to get any new work done. It is, perhaps, a self-limiting disorder since those most involved must eventually run out of things to talk about. In any case, we need not feel that limitations on the size of our programs significantly diminish the service to our members and guests. I believe our aims are best served by continuing to hold a program of the highest quality and limited size and continuing to provide the opportunity for less formal communication, both technical and otherwise, which traditionally goes on outside this hall.

The other problem that the Society must face is one for which the solution is less apparent and which will not be adequately handled by simple maintenance of the *status quo*. That problem concerns the election of new members. The problem has been very clearly outlined by Dr. Dole and his associates in the material circulated in support of the constitutional amendment which they have proposed. The amendment would eliminate from consideration all candidates for election to the Society who have reached the age of 40. If the amendment alone or in combination with a moderate increase in the rate of election of new members would restore the balance between candidates proposed and those elected, it would be a welcome forward step. However, I do not believe that it would adequately meet this problem. It is true that there would be a sharp decrease in the candidates for election next year if all those over 40 were dropped from consideration and their elimination from consideration were on such an arbitrary basis that it could not be considered a reflection on them personally. Of the 193 candidates for election this year, 86 would not be eligible for consideration next year solely on the basis of age. An additional 34 candidates from the group nominated in 1958 would either have been elected or dropped from consideration, bringing the total number carried over from this year to something under 73 and most likely around 50. If we could then anticipate next year a number of new proposals not greater than the average of recent years, the list to be considered would be at its lowest level in the last ten years. However, I doubt that this sitaution could last very long. An extraordinarily large number of candidates was proposed in 1958—so many, in fact, that even after reduction of the list by two previous elections there were still more candidates up for election this year from those nominated in 1958 than the totals proposed in either 1959 or 1960. Furthermore, of the candidates proposed this year the unusually large number of 26, or 39 per cent, would not be eligible next year under the proposed amendment, 18 of these 26 being already past the age of 40. Thus the number who would be dropped this year is unusually large in several respects. It seems clear that the membership, anticipating possible adoption of the amendment, has proposed the names of a large number of candidates for whom it might be the last chance. It seems virtually certain, however, that in the future, in view of the limited period of eligibility, candidates will be proposed in equal numbers but at an earlier age, when it may be even more difficult to evaluate their independent contributions.

In seeking a satisfactory policy for the election of new members, it is essential that we have a clear idea of what we wish to accomplish by that policy. In other words, what is to be the significance of membership in the Society? It seems to me that election to membership represents primarily recognition of the accomplishment of significant original and independent research in clinical and related fields. It seems, furthermore, that election to membership is, more or less, an end in itself. The rights and privileges which pertain to membership are otherwise relatively trivial. A regrettably small minority is privileged to hold office or serve on the committees of the Society and thus to play an active role in the conduct of its business. While only members, active or emeritus, may introduce papers at the meetings, it is unusual for any investigator with worthy material to be unable to find a sponsor. As for the sponsorship of candidates for membership, the problem is only too clear. I therefore find it difficult to believe that most candidates over the age of 40 have failed of election only because the Councils have preferred persons who could participate as active members for five or more years.

If election is to be considered recognition of accomplishment in research, however, it behooves us to make certain that those elected are those whose accomplish-

ments best warrant the distinction. If we can have complete confidence that this is the case, we can live with any arbitrary limitation of the number elected and not feel that any injustice has been done those rejected, although we should certainly adjust the arbitrary limitation to the number of really highly qualified candidates. The question we then must ask ourselves is whether our evaluation of candidates is adequate to provide complete confidence that those most qualified have been elected. I must frankly admit that I do not feel that our present system uniformly warrants such confidence. Let me hasten to say that I believe the Council makes every effort to base its decisions solely upon the significance, originality and independence of an individual's contributions. But the ability of the Council to evaluate these on the basis of the information available to it is limited. The high degree of specialization of which we spoke earlier makes it unlikely that any Council member will be familiar with the details of the work of many candidates outside of his own field of interest. The short time available and the large number of candidates to be considered make it difficult for most Council members to read more than a small selection of the papers of many of the candidates. Consequently they must rely largely upon the letters of the nominators, which rarely are restrained in their support of the candidates, upon an attempt to evaluate the bibliographies from the titles of the papers and the reputation of the journals in which they appear, upon the advice of colleagues who may be familiar with the work of some of the candidates, and upon the letters received from members. The latter are not too helpful, since they so rarely offer a comparative evaluation of various candidates and so often represent a good word for a nominee solicited by his sponsors. I have painted the worst side of the picture. Despite the limitations, I believe the Council makes the best of a very difficult situation and, by and large, recommends for election each year those best qualified for membership. However, I feel that the process could be considerably improved, the burden on the Council somewhat relieved, and confidence in the adequacy of the selection process strengthened. This end might be served by the appointment of a qualifications committee, composed of several representatives of each of the various specialized areas of investigation. Such a group might review the work of each candidate in sufficient detail to be able to provide to the Council an evaluation based upon a familiarity both with the field and the standing of the candidate within it. The final selection of those to be recommended to the Society for election would remain the responsibility of the Council, which would not necessarily be bound by the recommendations of the committee and would be expected to maintain a reasonable balance among the various fields of investigation. The number of individuals recommended by the committee as highly qualified might also be of value in providing to the Council some guide as to the desirable annual rate of election. Whether or not such a procedure is adopted I feel that it is essential that the nomination date be advanced so as to provide, to those who are to evaluate the candidate, sufficient time to familiarize themselves more thoroughly with his work. With some such assurance of the adequacy of our selection process and with a clear understanding among the proposers of new members of the criteria for election, we need have little concern about the number or age of the candidates for election.

PAPERS PRESENTED AT THE FIFTY-SECOND ANNUAL MEETING 1960

1. The Induction of Heterologous Immunity to Influenza by Aerosol Administration of Inactivated Virus. JEROME L. SCHULMAN and EDWIN D. KILBOURNE,* New York, N. Y. (1026)

2. On the Role of Virus Sulfhydryl Groups in the Attachment of Enteroviruses to Cells. LENNART PHILIPSON and PURNELL W. CHOPPIN, New York, N. Y. (introduced by Igor Tamm). (1017)

3. The Capacity of Tubercle Bacilli to Assume a Latent State *In Vivo*. ROBERT M. MCCUNE, New York, N. Y. (introduced by Walsh McDermott). (1009)

4. Studies on the Mechanism of Fever Following Intravenous Inoculation of Staphylococci. ELISHA ATKINS * and LAWRENCE R. FREEDMAN, New Haven, Conn. (969)

5. Relationship of Phagocytosis to the Fall in Spinal Fluid Glucose in Experimental Meningitis. ROBERT G. PETERSDORF, Baltimore, Md. and Seattle, Wash. (introduced by W. M. M. Kirby). (1016)

6. Studies of Granulocyte Kinetics. JOHN W. ATHENS, ALVIN M. MAUER, SPENCER O. RAAB, OTTO P. HAAB and GEORGE E. CARTWRIGHT,* Salt Lake City, Utah. (969)

7. Presence of an Altered Amino Acid Sequence in Hgb M (Boston Type). PARK S. GERALD, MARY L. EFRON and MARGARET J. PEASE, Boston, Mass. (introduced by Louis K. Diamond). (989)

8. Oxidative Hemolysis and Precipitation of Hemoglobin: Heinz Body Anemias as an Accelerated Form of Red Cell Aging. JAMES H. JANDL * and DAVID W. ALLEN, Boston, Mass. (1000)

9. The Enzymatic Defect of Orotic Aciduria. LLOYD H. SMITH, JR. and CHARLES M. HUGULEY, JR., Boston, Mass. and Atlanta, Ga. (introduced by Anne P. Forbes). (1029)

10. Relation of Hormone-Receptor Bonding and Antidiuretic Action of Vasopressin. IRVING L. SCHWARTZ,* HOWARD RASMUSSEN, MARY ANNE SCHOESSLER, CONRAD T. O. FONG and LAWRENCE SILVER, Upton, N. Y. (1026)

11. Micropuncture Study of Net Transtubular Movement of Water and Urea in the Rat Kidney. WILLIAM E. LASSITER, CARL W. GOTTSCHALK * and MARGARET MYLLE, Chapel Hill, N. C. (1004)

12. Effect of Acute Uremia on Arterial Blood Pressure in Rabbits. CARMELO GIORDANO, HERMAN WEINSTOK and JOHN P. MERRILL,* Boston, Mass. (990)

13. A "Precursor Solution" of Pancreatic Juice: Evidence for an Exchange Mechanism. H. D. JANOWITZ * and D. A. DREILING, New York, N. Y. (1000)

14. The Effect of Wheat Instillation into the Proximal Ileum of Patients with Idiopathic Sprue. CYRUS E. RUBIN, LLOYD L. BRANDBORG, ARNOLD L. FLICK, CHERILL PARMENTIER, PATRICIA PHELPS and SALLY VAN NEIL, Seattle, Wash. (introduced by Clement A. Finch). (1023)

15. The Effect of Changes in Atrial Systole on the Relation Between Mean Atrial Pressure and Stroke Work. STANLEY J. SARNOFF,* JERE H. MITCHELL and JOSEPH P. GILMORE, Bethesda, Md. (1025)

16. Metabolic Regulation of Cardiac Output. WILLIAM E. HUCKABEE,* Boston, Mass. (998)

17. On the Mechanism of Carotid Sinus Function. LYSLE H. PETERSON,* ERIC O. FEIGL and PETER GOURAS, Philadelphia, Pa. (1017)

18. Effect of Antihypertensive Treatment in the Rat on the Potentiation of Atherogenesis by Experimental Hypertension. QUENTIN B. DEMING,* MARIE M. DALY, JAIME BLOOM, LILI BRUN and RUTH KAPLAN, New York, N. Y. (980)

19. Chromosome Constitution in Human Gonadal Disorders. AVERY A. SANDBERG,* THEODORE S. HAUSCHKA, EDWIN GORDY and GEORGE F. KOEPF, Buffalo, N. Y. (1024)

20. Transmanganin, the Specific Manganese-Carrying Protein of Human Plasma. GEORGE C. COTZIAS * and ALBERT J. BERTINCHAMPS, UPTON, N. Y. (979)

21. Inhibition of Aromatic Amino Acid Decarboxylation in Man and Associated Pharmacological Effects. JOHN A. OATES, JR., LOUIS GILLESPIE, JR., J. RICHARD CROUT and ALBERT SJOERDSMA,* Bethesda, Md. (1015)

22. Immunoassay of Plasma Insulin Concentrations in Normal and Diabetic Man: Insulin Secretory Response to Glucose and Other Agents. ROSALYN S. YALOW and SOLOMON A. BERSON,* Bronx, N. Y. (1041)

23. The Krebs Cycle and Diabetic Ketosis. DANIEL W. FOSTER and MARVIN D. SIPERSTEIN,* Dallas, Tex. (986)

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* Member.

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24. Mannose Metabolism, an Index of Glucose Utilization. GEORGE F. CAHILL, JR., FRANCIS C. WOOD, JR., BERNARD LEBOEUF and ALBERT E. RENOLD,* Boston, Mass. (976)

25. Stimulation of Hepatic Fatty Acid Synthesis by Ethanol *in Vivo* and *in Vitro*. CHARLES S. LIEBER, LEONORE M. DECARLI and RUDI SCHMID,* Boston, Mass. (1007)

26. The Effect of Cell Structure and Growth Hormone on Protein Synthesis in Striated Muscle. DAVID M. KIPNIS and ERIC REISS, St. Louis, Mo. (introduced by Carl V. Moore). (1002)

ABSTRACTS

Antidiuretic Action of Oxytocin. R. ABDUL-KARIM and N. S. ASSALI, Los Angeles, Calif. (introduced by Joseph F. Ross).

It is generally believed that oxytocin exerts a minimal antidiuretic action when compared to vasopressin. Actually, oxytocin increases water and electrolyte excretion in the rat and may even antagonize the antidiuretic action of vasopressin.

To test the renal action of oxytocin in man, studies were performed on 12 females (5 pregnant and 7 nonpregnant) and 5 males. One natural (Pitocin) and 2 synthetic (Syntocinon and DuVigneaud's polypeptide) preparations were infused intravenously in doses varying from 6.5 to 315 mU per minute. In each instance, oxytocin induced a marked fall in urine flow which lasted for the duration of the infusion. Renal plasma flow (PAH), glomerular filtration rate (inulin), electrolyte excretion and osmolal clearance did not change significantly, whereas free water clearance fell and often became negative. In the pregnant subjects who received oxytocin for labor induction, the antidiuretic action became evident before the oxytocic effect.

To investigate the possibility that oxytocin antidiuresis might be mediated through stimulation of ADH secretion, oxytocin was infused into 2 patients with diabetes insipidus and 3 normal subjects who ingested 180 ml of whiskey. The same fall in urine flow and in the free water clearance occurred in these subjects, suggesting a direct action of oxytocin on the kidney. It is concluded that in man oxytocin has antidiuretic properties closely similar to those of vasopressin.

Studies of Granulocyte Kinetics. JOHN W. ATHENS, ALVIN M. MAUER, SPENCER O. RAAB, OTTO P. HAAB and GEORGE E. CARTWRIGHT,* Salt Lake City, Utah.

In these studies blood was withdrawn from each subject into a plastic bag, the granulocytes labeled with radioactive diisopropyl fluorophosphate (DFP^{32}) and the labeled cells returned to the circulation. The size of the pool into which the labeled cells are infused, the total blood granulocyte pool (TBGP), has been measured with this method. The granulocytes have been shown to leave the TBGP in a random manner and the time in which half of the cells leave ($T_{\frac{1}{2}}$) has been determined. From the size of the TBGP and the $T_{\frac{1}{2}}$, the granulocyte turnover rate (GTR) has been calculated.

In 40 normal subjects the mean values for these parameters were: TBGP 0.83×10^9 cells per kg, $T_{\frac{1}{2}}$ 6.9 hours and GTR 2.1×10^9 cells per kg per day.

In 6 patients with chronic myelocytic leukemia in relapse the mean values were: TBGP 20×10^9 cells per kg, $T_{\frac{1}{2}}$ 49.2 hours and GTR 5.7×10^9 cells per kg per day. In 3 patients with chronic myelocytic leukemia in remission after treatment with Myleran these values had returned almost to normal.

In 4 subjects with infection and leukocytosis the values were increased over normal but only moderately so. In two subjects with chronic infections and no leukocytosis the values were within the normal range.

The TBGP has been shown to consist of two compartments in rapid equilibrium with each other, the circulating granulocyte pool (CGP) and the marginal granulocyte pool (MGP). The size of the CGP is calculated from the blood volume and the absolute granulocyte count. The MGP is the difference between this value and the TBGP. The distribution of granulocytes in these two compartments of the TBGP has been studied in the subjects described above. Thirty-five to 46 per cent of the granulocytes in the TBGP was found to be in the CGP. Cells shift from the MGP into the CGP after epinephrine injection or exercise.

Studies on the Mechanism of Fever Following Intravenous Inoculation of Staphylococci. ELISHA ATKINS* and LAWRENCE R. FREEDMAN, New Haven, Conn.

Although gram-positive microorganisms produce some of the most pyrogenic infections in man, the sequence of events leading to fever with these bacteria has remained obscure except where purulent foci have been established. There has been conflicting evidence as to whether staphylococci are capable of causing fever after intravenous injection since they do not possess endotoxins which are present in gram-negative bacteria. In the experiments to be described, injection of either live or autoclaved staphylococci resulted in fever after a characteristic latent period of 1 hour. A similar latent period is seen with fever produced by inoculation of either influenza virus or antigen in specifically sensitized recipients. By studies involving passive serum transfer, the fever was shown to be directly caused by a circulating endogenous pyrogen (EP). The whole microorganism rather than one of its products liberated into the culture medium appeared to be essential in releasing this endogenous material. No cross-tolerance to bacterial endotoxin was demonstrable although there was a diminished response to other heterologous pyrogens after injection of staphylococci. Tolerance in this situation appears to be due to a temporary inability of the recipient given staphylococci to mobilize further amounts of EP to another stimulus.

The experiments suggest that a number of unrelated agents, including bacterial cells and other substances of large molecular size, produce fever by releasing an EP. The interaction of these substances with circulating polymorphonuclear leukocytes is believed to be the initial step in this process and may therefore be essential to the de-

* Member.

velopment of fever in a wide variety of different situations, both clinical and experimental.

Preservation of Platelet Viability at 4° C. MARIO BALDINI and SHIRLEY EBBE, Boston, Mass. (introduced by William Dameshek).

The effectiveness of platelets in promoting hemostasis depends chiefly upon their viability. In the present study the viability of platelet concentrates preserved in different media at 4° C was measured.

A rabbit model was first adopted, and viability of stored rabbit platelets labeled *in vitro* with Cr^{51} or *in vivo* with P^{32} was investigated by following platelet radioactivity in the circulation of normal recipients and also by enumerating the circulating platelets after infusion into thrombocytopenic animals. Two parameters were used to determine viability: *1*) the platelet lifespan and *2*) the maximum platelet recovery in the recipient's circulation. From these, the platelet "viability index" was calculated and was expressed in per cent of the value obtained with fresh platelets.

Platelet concentrates stored for 24 hours in saline had a viability index of less than 2 per cent. When stored in gelatin the viability index was less than 1 per cent. A new storage medium containing inosine, adenine, glucose, plasma and phosphate buffer was then investigated. With the use of this medium, the viability index of platelets was 37 per cent after 24 hours and fell to 11 per cent only after 6 days of storage. Removal of single components from this medium demonstrated that its favorable effect on platelet viability was due mainly to *1*) plasma, and *2*) the purine metabolites. The effect of the latter was more striking after the third day of storage. There was evidence that the purine metabolites delayed the natural aging process of the stored platelets.

Experiments with human platelets preserved as Cr^{51}-labeled concentrates confirmed the favorable effect of the medium containing plasma and the purine metabolites. After 48 hours of storage, platelet viability was 3.3 times higher than in the medium containing only plasma, and was 15 times higher than in the saline medium.

The Influence of Anion Mobility on Urinary Acidification and the Excretion of Titratable Acid. NORMAN BANK and WILLIAM B. SCHWARTZ,* Boston, Mass.

The influence on acid excretion of anions of different mobility (membrane-penetrating ability) has been studied in dogs whose sodium and chloride excretion had been reduced by dietary restriction. It was demonstrated that administration of the sodium salt of phosphate (pH 7.4), a buffer anion of low mobility, leads to a marked reduction in urine pH and the excretion of near theoretical maximum amounts of titratable acid. These results suggest that in the nonacidotic dogs a disproportion between sodium and anion reabsorption was the critical factor in determining not only the pH gradient between blood and urine but also the excretion of titratable acid. This hypothesis is supported by the observation that infusion of the more mobile anions, chloride or thiocyanate, into phosphate-loaded dogs results in progressive elevation of urine pH and reduction in titratable acid excretion, while administration of the less mobile anions, sulfate or ferrocyanide, is associated with sustained or increased urine acidity and excretion of near theoretical maximum amounts of titratable acid.

As a possible mechanism to account for these findings, it is proposed that the various anions affected urine pH and acid excretion through their influence on the electrical gradient established by active sodium transport across the renal tubular cells. According to this hypothesis, the magnitude of the gradient is determined by the mobility of the available anions, those which penetrate the tubular epithelium more readily having a greater tendency to follow sodium and thus to reduce the potential difference. Changes in urinary pH and titratable acid excretion have tentatively been attributed to the passive movement of hydrogen ions in response to variations in the transtubular electrical gradient. Although active transport of hydrogen may also be involved in the overall acidifying process, the present data suggest that passive diffusion of hydrogen plays an important role in the mechanism governing urinary acidification and the renal excretion of acid.

An Alternate Schedule for Poliomyelitis Immunization. EUGENE V. BARNETT and SAMUEL BARON, Rochester, N. Y. and Bethesda, Md. (introduced by Howard B. Slavin).

The current dosage schedule of three 1 ml injections of poliomyelitis vaccine over 8 to 12 months produces an adequate antibody response in most nonimmunes following the second or third dose. The present study was undertaken to determine a dosage schedule which would provide rapid immunization, maintain detectable antibody levels throughout the immunization period and produce the high levels of antibody which have been correlated with long duration of immunity. Such a schedule would be particularly desirable for those who seek rapid immunization, such as contacts of paralytic cases.

One group of nonimmune children was given vaccine of low antigen content according to the current schedule of three 1 ml doses over 7 months. Another group of nonimmune children was given an initial dose of 10 ml of the same vaccine followed by a 1 ml booster at 7 months. A group of adults was given an initial 10 ml dose of vaccine of high antigen content followed by 1 ml doses at 1 and 7 months. The results may be summarized as follows: *1*) Significant quantities of antibody to the 3 poliovirus types appeared between the second to sixth day following 10 ml of high antigen-content vaccine. *2*) Antibody to Type 1 and 3 polioviruses followed inoculation of a single 10 ml dose or two 1 ml doses but soon declined to undetectable levels. It was usually boosted by a third inoculation at 7 months. *3*) Antibody which resulted from an initial 10 ml dose followed by a 1 ml dose at 1 month, remained detectable throughout the immunization period and was boosted to higher levels by a 1 ml dose at 7 months.

The Significance of the Instantaneous Aortic Blood Ejection Velocity. G. OCTO BARNETT, SAMUEL M. FOX, 3RD, ALEXANDER J. MALLOS, JOSEPH C. GREENFIELD, JR. and DONALD L. FRY, Bethesda, Md. (introduced by James H. Baxter).

The time relationships between pressure and blood velocity in the ascending aorta can be used to define the mechanical function of the myocardial pumping mechanism. To the extent that mechanical function is altered early in disease, evaluation of these pressure-velocity relationships has important biophysical and clinical implications. Recent evidence indicates that changes of blood velocity may be a sensitive index of altered mechanical function. It was the purpose of this study to determine to what extent certain disease processes, pharmacological agents, and experimentally produced myocardial ischemia altered the pressure-velocity relationships. These relationships were evaluated by the simultaneous estimate of central aortic pressure and instantaneous blood velocity obtained by the computed pressure gradient technique using a catheter system developed in this laboratory. These observations were made on the intact unanesthetized man and dog.

Diffuse myocardial disease in man and experimentally produced myocardial damage in the dog were associated with changes in the blood velocity curve, although the blood pressure remained essentially constant. These changes in blood velocity consisted of a decreased initial acceleration and a diminished peak velocity. In man with myocardial disease, and in dogs, acetyl strophanthidin, norepinephrine, and isoproterenol caused an increase in ejection velocity. In the dog, methoxamine, which has predominantly a peripheral vasoconstrictor action, caused a decrease in ejection velocity.

This approach, using the computed pressure gradient technique, would appear to open new avenues to a more sensitive evaluation of cardiovascular dynamics, such as the computation of cardiac power and work, in the intact man.

A Pattern of Sodium Excretion in Patients with Decompensated Cirrhosis of the Liver Who Recover from Their Edematous State. EUGENE Y. BERGER, RENE PECIKYAN and GRACE KANZAKI, New York, N. Y. (introduced by J. Murray Steele).

Daily urinary sodium was measured in 5 patients with decompensated Laennec's cirrhosis of the liver who excreted less than 2 mEq of sodium per day and who, without the use of diuretic agents or a low sodium diet, went on to exhibit a natriuresis, lose their ascites and recover from their edematous state. From the onset of ascites which required paracentesis, to the onset of the natriuresis, 3 to 11 months elapsed during which 5 to 14 paracenteses were performed. Two of the 5 patients had febrile episodes at the onset of natriuresis; the third patient received 50 mg of Δ^1-androstene-3,17-dione intramuscularly and developed localized tenderness at the site with associated fever; in the remaining 2 patients there was no other evident change or event in the clinical course. Excepting one subject, who developed ascites terminally 7 years later, edema fluid did not recur in a subsequent observation period of 3 to 7 years.

The onset of natriuresis occurred slowly over several days with urinary sodium increasing from tenths to 1 to 5 mEq per day. This early increment represented a several-fold increase in sodium excretion but was insufficient to influence either urinary volume or the patient's weight. As the natriuresis continued, it followed the same pattern in all 5 patients. The daily urinary sodium excretion doubled every 3 days (approximately) until normal sodium excretion was achieved. At this rate, on a diet of 50 to 150 mEq of sodium, a month elapsed from the beginning of the natriuresis until its effect was reflected in a decrease in weight. The continued increase of urinary sodium from tenths of a mEq to over 100 mEq per day was in keeping with the concept of a first order process. One possible explanation for this pattern is an increase in hepatic aldosterone clearance.

Changes in Gross Body Composition During Controlled Weight Loss. NATHANIEL I. BERLIN and DONALD M. WATKIN, Bethesda, Md. (introduced by Charles G. Zubrod).

Three obese patients were placed on an 800 calorie, 8 g N per day metabolic balance regimen for 60 to 120 days. Changes in gross body composition [fat, water and dry lean tissue (minerals and proteins)] were calculated from serial measurements of total body water with tritiated water and body density, determined with a helium dilution device, and from N, Na and K metabolic balance data.

Following a phase of initial rapid weight loss of 5 to 10 days' duration, due primarily to water loss, all 3 subjects lost weight and fat at an approximately constant rate, proportional to initial body fat content. In 2 patients fat loss calculated from metabolic balance data and body water and density measurements were in good agreement; in the third patient the fat loss could be calculated from the N balance and body water data, but not from N, Na and K balances. The measured changes in dry lean tissue constituents varied from negligible to 1.25 kg and were in good agreement with those calculated from body water and density data.

The ratios of caloric expenditures, calculated by the Newburgh insensible water loss technique, to that calculated from the difference in caloric intake and caloric content of feces and urine plus 9.2 calories per g fat loss per day, were 1.40, 1.32 and 1.07, the last occurring in a patient with steroid diabetes.

The combination of metabolic balance data with body water and density measurements permits a satisfactory measurement of changes in body composition. An 800 calorie, 8 g N per day intake in obesity results in a rate of fat loss proportional to initial body fat content, with a small but variable change in lean tissue mass.

The Demonstration of Three Types of Inhibitors and One Type of Stabilizer of the Latex Fixation Test for Rheumatoid Arthritis. GERSON C. BERNHARD and DAVID W. TALMAGE,* Denver, Colo.

The demonstration of three distinct types of inhibition and one type of stabilization of the latex fixation system has been possible by varying the order of addition of the inhibitor and the reagents in the standard test procedure. The Singer method for latex agglutination with a positive rheumatoid serum was performed in cuvets. Optical densities were measured photometrically before incubation and subsequent to incubation and centrifugation. The per cent optical density (A) remaining in the supernatants after centrifugation was plotted against the dilutions of rheumatoid serum. Standard curves without inhibitor were compared with those obtained when conditions were altered by addition of an inhibitor at various steps in the procedure. Incubating bovine serum albumin (BSA) or heat-inactivated normal human serum (NHS) with latex prior to coating the latex with human gamma globulin (HGG) gave curves showing both stabilization (increased per cent A remaining in supernatants of controls) and inhibition (reduced agglutination by rheumatoid factor). Neither BSA nor heated NHS produced inhibition when added after latex was coated with HGG. However, *fresh* NHS produced inhibition when added at this stage. The inhibition by *fresh* NHS was greatest when it was incubated with HGG-coated latex before the addition of rheumatoid factor. Heating to 56° C at this stage did not destroy inhibition. However, *fresh* NHS preincubated with rheumatoid factor and then heated to 56° C before contact with HGG-coated latex was not inhibitory. Therefore, the inhibitor in *fresh* NHS appears to act on HGG-coated latex. Aggregated HGG and certain antigen-antibody precipitates are known to remove rheumatoid factor from serum. Thus inhibitors can cause reduced agglutination by reacting with: *1*) the uncoated latex; *2*) the HGG-coated latex; *3*) the rheumatoid factor. In addition, stabilizers can be distinguished from inhibitors by their effect on the latex suspension in the absence of rheumatoid factor.

Metabolic Turnover of the Ribonucleic Acid of Mammalian Reticulocytes During Maturation In Vitro. JOHN F. BERTLES, CHARLES J. WILTZ and WILLIAM S. BECK,* Boston, Mass.

The development of reticulocytes in mature erythrocytes involves the progressive loss of certain biosynthetic capacities and of ribonucleic acid (RNA). Little experimental evidence is available on the fate of the RNA and on the question of whether RNA synthesis continues during the period when net loss of RNA is taking place. Both questions bear on the overall problem of cellular differentiation; the latter relates to the current controversy on the cellular sites of RNA synthesis. The present investigation shows incorporation of certain labeled nucleic acid precursors into reticulocyte polyribonucleotide and reveals the fate of a portion of the RNA during maturation.

Reticulocytes were obtained from anemic rabbits, washed, and incubated in serum-bicarbonate buffer (pH 7.4) under a continuous flow of 95 per cent O_2, 5 per cent CO_2. Glucose concentration in the medium and pH were maintained by continuous addition of glucose-bicarbonate solution under pH-stat control. Adenine-8-C^{14} and sodium formate-C^{14} were added separately to individual flasks at zero time, and samples were taken up to 24 hours. The washed cell mass of each sample was dissolved in sodium dodecyl sulfate, and polynucleotides were precipitated with ethanol or perchloric acid (PCA). After hydrolysis with alkali or hot 70 per cent PCA, the nucleotides or bases were separated by paper chromatography. The extracellular fluid of each aliquot was dialyzed and desalted on Dowex-1 resin. Purine and pyrimidine components were isolated by paper chromatography.

Three compounds were isolated from the extracellular fluid at 24 hours and tentatively identified as hypoxanthine, cytidine and uracil. Both adenine and formate labeled cell polynucleotide adenine, but formate also labeled polynucleotide guanine. The results suggest that maturing reticulocytes synthesize purine nucleotides and polyribonucleotide *de novo* during the period of net RNA loss; and as maturation proceeds, products of nucleic acid breakdown appear outside the cell.

Oral Glutamate Therapy in Ammonemia due to Blood in the Gastrointestinal Tract. ALICE N. BESSMAN, Baltimore, Md. (introduced by George S. Mirick).

It is generally accepted that elevated blood ammonia levels play a significant role in the production of confusion and coma in many cases of hepatic decompensation. Intravenous sodium (or sodium-potassium) glutamate administration has been shown to lower blood ammonia levels, but the amount of glutamate that can be administered is limited by the large quantities of intravenous fluid and electrolyte that had to be administered. In the recent literature oral sodium glutamate has been reported to be an effective adjunct to low protein diet in lowering elevated ammonia levels in patients with liver disease.

The greatest unanimity of opinion regarding the effectiveness of intravenous glutamate therapy has concerned those cases precipitated by gastrointestinal bleeding. We have studied the effectiveness of orally administered sodium glutamate in patients with liver disease who had ingested test meals of whole blood. Six patients with Laennec's cirrhosis, each serving as his own control, ingested *1*) whole blood containing a known amount of protein nitrogen and *2*) the equivalent amount of blood plus 25 g of sodium glutamate. Arterial and venous blood ammonia levels were followed over a 5 hour period. All patients showed a rise in blood ammonia levels following ingestion of test loads. In *no* patient did the addition of sodium glutamate diminish this rise and in some patients there was greater ammonemia after blood plus glutamate than there was after blood alone. It was

concluded that oral administration of sodium glutamate in the treatment of ammonia intoxication resulting from blood in the gastrointestinal tract is of no value.

Variability of Response of Human Tumor Cells to Chemotherapeutic Agents. H. R. BIERMAN* and G. J. MARSHALL, Los Angeles, Calif.

The response of patients with neoplastic diseases to chemotherapeutic agents is often highly variable despite the identical histologic pattern of the tumor. Quantitative cytometric and cytologic measurements of normal and neoplastic cells in effusions of 15 patients were performed following 40 single or multiple intracavitary administrations of tracer amounts of 2 classes of alkylating agents (amine mustards and Myleran).

Prompt decrease in the neoplastic cell population associated with cytoplasmic, nuclear and nucleolar changes without similar damage to normal cells was usually correlated with beneficial clinical response when full therapeutic doses were employed. However, identical tumor cell types in 5 patients failed to exhibit these changes at concentrations of the chemotherapeutic agent which caused profound changes in the normal cell population. Intracellular incorporation of H^3-thymidine showed variation in uptake between tumor cell types and a variation in uptake prior to and following the chemotherapeutic agent. Combined chromosome, microsome and membrane changes in a decreasing tumor cell population with decrease in H^3-thymidine uptake are indicative of significant alterations in intracellular metabolism. Nuclear changes alone proved inadequate in predicting ultimate chemotherapeutic efficacy. Resistant cell populations became evident in 4 of the 15 patients and could not be eliminated at the highest chemotherapeutic concentrations employed.

The data suggest that the convenient term "biologic variation" as applied to these agents refers in part to subtle differences in intracellular biochemical mechanisms of neoplastic cells undisclosed by conventional histopathologic technics. Assays of chemotherapeutic agents on neoplastic cells in the human host aids in the selection of effective agents and affords an opportunity to study the mechanism of DNA synthesis and duplication.

Effects of Antirheumatic Agents on Connective Tissue Metabolism. ALFRED JAY BOLLET,* Charlottesville, Va.

The metabolic effects of agents used in therapy of rheumatic diseases are under investigation in an attempt to elucidate changes which occur during suppression of inflammation. Thus far, these studies have been concerned primarily with two enzymes, the transamidase which synthesizes glucosamine-6-phosphate from fructose-6-phosphate and glutamine and DPNH cytochrome C reductase, and with two tissues, liver and the connective tissue which forms in polyvinyl sponges in rats.

Gold chloride almost completely inhibited the activity of the enzyme which synthesizes glucosamine-6-phosphate in a concentration of 50×10^{-5} M in liver homogenates, and 5×10^{-5} M in connective tissue homogenates. The latter concentration is in the range found in the serum of patients receiving gold therapy. Administration of 10 mg of gold sodium thiomalate to rats one week prior to assay resulted in a 70 per cent decrease in the specific activity of the glucosamine synthesizing enzyme in connective tissue, but no change in activity of the enzyme in liver. A decrease in the acid mucopolysaccharide content of the connective tissue in the sponge implants also occurred after gold sodium thiomalate administration.

Sodium salicylate also inhibited the activity of the glucosamine synthesizing enzyme in both liver and connective tissue homogenates. Twenty per cent inhibition occurred with a concentration of 2.1×10^{-3} M, but very high concentrations were needed to cause complete inhibition. A decrease in the activity of this enzyme occurred in both liver and connective tissue following hydrocortisone administration to rats, but this steroid had no effect *in vitro*.

DPNH cytochrome C reductase was inhibited by gold chloride and salicylate *in vitro* in concentrations similar to those which inhibited the glucosamine synthesizing enzyme in each type of homogenate. Quinacrin also inhibited the DPNH cytochrome C reductase *in vitro* in a concentration of 12.5×10^{-3} M, but this concentration did not affect the glucosamine synthesizing enzyme.

The Alveolar Lining: A Method of Extraction; The Surface Tension Lowering Effect of Cigarette Smoke. STUART BONDURANT, Indianapolis, Ind. (introduced by John B. Hickam).

The stable coexistence of alveoli of varying size in continuity with the trachea has been attributed to a decrease of surface tension of the alveolar lining layer as surface area is decreased. The surface tension of films obtained by compressing the lung in saline is 35 to 45 dynes per cm^2 (T_1) decreasing to < 20 dynes per cm^2 (T_2) when surface area is decreased by 80 per cent. (Saline $T_1 = T_2 = 72$ dynes per cm^2.) There is strong evidence that the alveolar lining layer has similar unique surface properties. Such films are not consistently obtained and cannot be easily harvested for further analysis.

Surface tension has been measured (Wilhelmy balance) on extracts prepared by 5 methods (5 rats each): compression of the lung in saline $T_1 = 39 \pm 8$, $T_2 = 15 \pm 13$; homogenized lung $T_1 = 40 \pm 10$, $T_2 = 24 \pm 4$; saline washing of trachea $T_1 = 46 \pm 4$, $T_2 = 22 \pm 7$; endotracheal bubbles produced by vascular perfusion with distilled water while the lungs are mechanically ventilated $T_1 = 40 \pm 10$, $T_2 = 20 \pm 4$; same procedure, saline perfusion, $T_1 = 34 \pm 7$, $T_2 = 11 \pm 4$.

The bubbles can be evaporated at room temperature to yield 75 to 100 mg of of amorphous white powder per rat. Ten to 100 mg of this powder added to a 90 cm^2 saline surface causes $T_1 = 35 \pm 7$, $T_2 = 15 \pm 4$. Human lungs similarly treated also yield large quantities of surface active material.

Exposure of the films to cigarette smoke for one minute causes a decrease in surface tension: T_1 from 46 ± 7 to 27 ± 7, T_2 from 15 ± 4 to 11 ± 4 (15 rats, both $p < 0.001$.) In 6 extracts from 2 normal human lungs,

T_1 decreased from 36 ± 13 to 24 ± 3 ($p < 0.05$) and T_3 decreased from 11 ± 5 to 9 ± 3 ($p > 0.05$) with application of cigarette smoke.

A method is described for extracting surface active material, probably the alveolar lining layer, from the lung. Exposure to cigarette smoke causes a decrease in the surface tension of this material. Such a change occurring *in vivo* would alter the stability of alveolar size favoring the development of overdistended alveoli.

Observations on the Operation of Starling's Law of the Heart in Man. EUGENE BRAUNWALD, ROBERT L. FRYE, JOSEPH W. GILBERT and MAURICE AYGEN, Bethesda, Md. (introduced by Robert P. Grant).

There has been considerable controversy concerning the applicability of the Frank-Starling mechanism to the human heart. This investigation was designed to determine whether left ventricular end-diastolic fiber length (EDFL) and end-diastolic pressure (EDP) are important determinants of ventricular contraction. Systemic arterial pressure and effective EDP were measured at operation in 20 adult patients with rheumatic mitral valve disease and atrial fibrillation. Continuous alterations of the length of a segment of left ventricular muscle were recorded simultaneously in 7 patients by means of a mercury-filled resistance gauge sutured to the surface of the left ventricle. The variability of diastolic length resulted in beat-to-beat alterations of ventricular filling which resulted in variations of EDFL and EDP. For each individual beat the peak systolic ventricular pressure was utilized as an index of maximum tension developed, the area beneath the left ventricular pressure curve (tension-time index) was employed as a measure of the total tension developed and the systemic arterial pulse pressure was utilized to indicate relative changes in stroke volume. In every patient alterations in EDFL and EDP correlated closely with changes in systolic pressure, tension-time index, and arterial pulse pressure. In order to determine whether these relationships also exist in intact unanesthetized subjects, effective EDP was measured by means of transseptal left heart catheterization in 4 patients. The correlations of EDP with systolic pressure, tension-time index, and pulse pressure were similar to those obtained in the patients studied at operation. These results are consistent with the view that when filling is altered abruptly, Starling's law of the heart operates in man and that EDFL and EDP are important determinants of the characteristics of the subsequent ventricular contraction.

The Effect of Hyperglycemia Without Glycosuria on Salt and Water Excretion. EMANUEL H. BRESLER and JERRY N. GILMARTIN, New Orleans, La. (introduced by Grace Goldsmith).

The forces involved in transtubular movement of glomerular filtrate have not been adequately defined. It is widely held that the tubular wall in the main forms an impermeable barrier with respect to diffusion of sodium chloride. Recently, an older concept advanced by Ludwig has been revived, in which it is conceived that glomerular filtrate is largely reabsorbed across a segment of the tubular barrier which is permeable to electrolytes as well as water, in answer to osmotic forces in the peritubular area. If this latter model is correct, then, as pointed out by L. E. Bayliss, active transport of glucose should contribute importantly to peritubular osmotic activity. Accordingly, 6 dogs were infused with normal saline at the rate of 5 cc per minute. In some instances a priming dose of 150 cc 5 per cent sodium chloride was infused over a 30 minute period. After several hours a steady state of urinary output was achieved with output of salt and water closely approximating input. Glucose in a concentration of 2 or 3 per cent was then added to the saline infusion for 30 minutes followed by resumption of normal saline infusion. Blood glucose was determined as well as urine sodium, chloride, and osmolal concentrations. In all cases the output of sodium chloride and water decreased markedly (circa 20 to 40 per cent) during periods of hyperglycemia (circa 160 mg per 100 ml) returning to baseline outputs as blood glucose returned to normal values. Inulin and PAH clearances appeared to show no significant changes. These data support the concept that a portion of glomerular filtrate is reabsorbed across some segment of the renal tubule permeable to electrolytes as well as water. In this area active reabsorption of glucose contributes importantly to effective peritubular osmotic activity.

Electrical and Osmotic Characteristics of the Isolated Turtle Bladder. WILLIAM A. BRODSKY* and THEODORE P. SCHILB, Louisville, Ky.

Electrical potential difference (P.D.) and ohmic resistance across the isolated turtle bladder were measured. Mounted in a chamber between two identical oxygenated Ringer's solutions, the interstitial side was positive in an external circuit with respect to the mucosal side by 100 to 120 mv, while the D. C. resistance (change of voltage for 1 to 10 μa of externally applied current) was 2,500 ohms per 0.3 cm^2 of tissue. Such levels of potential and resistance were maintained for 24 to 48 hours with the tissue in oxygenated media. Nitrogenation of the bathing solutions caused a decrease of P.D. to 60 to 65 mv, and an increase of resistance to 4,200 ohms per 0.3 cm^2 of tissue, which levels were maintained for 4 to 6 hours of nitrogen bubbling. We attempted to relate the electrical properties of the bladder with its ability to transport water and solute. Whole bladders, filled with 1/3 Ringer's, were immersed in full-strength Ringer's. Rate of weight loss of such "bladder bags" was 0.20 to 0.25 g per hour per 10 to 30 g of total weight of bladder plus content. Osmotic activity of inside solution, initially 60 mOsm per kg, decreased to values of 40 to 50 mOsm per kg after 4 to 24 hours of incubation. Addition of vasopressin, 0.1 unit per 1 ml of external solution, accelerated the water efflux in one experiment, and induced but little change in another. Dilution of the internal fluid was observed with or without the hormone. This demonstrates directly a movement of solute (NaCl)

against the chemical potential gradient, and probably, the movement of sodium ion against the electrochemical potential gradient.

Serum Factors in Acquired Hemolytic Anemia in Patients with Lymphoma and Leukemia. JEROME I. BRODY and STUART C. FINCH,* New Haven, Conn.

The purpose of this study was to determine whether or not the erythrocyte-coating globulin found in the sera of patients with acquired hemolytic anemia could be characterized as erythrocyte antibody, using the technique of immune adherence. This method was chosen because immune adherence occurs only as a consequence of an antigen-antibody reaction and would not occur if the globulin was not erythrocyte antibody.

Sera obtained from lymphoma and leukemia patients with an indirect Coombs positive reaction were compared immunologically to ABO-specific isologous and heterologous erythrocyte antibody. The technique consisted of incubating serial dilutions of test serum with known concentrations of human red cells, human complement, and freshly washed guinea pig platelets. Immune adherence of red cells to the indicator platelets developed in the presence of complement and red cell antibody. This produced both macroscopic and microscopic flocculation of the red cells. It was found that either type of group specific erythrocyte antibody reacted strongly in immune adherence with normal red cells in this system. Coombs positive red cells from patients with lymphoma and leukemia also reacted strongly in immune adherence in the presence of rabbit antihuman globulin. These reactions did not take place in the absence of complement. In contrast, Coombs indirect positive sera from nontransfused patients with acquired hemolytic anemia did not react in immune adherence with either their own or group-compatible homologous erythrocytes.

These studies demonstrate that in these patients with lymphoma and leukemia the erythrocyte-coating globulin of acquired hemolytic anemia is not characterized as erythrocyte antibody by the immune adherence technique. It is believed that these abnormal globulins, with affinity for the red cell surface, are nonimmunologic and may represent a form of dysproteinemia. The data also indicate that heterologous and isologous group-specific red cell antibody reactions are complement-dependent in immune adherence.

Renal Handling of Estrogens in Late Pregnancy. C. HARMON BROWN, BENJAMIN D. SAFFAN and JOHN R. K. PREEDY,* Atlanta, Ga.

Analysis of urinary and plasma estrogen levels in late pregnancy have indicated marked differences between the U/P ratios of estrone and estradiol-17β on the one hand, and of estriol on the other, suggesting differences in the renal handling of these steroids. To investigate this further, 7 women in the thirty-sixth to fortieth week of normal pregnancy were given sufficient water to establish a diuresis of 3 to 10 ml per minute. Urine was collected for 90-minute periods, in the middle of which a plasma sample was drawn. Estrone, estradiol-17β, and estriol were determined in plasma and urine samples by a chemical method which measures conjugated and unconjugated forms of each estrogen together.

Mean clearance rates for estrone and estradiol-17β were 15.6 ml per minute (SD 5.0) and 12.0 ml per minute (SD 0.2), respectively. In contrast, the mean estriol clearance was 296 ml per minute (SD 46.0). The mean U/P ratios of estrone, estradiol-17β, and estriol were 3.3, 2.5, and 64.2, respectively. The differences in clearance rates are unlikely to be due to technical factors. Recoveries of each of the 3 estrogens added to both urine and plasma were similar (75 to 85 per cent).

The low clearance rates of estrone and estradiol-17β may be due to the physicochemical state of these hormones in plasma, or possibly to glomerular filtration and tubular reabsorption. The clearance rate of estriol on the other hand is approximately twice average published figures for glomerular filtration rate in late pregnancy. This is supported by simultaneous endogenous creatinine clearance rates obtained in some of our subjects. Thus, it appears that in late pregnancy some component of the total estriol of plasma is secreted by the renal tubule. This component is likely to be estriol glucosiduronate.

Thyroidal Iodide Secretion. BELTON A. BURROWS,* D. WARD SLINGERLAND, ANTHONY LIUZZI, ELIZABETH S. DELL and DOROTHY E. GRAHAM, Boston, Mass.

Thyroidal secretion of nonthyroxine iodide has been suggested by previous studies in normal subjects, hyperthyroid patients, and experimental animals. In the present study discrepancies in the conventional model of iodine turnover (iodide to thyroid iodine to circulating hormonal iodine) are demonstrated and the proportion of nonthyroxine iodine released by the thyroid is estimated.

Intravenous tracer doses of radioiodine given to hyperthyroid patients virtually disappeared within one day from the extrathyroidal iodide space, as shown by an initial rapid decrease in both the plasma inorganic radioiodide concentration and the rate of urine radioiodide excretion. Therefore, after Day 1, radioiodide appearing in plasma or urine had made one or more passages through the thyroid. For the next several days, plasma inorganic radioiodide concentration and the rate of urinary radioiodide excretion decreased, even though serum protein-bound radioiodine concentration increased twofold. If the urine radioiodine were derived exclusively from the degradation of labeled thyroid hormone, the rate of urinary radioiodine excretion could be expected to increase, rather than decrease, with increased serum protein-bound radioiodine levels. Therefore, the contribution to urinary radioiodine excretion of thyroidal radioiodine in some form other than thyroxine is indicated.

Urine iodine specific activity was as much as 3 times greater than serum protein-bound iodine specific activity during this interval, and showed considerable day to day variation. With the administration of blocking doses of Tapazole, this variation in urine iodine specific activity

was reduced, and the values were less than serum protein-bound iodine specific activity to a degree consistent with daily dietary increments of stable iodine. It would appear that prior to Tapazole, nonthyroxine iodide of varying specific activities, and in amounts at least comparable to thyroxine iodine, was being released by the thyroid.

Mannose Metabolism, an Index of Glucose Utilization. GEORGE F. CAHILL, JR., FRANCIS C. WOOD, JR., BERNARD LEBOEUF and ALBERT E. RENOLD,* Boston, Mass.

Intravenous administration of D-mannose or D-glucose to normal humans (0.5 g per kg given in 3 to 4 minutes) resulted in similar disappearance rates. Injection of insulin (0.1 unit per kg) with mannose increased equally the disappearance rates of both endogenous glucose and infused mannose. Injection of mannose, unlike glucose, did *not* increase serum insulin activity as measured by adipose tissue bioassay (in agreement with pancreatic infusion data from experimental animals). Thus, mannose is "insulin-responsive" similar to glucose, but unlike glucose, does not stimulate the β-cell to secrete insulin. Since there is no significant endogenous source of free mannose, mannose disappearance is an index of removal alone, and being insulin-responsive, reflects insulin activity. Recycling as with C^{14}-labeled substrate is obviated.

Prolonged mannose infusions in two normal humans (0.5 g per kg per hour for 10 hours) resulted in a marked and persistent reduction in blood glucose to 10 and 23 mg per 100 ml (without hypoglycemic symptoms, since brain presumably utilizes mannose) suggesting a marked suppression of hepatic glucose output. Transient elevation in serum bilirubin was noted without alteration in other hepatic function tests. In a mild diabetic, blood glucose levels were suppressed by a 5 hour mannose infusion, while in a severe ketotic diabetic off insulin, there was no change in blood glucose. Disappearance rates of mannose acutely infused into diabetics were depressed similar to those of glucose, reflecting a decreased utilization of both sugars. In two patients with Cushing's syndrome, mannose levels fell at a considerably faster rate than administered glucose, suggesting that the level of the latter was sustained by accelerated hepatic gluconeogenesis. Thus mannose is able to serve as a "marker" for glucose "utilization" or "turnover."

Additional studies with mannose-C^{14} in isolated tissues corroborated the above findings, namely, a parallel decrease in glucose and mannose metabolism in diabetic tissues including adipose tissue, kidney and liver. Insulin *in vitro* equally stimulated glucose and mannose metabolism in adipose tissue as measured by recovery of label in CO_2, fatty acids and glyceride-glycerol.

Circulating Antinuclear Globulins in Patients with Chronic Liver Disease. PAUL CALABRESI and MORTIMER GREENBERG, New Haven, Conn. and Boston, Mass. (introduced by S. R. Lipsky).

The sera of 32 patients with chronic liver disease, 24 alcoholics with cirrhosis and 8 individuals with postnecrotic cirrhosis, were tested for circulating antinuclear globulins by the fluorescent antiglobulin technique. Fixed homologous leukocytes in normal peripheral blood smears were the source of nuclear material. No attempt was made to quantitate the titer of antinuclear globulins in this study.

Of 10 patients with positive fluorescent antinuclear tests, 7 had nutritional cirrhosis and 3 had postnecrotic cirrhosis. The latter group included 2 women with transiently positive lupus erythematosus (L.E.) cell preparations who had been clinically designated "lupoid hepatitic." Of particular interest was the finding that 7 (50 per cent) of the 14 women in this series had positive antiglobulin tests as compared to only 3 (17 per cent) of the 18 men.

As a group, the patients with positive tests did not differ clinically from the others in any respect, including history of alcoholism when present, duration and severity of liver disease, age or physical findings. Liver function tests, serum protein levels and total leukocyte counts were also comparable in both groups but the latex fixation test was positive in every case with a positive antinuclear test.

These findings indicate that an abnormal circulating globulin with nuclear affinity may be present in the sera of certain patients with chronic liver disease. This factor was demonstrable in both patients with positive L.E. cell preparations. In the present study antinuclear globulins were encountered more frequently in women.

The Effect of Partial and Total Denervation of the Heart on Left Ventricular Function. CARLETON B. CHAPMAN,* TOM A. BRUCE, ORLAND BAKER and JOSEPH N. FISHER, Dallas, Tex.

The role of intrinsic myocardial reactivity, the central feature of Starling's Law, is difficult to assess if cardiac innervation is intact. For this reason cardiac sympathectomy (removal of both chains from middle cervical ganglion to T-5) was done in 4 normal dogs. Ten days later, LV volume and pressure curves were recorded at rest and during light exercise in intact anesthetized animals using biplane cinefluorographic methods. Bilateral vagal section was then done and the studies repeated. Three dogs were studied before and after vagal section alone.

In unoperated anesthetized animals (9 dogs), exercise produced 22 and 6 per cent increase in pulse rate and stroke volume, respectively. Cardiac output and minute work rose 33 and 51 per cent, respectively. With total cardiac denervation, exercise produced no change in pulse rate, which remained at 125, but stroke volume rose 21 per cent. Cardiac output and minute work increased 18 and 26 per cent, respectively. Vagectomy alone caused marked resting tachycardia; exercise produced modest further increase in rate (180 to 187) and in stroke volume. Similar changes occurred with sympathectomy alone except that rates were lower (108 to 115) and increase in stroke volume larger. Decrease in end-diastolic volume during exercise occurred in normal dogs but

slight increase was the rule with partial or total denervation.

Although total denervation depresses cardiac response to exercise in absolute terms, considerable increase in stroke volume and work is still possible. Increase in rate, the dominant mechanism in normal dogs, makes no contribution if the heart is denervated. The behavior of end-diastolic volume during exercise in denervated animals opposes the view that residual response is due to adrenal medullary release. The most likely stimulus to the left ventricle under these circumstances is increased filling.

Erythropoiesis and Blood Destruction in Congestive Heart Failure. ROBERT B. CHODOS, WILLIAM CHAFFEE and RAYMOND WELLS, JR., Syracuse, N. Y. (introduced by B. V. Jager).

The red cell volume, as well as the plasma volume, may be increased in congestive heart failure. Following compensation, red cell volume may decrease in certain patients. The mechanism responsible for this decrease has not been demonstrated. Studies have therefore been undertaken to determine whether this decrease is due to depression of erythropoiesis, an increase in destruction of red cells, or a combination of these processes.

Red cell and plasma volumes were measured independently during failure and after compensation, employing radiochromium (or radiophosphorus) and Evans blue methods, respectively. Erythropoiesis was estimated by radioiron turnover techniques. Destruction of red cells was evaluated by radiochromium red cell survival and by estimation of fecal urobilinogen excretion.

Red cell volume decreased significantly in 5 of 10 patients studied, plasma volume in 6 of 10, and total blood volume in 8 of 10. There was no evidence of increased blood destruction. Red cell survival was normal in all patients and there was no increase in fecal urobilinogen. During cardiac decompensation, the half-time of plasma iron disappearance was rapid (0.33 to 1.15 hours) and plasma and red cell iron turnover were both consistently increased (43 to 182 mg per day). After compensation the half-time of plasma iron disappearance increased (1.0 to 2.0 hours) and plasma and red cell iron turnover both decreased strikingly. Control studies in normal subjects did not show significant changes in iron turnover.

These data indicate a definite decrease in iron turnover following compensation from congestive failure. It is suggested that the mechanism responsible for any decrease in red cell volume is a compensatory decrease in erythropoiesis.

Correction by Heparin of Coagulation Abnormalities Due to Intravascular Clotting. DALLAS V. CLATANOFF, Madison, Wis. (introduced by Robert F. Schilling).

Low fibrinogen may be the result of intravascular clotting with or without demonstrable increase in fibrinolytic activity. The intravenous infusion of homologous dog brain thromboplastin produces a prompt and severe reduction in fibrinogen, Factor V, prothrombin, antihemophilic globulin, and platelets. When the same quantity of thromboplastin was given to heparinized dogs no significant decrease in these coagulation factors occurred. Histologic sections of lung tissue showed fibrin deposits in small vessels of the lung in the dogs receiving only thromboplastin, while none was observed in dogs which were heparinized prior to the thromboplastin infusion. The amount of intravascular fibrin deposition was greatly increased in dogs which were given homologous fibrinogen during the thromboplastin infusion.

Patients with visceral neoplasms demonstrating abnormalities of the coagulation mechanism due to intravascular clotting were studied. One patient with hemorrhagic manifestations and three patients with thromboembolic phenomena had low fibrinogen (110 to 150 mg). Additionally, in these patients, other coagulation abnormalities of variable degree included platelet counts as low as 90,000 per cu mm, prothrombin levels to 40 per cent, and Factor V levels to 40 per cent. *In vitro* evidence for increased fibrinolysis could not be demonstrated in any of the patients. When given parenteral heparin there was significant improvement or complete return to normal of these alterations in a period of 3 to 7 days. Relapse occurred in the two patients in whom heparin was discontinued.

It is concluded that heparin therapy can control the manifestations of intravascular clotting and that fibrinogen administration alone might be hazardous if thromboplastic substances are continuing to enter the circulation.

Serial Changes in Body Water, Exchangeable Sodium, Exchangeable Potassium and Serum Electrolyte Concentrations During the Treatment of Ascites. BERNARD F. CLOWDUS, JOHN A. HIGGINS, A. L. ORVIS and W. H. J. SUMMERSKILL, Rochester, Minn. (introduced by H. R. Butt).

Measures used in treating ascites have been held responsible for sodium and potassium depletion, with subsequent hyponatremia, uremia and coma, in patients with liver disease. Serial determinations of total body water (TBW), exchangeable sodium (Na_E), and exchangeable potassium (K_E) in relation to serum electrolyte concentrations were, therefore, made in 10 patients with cirrhosis and ascites during therapy. The latter comprised a low sodium diet and treatment with diuretics (mercaptomerin, chlorothiazide, prednisone and/or Aldactone). External sodium and potassium balances were carried out in 9 instances.

Before treatment, TBW and Na_E were high in every patient; in 4 patients judged to be free from excess fluid after therapy, mean initial values were 13 and 50 per cent, respectively, above the final readings. During treatment, weight loss followed water loss, but the latter correlated poorly with sodium loss; the ratio of sodium to water (Na_E/TBW) varied widely, both initially (100 to 72

mEq per L) and in its change with treatment (+1.4 to −23.0 mEq per L). K_E and potassium balance did not alter significantly throughout the studies.

Serum sodium (Na_S) and potassium (K_S) concentrations had no direct relationship to Na_E and K_E, and sodium or potassium depletion was not observed. As changes in Na_E correlated with the external sodium balance, fluctuations in Na_S appeared to reflect movements of sodium between intracellular and extracellular compartments, rather than its "sequestration" in or "mobilization" from the skeleton as a result of treatment.

Hyponatremia and uremia in patients with ascites were associated with high rather than low Na_E values. Low-normal Na_E values were found only after effective treatment with an aldosterone antagonist, and in two patients slight hyponatremia and uremia developed under these circumstances.

The In Vivo Glucuronide Conjugation of Radioactive Steroids by the Dog Kidney. GEORGE L. COHN and MICHAEL HUME, New Haven, Conn. (introduced by David Seligson).

It has been established that the liver is the main site of steroid glucuronide formation, but it is not known whether or not the kidney can carry out a similar function. Dutton has observed that kidney tissues contain enzyme systems which can form glucuronides. In order to test the hypothesis that the dog kidney can form glucuronides *in vivo,* the following experiments were carried out. With a Potts clamp on the renal vein at its junction with the inferior vena cava, radioactive 17-ketosteroids were injected into the renal artery and blood was obtained simultaneously from the catheterized renal vein and internal jugular vein. Urine formed during the course of the perfusion was also collected. Nephrectomy was carried out at the conclusion of the experiment. Steroids were isolated by organic solvent extraction, paper electrophoresis and paper chromatography. It was found that during the perfusion of the radioactive steroid, a small proportion was converted to the glucuronide. When a tracer quantity of etiocholanolone-H^3 was injected, approximately 8 per cent was found in the free fraction, while 16 per cent was converted to the glucuronide. When androsterone-H^3 of the same specific activity was injected, 28 per cent was found in the free and 14 per cent in the conjugated form.

The glucuronides were characterized by their mobility on paper electrophoretograms and paper chromatograms using pure reference standards, and by hydrolysis with β-glucuronidase and subsequent paper chromatography of the free 17-ketosteroids. Radioactivity measurements were performed throughout the procedures. No significant radioactivity could be found in the urine, internal jugular vein blood, or in the organic solvent extractions of the kidney homogenates. No sulfates or phosphates were detected. It is concluded that the dog kidney can convert 17-ketosteroids *in vivo* to their respective glucuronides. The implications of these experiments will be discussed.

Conjugation of Sulfobromophthalein Sodium (BSP) with Glutathione by an Enzyme in the Supernatant Fraction of Liver. BURTON COMBES and GENEVA SUE STAKELUM, Dallas, Tex. (introduced by Gladys Fashena).

Conjugation of BSP with glutathione or cysteine, in the liver, is now considered to account for the ninhydrin-reacting BSP compounds excreted in bile of man, rat and dog. The present studies indicate that conjugation of BSP is catalyzed by an enzyme in the supernatant fraction of liver. Furthermore, glutathione is by far the preferred substrate.

Incubation of BSP with rat liver homogenate (prepared in 0.1 M phosphate buffer, pH 7.8) at 37° C, in room air, yields a ninhydrin-reacting BSP compound identical chromatographically and electrophoretically with the major BSP compound in rat bile. When S^{35}-labeled glutathione is added to the homogenate-BSP mixture, radioactivity is incorporated into the synthesized BSP compound. Hydrolysis of this compound (5.9 N HCl, 100° C, 16 to 18 hours) yields glycine, glutamic acid and alanine. The latter has been shown to be cysteine minus its sulfhydryl group. It is concluded, therefore, that the compound formed is a BSP-glutathione conjugate. Conjugate is not formed by liver homogenates previously heated at 100° C for 5 minutes. All of the enzyme activity can be accounted for in the supernatant fraction of liver; none is demonstrable in microsomes or mitochondria.

Capacity to synthesize conjugate was restored to supernatant fraction previously dialyzed in distilled water or phosphate buffer, by the addition of glutathione, cysteinyl glycine or cysteine. The relative quantity of conjugate formed by rat liver with equimolar amounts of each of these substrates was 15:2:1, respectively. BSP conjugate was not formed with glycine, glutamic acid or alanine. No requirement for cofactor has been demonstrated.

Conjugating enzyme has also been found in homogenates of liver obtained from man, dog, guinea pig, mouse and cat. The observation that glutathione is the optimal substrate in these species provides strong evidence that conjugation of BSP with glutathione is the major pathway of BSP metabolism.

Some Studies on the Nature of Quinidine-Albumin Interaction. HADLEY L. CONN, JR.* and ROBERT J. LUCHI, Philadelphia, Pa.

The fundamental nature of quinidine action on the heart remains unknown. From earlier data, the authors have postulated that the prime tissue reaction of quinidine is a selective binding to certain protein sites. Some of these contribute to the normal "binding" of ions. If the binding involves a "critical" cell protein, the result is a pharmacologic or toxic effect. If it involves a "non-critical" protein such as albumin, drug activity is reduced. As a start toward collecting data pertinent to this hypothesis, the nature of quinidine-albumin interaction was studied in a dialysis equilibrium system.

The results showed a binding of 1 mole of quinidine per mole of albumin at pH 7.4 and an association constant of 1.3×10^4, indicative of tight and rather specific binding. With increasing pH, binding increased. Furthermore, at pH 10 the mole ratio became 3 quinidine to 1 albumin, suggestive of alteration in albumin structure. Ionic effects in addition to that of hydrogen ion were investigated. Chloride weakly and calcium ions strongly interfered with quinidine-albumin binding. Studies of competition for albumin sites between various fragments of and the total quinidine molecule revealed that the two major quinidine binding sites involve the quinoline ring nitrogen and the bridging secondary alcohol. The reactive sites in albumin were investigated by studying binding after preliminary combination of the protein with various compounds known to react with specific groups. The results suggest that the albumin sites involved are a lysine ϵ amino group and a spatially adjacent carboxyl group.

Conclusions are that *1*) albumin-quinidine binding is of a rather specific nature; *2*) in so far as this reflects interference with ion metabolism, hydrogen and calcium seem most affected. It is of special interest to discover whether quinidine-albumin binding is a prototype of all quinidine-protein interactions.

Stimulation of Steroid Formation of Adrenocortical Homogenates by Medulla and Catecholamines. D. Y. Cooper, Otto Rosenthal and W. S. Blakemore, Philadelphia, Pa. (introduced by Robert E. Forster).

In a study of *in vitro* corticosteroid synthesis by adrenal slices we noticed that slices containing medulla and cortex produced more steroids than slices containing cortical tissue alone, although medullary slices by themselves were inactive. To explore the mechanism of this phenomenon we have studied the effect of medullary homogenates upon conversion of progesterone to 17,21-dihydroxycorticosteroids (Silber-Porter chromogens) by bovine cortical homogenates in the following system: Krebs Ringer-bicarbonate-2 per cent albumin (7 ml); progesterone (1 μmole); glucose-6-phosphate (24 μmoles); TPN (2.4 μmoles); cortical homogenate (approximately 100 mg); and gas phase 5 per cent CO_2 in O_2; 37° C; 4 hours. Addition of 15 mg of medulla increased corticoid formation by 30 to 100 per cent. Larger additions (up to 120 mg) produced additional though smaller increments. The supernatant fraction of centrifuged medullary homogenate from 15 mg tissue was as effective as the whole homogenate. However, larger amounts did not produce additional increments. Catecholamines (0.3 μmole of epinephrine, norepinephrine or isoproterenol) produced stimulation indistinguishable from that of the medullary supernatants. These results show that acceleration of corticoid formation by medullary tissue is due in part to catecholamines. Paper chromatographic analysis of the hydroxylation products of progesterone, 17α progesterone, 11-desoxycorticosterone and 11-desoxycortisol indicate catecholamines enhance hydroxylation at C-21 and perhaps C-17 but not at C-11.

Transmanganin, the Specific Manganese-Carrying Protein of Human Plasma. George C. Cotzias* and Albert J. Bertinchamps, Upton, N. Y.

We have shown earlier that the body's manganese exists largely as readily dissociable complexes. In spite of loose binding, manganese follows a highly specific pathway leading chiefly through the mitochondria. The specificity of this element's behavior *in vivo* was discordant with the lack of specificity it displayed *in vitro*. This discord suggested the existence of a specific manganese-carrying plasma protein (transmanganin).

Bound Mn^{54} and Fe^{59} localized on the β_1-globulins electrophoretically. The metals exchanged with their own isotopes but not with each other, a fact confirmed with ultrafiltration. Purified transferrin was avid for iron, not for manganese.

The preponderant valence of transmanganin-bound manganese was intermediate between valences 2^+ and 4^+. Therefore most probably it is valence 3^+, which is unstable when not bound. This was shown by extensive tests employing aerobiasis, anaerobiasis, oxidation and reduction for breaking and formation of the complex. Magnesium competed only with divalent manganese.

Serial ultrafiltrations of the same sample (fractional ultrafiltration) showed a nonartifactual, striking, progressive fall of the Mn^{54} concentration in the ultrafiltrates. On the basis of the mass law, this meant the linking of more than one globulin molecule per manganese atom. Similar behavior was displayed by insulin-bound Zn^{65}, plasma- and transferrin-bound Fe^{59}. Control systems using some of these same metals showed the classical stability on fractional ultrafiltration.

These experiments reconcile the *in vitro* with the *in vivo* behavior of manganese: the physiologically transported trivalent manganese necessitates stabilization by binding to transmanganin. This globulin exemplifies a novel group of metalloproteins in which the metal binds more than one protein molecule. The ambient redox potential controls association and dissociation, thus determining specificity and direction of transport. Such behavior should be looked for intracellularly (where manganese concentrates) in order to define the biochemical specificity of this important cofactor.

Red Cell Shape and Red Cell Life Span in Hereditary Spherocytosis. William H. Crosby* and Marcel E. Conrad, Washington, D. C.

Subjects of the study were two healthy young men with hereditary spherocytosis (HS). Both had a well compensated hemolytic disease (short red cell life span without anemia). They were phlebotomized each week until they developed iron deficiency anemia and their erythrocytes became hypochromic and flattened. The abnormal behavior of the HS red cells in fragility tests was greatly improved and in one case became normal. However, the short life span of the cells was not improved by the change in shape. $T_{\frac{1}{2}}$ of Cr^{51}-labeled red cells before and after phlebotomy was 9 days in one patient and 15 days in the other. Later, splenectomy corrected the

hemolytic disease in both patients. In one of them the iron deficiency was not treated, but after the operation the hypochromic cells survived normally in his circulation. In the other one the administration of iron restored the red cells' spheroidal shape, and they too survived normally after splenectomy.

The shape of the red cell in HS does not appear to be responsible for its premature destruction by the spleen. Iron deficiency corrects the spherocytosis but it does not correct the hemolytic disease; splenectomy corrects the hemolytic disease but it does not correct the spherocytosis.

Physiological Studies in a Family with Nephrogenic (Vasopressin-Resistant) Diabetes Insipidus (N.D.I.). RALPH CUTLER, CHARLES R. KLEEMAN,* J. THOMAS DOWLING and MORTON H. MAXWELL, Los Angeles, Calif.

Three adults and one child (age 11) of a family with hereditary nephrogenic diabetes insipidus (13 per cent affected members in 7 generations) were studied. Urinary output averaged 15 to 20 and 3 L per day in the adults and child, respectively. Maximal urinary osmolalities remained markedly hypotonic (60 to 100 mOsm per kg) during vasopressin and 5 per cent saline infusions. Dehydration (2 kg weight loss) *did not* enhance maximum osmolalities except in the child who attained 442 mOsm per kg. Cortisol administration (180 mg daily $\times 5$) augmented all parameters of maximal water diuresis (M.W.D.); isoncotic expansion of plasma volume did not augment M.W.D.

A 20 to 50 per cent reduction in GFR caused marked *antidiuresis* with minimal *increase* in urinary osmolality. During M.W.D. in one adult (upright position) a hexamethonium infusion decreased GFR to less than 15 per cent of normal and urinary volume to 0.5 ml per minute *without producing hypertonic urine* (max. osm 270). In adult cases chlorothiazide (1.5 g per day) caused a 50 to 75 per cent decrease in daily urinary volume *without producing hypertonic urine* (max. osm 174), a reversal of the normal diurnal pattern of water execretion, and at least a 30 per cent reduction in GFR. When the negative salt balance induced by chlorothiazide was prevented by a high salt intake daily, urinary volumes *did not decrease.* Chlorothiazide did not enhance the renal (tubular) response to vasopressin infusions.

Conclusions: *1)* In the adult subjects, dehydration did not concentrate urine greater than vasopressin. *2)* Chronic cortisol administration augments M.W.D. *in absence of any tubular response to vasopressin,* while plasma volume expansion did not augment M.W.D. *3)* In N.D.I. a hypertonic urine could not be produced by a marked GFR reduction during M.W.D. This contrasts with animal results (Berliner et al. J. clin. Invest. 1956, **35,** 690) and in vasopressin-sensitive diabetes insipidus (Kleeman et al. Proc. Soc. exp. Biol (N. Y.) 1957, **96,** 189). *4)* Chlorothiazide-induced antidiuresis in N.D.I. is probably due to altered body sodium and renal hemodynamics rather than to a direct effect on the concentrating segment of the nephron.

Hormonal Control of Collagen Synthesis by Cartilage Studied In Vitro. WILLIAM H. DAUGHADAY* and IDA KOZAK MARIZ, St. Louis, Mo.

Collagen and chondroitin sulfate, as chondromucoprotein, are the principal extracellular products of growing chondroblasts. Cartilage from hypophysectomized rats forms chondroitin sulfate at a reduced rate and this has been demonstrated in an *in vitro* system. Moreover, synthesis is greatly increased by adding normal serum to the medium. To study the parallel process of collagen synthesis, the conversion of proline to collagen hydroxyproline has been measured. No other source of this unique amino acid is known.

Pools of 15 costal cartilage segments were incubated in enriched medium containing essential amino acids (Eagle) and proline-U-C^{14}. After 24 hours, cartilage hydroxyproline was isolated in satisfactory radiopurity by Dowex-50 chromatography. The hydroxyproline from cartilage of young rats, hypophysectomized 2 to 3 weeks previously, had a specific activity of half that obtained from normal rats. In other experiments, costal cartilages from 6 hypophysectomized rats were distributed randomly into four flasks; the medium for two flasks contained 18 per cent normal rat serum and for the other two, 18 per cent hypophysectomized rat serum. Hydroxyproline isolated from cartilage incubated with two separate pools of normal serum had a mean specific activity of 68 per cent (range 25 to 106) greater than hydroxyproline from incubations with hypophysectomized rat serum. The results have been corrected for slight differences in initial medium proline pools determined by isotope dilution chromatographic methods. Conversion of medium proline to cartilage hydroxyproline was 1.7 and 1.7 μg per 100 mg of cartilage in two incubations with hypophysectomized rat serum and 2.6 and 3.0 with normal rat serum.

These changes in hydroxyproline formation *in vitro* are evidence of impaired collagen synthesis after hypophysectomy and its partial correction by humoral factors in normal serum. It is unknown whether the same factors stimulate both collagen and chondroitin sulfate synthesis.

Effect of Antihypertensive Treatment in the Rat on the Potentiation of Atherogenesis by Experimental Hypertension. QUENTIN B. DEMING,* MARIE M. DALY, JAIME BLOOM, LILI BRUN and RUTH KAPLAN, New York, N. Y.

In man, dog, rabbit and rat, the hypertensive state is accompanied by increased severity of atherosclerosis. Whether nonspecific control of blood pressure would prevent this effect has not been known.

In the present experiments, atherosclerosis has been produced in rats by suppression of the thyroid combined with addition of cholesterol and cholic acid to the diet. Hypertension has been produced by constriction of one renal artery. Operated animals whose blood pressures failed to rise have been used as normotensive controls. In the same length of time on diet, hypertensive rats develop more extensive atherosclerosis than normotensives.

Comparison was made between normotensive animals, hypertensive animals and hypertensive animals rendered normotensive by adjusted doses of reserpine, hydralazine, mecamylamine and chlorothiazide given concurrently. The same dietary program was maintained in the three groups.

Pharmacological control of established renal hypertension resulted in a degree of atherosclerosis significantly less than that occurring in matched, untreated hypertensive animals and approximating that in untreated normotensive animals.

Serum Thrombotic Accelerator (STA) Activity as a Measure of the Antithrombotic Effect of Dicumarol. DANIEL DEYKIN, STANFORD WESSLER* and STANLEY M. REIMER, Boston, Mass.

Experimentally-induced intravascular thrombosis can be initiated through the "intrinsic" clotting system (in the absence of tissue thromboplastin). The administration of coumarin-type drugs results in the depression of clotting factors known to be required components of this pathway. There is no experimental evidence, however, to indicate that these drugs can inhibit *in vivo* thrombosis mediated through this intrinsic system. The quantitative bioassay of the STA activity of thrombin-free mammalian serum, previously demonstrated in this laboratory, is a measure of experimental *in vivo* thrombosis mediated through the intrinsic pathway. This assay permitted exploration of the intrinsic system as a possible site for an antithrombotic action of Dicumarol. Nine dogs received Dicumarol in doses adequate to maintain the one-stage prothrombic activity at less than 10 per cent of the control values for 2 to 48 days without inducing hemorrhage in any animal. Three dogs served as controls. The following parameters were followed serially in all animals: weight, temperature, hematocrit, *in vitro* assays of one-stage prothrombic activity, clotting factors II (prothrombin), V (ac-globulin), VII (convertin), VIII (antihemophilic globulin), IX (PTC), X (Stuart), Hageman and PTA; and an *in vivo* assay of STA activity. A statistically significant antithrombotic effect as measured by the loss of STA activity was observed in the sera of 8 of the 9 treated animals. This was related, in part, to the dose of anticoagulant and was independent of the depression of any clotting factor, singly or in combination, known to be affected by Dicumarol. This is considered to be the first experimental demonstration of an *in vivo* antithrombotic action of Dicumarol mediated through the intrinsic clotting system.

Hypoaldosteronism in Panhypopituitarism. JOSEPH F. DINGMAN, EDUARDO GAITAN, MAX C. STAUB, AKIRA ARIMURA and RALPH E. PETERSON,* New Orleans, La. and New York, N. Y.

There are several reports of hypoaldosteronism with selective adrenocortical insufficiency but hypoaldosteronism from adenohypophyseal destruction has not been described. Of two untreated patients with documented postpartum pituitary necrosis, one had two adrenal crises two years ago, with hyponatremia and shock precipitated by chronic bleeding and water intoxication, respectively. Urinary aldosterone, determined by double isotope derivative assay (normal range 5 to 20 μg per day), was 0.6 and 2.8 μg on normal salt diet prior to therapy; only 5.0 and 8.0 μg in two studies on 10 mEq Na diets plus 25 mg cortisone, during which adequate Na retention occurred without decompensation; and 10.0 and 15.0 μg on ACTH, which also increased urinary steroids to normal levels. The patient did well on cortisone, thyroid and liberal salt intake. Persistent aldosterone defect was highlighted by a recent crisis off cortisone during which urinary aldosterone was only 1.0 μg. Normal salt balance is now maintained on ACTH alone. The second patient had hyponatremia and coma with aldosterone value of only 1.2 μg per day but now maintains sodium balance on 25 mg cortisone alone. A third patient with breast cancer and cranial metastases had mild hypopituitarism after androgen therapy and achieved aldosterone levels before hypophysectomy of only 3.5 μg on low salt diet despite normal Na retention, and 6.5 μg on ACTH. Postoperative hyponatremia required both cortisone and mineralocorticoid therapy, and withdrawal of either steroid has precipitated mild adrenal crises during which aldosterone values were 2.5 and 2.7 μg, respectively. These studies show preservation of sodium homeostasis in hypoaldosteronism by some protective or potentiating action of cortisone. Although basal aldosterone secretion may continue in hypophyseal disorders, increased secretion may require aldosterone-stimulating actions of ACTH, the absence of which may result in relative hypoaldosteronism and sodium loss in hypopituitarism during stress.

Enzymatic Activity of Isolated Kidney Glomeruli in Two Types of Experimental Nephrosis. ULRICH C. DUBACH and LILLIAN RECANT,* St. Louis, Mo.

In nephrosis, attention has been focused upon specific alterations in glomerular structure. The foot process lesion identified by electron microscopy has been found in many varieties of this syndrome. Since it seemed possible that specific enzymatic alterations might be associated with this lesion, microbiochemical analyses of single glomeruli from normal nephrotic animals were made. Previous reports from this laboratory have described certain enzymatic activities in the normal glomerulus. In fully established aminonucleoside nephrosis, enzymatic analyses revealed a decrease in alkaline phosphatase and an increase in glucose-6-phosphate dehydrogenase activity.

In the present study, an attempt was made to trace the development of the changes in these enzymes in preproteinuric (6 animals) and proteinuric (9 animals) phases of aminonucleoside disease. Similar studies were made of antikidney serum nephrosis (6 animals). In addition, enzymatic analyses of liver and pancreas were carried out to assess specificity of renal enzyme changes. During maximal proteinuria in aminonucleoside nephrosis,

glomerular phosphatase decreased 40 per cent (mean normal 3.12 moles per kg dry weight per hour), while dehydrogenase increased 40 per cent (mean normal 1.15 moles per kg per hour). On the fourth and seventh day of aminonucleoside injection (prior to proteinuria), neither phosphatase nor dehydrogenase activities were altered. Nephrosis induced with antikidney serum produced similar changes. Enzymatic analyses of kidney homogenates showed changes similar to those noted in glomeruli in both types of nephrosis. Analyses of liver and pancreas homogenates were variably altered in both types.

These observations indicate that two etiologically different nephroses result in specific, similar enzymatic activity changes for glomeruli as well as for whole kidney homogenates. These findings appear to correlate with the magnitude of proteinuria. It is suggested that they represent the "deleterious" effects of continued protein transport through the glomerular structures.

Binding of Magnesium to Microsomal Nucleoprotein (RNAP) and Ribonucleic Acid (RNA). I. S. EDELMAN,* P. O. P. TS'O and J. VINOGRAD, San Francisco and Pasadena, Calif.

Thorough understanding of the mechanisms of amino acid assembly in RNAP in the process of protein synthesis requires detailed knowledge of the structural organization of microsomes. Earlier studies have shown that the structural integrity of microsomal RNAP is critically dependent on the attachment of an optimal number of Mg ions in the particle. We therefore undertook to study the details of the binding of Mg to RNAP and RNA.

Microsomal particles were isolated by differential ultracentrifugation from reticulocytes obtained from rabbits with phenylhydrazine anemia. The RNA was extracted from the same lot of microsomal RNAP. Exchange studies were carried out with Mg^{28} using dialysis chambers in one set of experiments and serial ethanol precipitation in another. Mg content was determined by eriochrome-EDTA titration and RNA content by ultraviolet absorption and by phosphorus (P) analysis. Particle size and homogeneity were evaluated by analytical ultracentrifugation.

Mg bound to RNAP and to RNA exchanged completely with buffer Mg^{28} in 30 minutes at 3° C. In 0.14 M KCl, 0.0015 M $MgCl_2$ buffer the Mg:P molar ratio was 0.18 and 0.23 for RNAP and RNA, respectively. In buffer of low K:Mg ratio, ribosomes consisted mostly of 80S particles which dissociated into subunits in buffer of high K:Mg ratio. Three lines of evidence were obtained to indicate that all of the Mg is bound by the RNA moiety: *1*) the kinetics of Mg exchange were indistinguishable in RNAP and in RNA, *2*) the Mg^{28} bound to RNAP was quantitatively recovered in the RNA moiety and, *3*) the Mg:P molar ratios of RNAP and RNA showed a parallel dependence on the buffer Mg concentration. Moreover, removal of the protein moiety uncovered about 30 per cent more binding sites for Mg. This may mean that the protein moiety and Mg compete for the same binding sites.

Studies Suggesting the Presence of an Extrahypothalamic ACTH-releasing Center in the Dog. RICHARD H. EGDAHL, Richmond, Va. (introduced by David M. Hume).

It is established that properly placed hypothalamic lesions markedly diminish or prevent the increased adrenal cortical secretion following operative trauma. The present experiments provide evidence that another, lower, CNS area has potential for regulatory function in ASTH release, and that this center is normally inhibited in the intact animal by nervous impulses from the cerebral cortex. Three types of CNS operative procedures were performed in dogs with chronic adrenal vein cannulas: total brain removal down to the inferior colliculus with intact pituitary ("isolated pituitary"); midbrain transection; bilateral decortication. Adrenal venous content of 17-hydroxycorticosteroids was determined by the method of Nelson and Samuels, and adrenal venous output per minute was calculated.

Results were as follows: *1*) All dogs with "isolated pituitaries" revealed elevated "resting" corticosteroid outputs 24 to 72 hours after surgery, and in two-thirds of these animals nerve stimulation resulted in further increased adrenal cortical secretion. *2*) Decorticate dogs demonstrated "elevated" resting levels of adrenal cortical secretion. Nembutal anesthesia prevented the high resting levels but left intact the increased adrenal cortical secretion following nerve stimulation. *3*) The response of dogs with midbrain transection was similar to that of the "isolated pituitary" dogs.

It is concluded: *1*) High resting levels and responses to stimulation in "isolated pituitary" dogs indicate that there is a lower CNS ACTH-releasing center, probably located in the hindbrain. A neurohumor (HBF) which results in ACTH secretion by the pituitary must be released from this area, inasmuch as the anterior pituitary is not innervated. *2*) Decorticate dogs have high resting corticosteroid outputs because the cerebral cortex is a principal inhibitor of the HBF-releasing area. The latter area can be depressed with Nembutal but a normal response to nerve stimulation is present because of an intact hypothalamic mechanism. *3*) Results of midbrain transection experiments indicate that cerebral inhibition of the HBF-releasing area is effected through nervous and not humoral pathways.

Bone Magnesium, Calcium and Citrate Interrelationships. LEONARD P. ELIEL,* JOSEPHINE HAWRYLKO and JOHN COMSTOCK, Oklahoma City, Okla.

Hypoparathyroid patients or intact dogs, given parathyroid extract, and patients with malignant osteolytic disease, display losses of Mg and Ca in a much higher Mg/Ca ratio than exists in bone. Blood and urine citrate generally parallel the changes in Mg and Ca balances in these conditions. Tissue analyses have not

revealed the site of Mg depletion. Dog bone and synthetic hydroxyapatite have been incubated with dog plasma or synthetic plasma ultrafiltrate to clarify the relationships between extracellular fluid Mg, bone Mg, bone Ca, and citrate concentration.

Powdered dog bone (1 g) incubated with plasma or ultrafiltrate (10 ml) removed Mg, Ca, P, and Na from the supernatant. Ultrafiltrate concentrations of these ions were reduced 0.6, 0.4, 0.6, and 9 mEq per L, respectively. Incubation with 0.01 M citrate resulted in contributions of these ions, by bone to ultrafiltrate, of 1.6, 3.6, 1.1, and 6 mEq per L, respectively; while 6.9 mEq per L of citrate was removed from the ultrafiltrate to bone. The increase in Mg was 10 times that anticipated from the normal Mg/Ca ratio in dog bone. Citrate-incubated bone, washed and re-equilibrated with ultrafiltrate alone, removed more Mg (1.0 mEq per L) than bone initially incubated without citrate. Synthetic hydroxyapatite (0.5 g) showed great Mg affinity, removing 1.6 mEq per L from the ultrafiltrate. Removal was prevented by addition of citrate.

These studies suggest that: *1*) Plasma or ultrafiltrate is supersaturated with Mg in respect to dog bone or hydroxyapatite. *2*) Citrate can accomplish, by ionic exchange, removal of Mg and Ca from bone in a much higher Mg/Ca ratio than exists in bone. *3*) The location of Mg in the hydration shell of bone crystals may make it more available than Ca for ionic exchange. *4*) The degree of Mg saturation of the hydration shell, local citrate concentrations and parathyroid activity may be significant factors governing Mg exchange and concentrations in extracellular fluid and bone.

Preferential and Active Transport of Linoleic Acid by Polymorphonuclear Leukocytes. PETER ELSBACH, New York, N. Y. (introduced by L. W. Eichna).

Previous experiments using acetate–1–C^{14} as an index of synthesis have shown that polymorphonuclear leukocytes, obtained from rabbit peritoneal exudates, synthesize most fatty acids *in vitro*. However, little or no radioactivity was found associated with linoleic, linolenic and arachidonic acid, following recovery of individual fatty acids after gas-liquid chromatographic separation. Despite this evidence that these three essential fatty acids were not synthesized, it was found that the composition of the fatty acids remained constant in leukocytes actively metabolizing in a medium containing serum lipids (ascitic fluid). It was therefore concluded that intracellular levels are maintained by preferential transport and/or relative exclusion from metabolic utilization.

The results of the present study indicate that preferential transport does take place and that the fatty acids accumulated are also metabolized. Leukocytes were incubated in ascitic fluid containing either linoleic acid–1–C^{14} (not synthesized by the leukocytes) or palmitic acid–1–C^{14} (actively synthesized). Linoleic acid was reproducibly concentrated in the cell-lipids at a rapid rate, while palmitic acid was incorporated erratically and usually at a much slower rate. The transport of both linoleic and palmitic acid into the cell was largely dependent upon anaerobic glycolysis, since iodoacetic acid and sodium flouride inhibited uptake of labeled fatty acids, whereas dinitrophenol and KCN had little or no effect. No transport occurred during incubation at 4° C. Metabolic utilization of accumulated linoleic–1–C^{14} and palmitic acid–1–C^{14} during incubation at 37° C was evidenced by appearance of increasing amounts of C^{14} activity: *1*) in CO_2 produced; *2*) in water-washes of lipid extracts of successive samples of cells and medium; *3*) in other fatty acids of lipids of cells and medium.

It is concluded that for maintenance of intracellular fatty acid composition, and leukocytes rely partly upon transport inward and that in the case of linoleic acid, transport is the sole mechanism.

Studies on the Equilibration of Thyroxine Between the Intravascular and Extravascular Spaces. NORMAN H. ENGBRING, EDWARD J. LENNON and WILLIAM W. ENGSTROM,* Milwaukee, Wis.

In an effort to define factors involved in the *in vivo* transport of thyroxine, the rate of disappearance of I^{131} L-thyroxine (T_4*) from the intravascular compartment of human subjects was acutely determined during the first hour after intravenous injection. A linear regression, log $Y = a + bx$, was found in each during the interval from 20 to 50 minutes after injection ($p = 0.01$). The mean half-time of disappearance for 16 normal, euthyroid subjects was very constant, 73.7 ± 6.6 minutes. Covariance analysis indicates parallel regression slopes. Studies on patients in other situations revealed: *1*) Greatly prolonged survival in spontaneous myxedema (9 cases), 110.7 ± 21.9 minutes, and in liver disease (10 cases), 163.6 ± 21.5 minutes—undoubtedly due to increased avidity of serum proteins for T_4* in the former and defective hepatic extraction in the latter. *2*) Markedly shortened survival in hyperthyroid Graves' disease (8 cases), 54.9 ± 9.0 minutes and in hypoproteinemic nephrosis, indicating that short T_4* survival and thyrotoxicosis are not necessarily related. *3*) Minimal effect on survival in 4 normal subjects fed 20 grains of dried thyroid, 67 ± 5.6 minutes. *4*) Lack of prolonged survival (68.6, 78.8 and 85.0 minutes) in three profoundly myxedematous patients after treatment of Graves' disease, indicating a persistence of a defect in equilibration. *5*) Estrogen administration to normals quickly (within 48 hours) prolonged T_4* survival, even before changes in protein-bound iodine (PBI), indicating very rapid effects on the serum proteins.

While correlation exists ($p = 0.01$) between level of PBI and T_4* survival among normals, those with spontaneous myxedema and untreated Graves' disease, observations 4 and 5 indicate T_4* survival may change independent of PBI.

This method is complementary to *in vitro* determinations of serum thyroxine-binding capacity and to long-term T_4* survival, the latter presumably measuring thyroxine degradation rate *after* equilibration among the binding compartments. The reported method is well

adapted to study factors involved in rate of egress of thyroxine from the circulation.

Inhibition of Incorporation of Orthophosphate into ATP by Fatty Acids. A. B. FALCONE, R. L. MAO and E. SHRAGO, Madison, Wis. (introduced by Ovid O. Meyer).

Numerous studies by others have demonstrated the rapid and considerable oxidation of fatty acids by various tissues. Pressman and Lardy (Biochim. biophys. Acta 1956, **21**, 458) reported that various fatty acids bring about a marked stimulation of mitochondrial ATPase activity.

In rat liver mitochondrial systems we have found that various saturated and unsaturated fatty acids inhibit the incorporation of P^{32}-labeled orthophosphate into ATP which occurs in the absence of oxygen uptake. This exchange reaction is thought to represent a portion of the coupling mechanism of the process of oxidative phosphorylation (Boyer, Luchsinger and Falcone, J. biol. Chem. 1956, **223**, 405). Inhibition by fatty acids was greatly augmented by blocking electron transport in the respiratory chain by inhibitors such as potassium cyanide, sodium azide, amytal, and antimycin-A. Strict anaerobic conditions had a similar effect. Significant inhibition was observed with concentrations of fatty acids which did not stimulate mitochondrial ATPase activity. Surface active agents such as desoxycholic acid and sodium lauryl sulfate were found to be inhibitory at concentrations one order of magnitude greater than that found for fatty acids.

The above findings point to a possible role of fatty acids as important regulatory agents in the overall energy economy of the cell.

The Effect of Sodium and Potassium Intake Upon the Natriuretic Response to Spirolactones. WILLIAM W. FALOON, FREDERIC F. TAYLOR, SAMUEL HELLMAN and SIMON OHANESSIAN, Syracuse, N. Y. (introduced by Eugene L. Lozner).

The effect of variations in electrolyte intake upon natriuresis during spirolactone administration has been studied in patients with hepatic cirrhosis and ascites. Spirolactone daily dosage was either 1 g of the 19-noranalog (SC 8109) or 400 mg of thioacetoxy analog (spironolactone) orally.

In two patients receiving SC 8109, average *net* daily sodium loss in comparable 6 day periods was 86.9 mEq during 10 mEq sodium intake compared to 8.6 mEq during 112 mEq sodium intake, the diet being otherwise constant. In four patients receiving SC 8109 with diets containing 10 mEq of sodium, infusion of hypertonic saline (1.8 mEq per kg of estimated dry weight) decreased the *net* sodium loss by 60 per cent or more on the infusion day. In four patients given spironolactone and low sodium diets (10 to 20 mEq) increasing potassium intake from 80 mEq daily to 160 mEq for 4 days increased sodium excretion by 50 per cent or more during the potassium supplement period. Urine sodium decreased upon withdrawal of potassium supplements. One of these patients subsequently given a higher sodium intake (76 mEq) showed no significant response to potassium supplementation. In four patients receiving low sodium diets with spironolactone, reducing daily potassium intake from 85 to 15 mEq yielded a decrease in urinary sodium of 50 per cent or more. Natriuresis was restored when the low potassium diet was supplemented with 78 mEq of potassium. In one patient exhibiting net sodium loss of 147 mEq daily during spironolactone with a low sodium diet, addition of 68 mEq to the sodium intake for 6 days reduced net loss to 96 mEq.

These studies indicate that high sodium or low potassium intake decreases and the reverse of these increases the net sodium loss during siprolactone administration. Maximum response appears to be obtained by a low ratio of sodium to potassium intake.

Evidence that Atheroma Fatty Acids Are in Flux. JOHN W. FARQUHAR, ROBERT L. HIRSCH and E. H. AHRENS, JR.,* New York, N. Y.

Assuming that human atheromata are not synthesized and degraded entirely *in situ,* we must postulate that their various lipid components exist in some sort of dynamic equilibrium with circulating lipids. In view of recent experiments in man with diets rich in unsaturated fats, it was asked whether such diets alter the fatty acid composition of atheromata.

Tissues were obtained at autopsy or exploration from 5 patients who had received corn oil as sole dietary fat (40 per cent of caloric intake) for 4, 5 and 9 weeks and 18 and 36 months. "Control" tissues were obtained at autopsy from 5 males of comparable age (31 to 55 years). Aortic atheromata were analyzed separately as small and thin (grade 1) and larger and thicker (grade 2) lesions; also, studies were made of "normal" aortic intima and media. Plasma and liver lipids were studied as examples of actively metabolizing fatty acid compartments. Concentrations of lipid classes (triglycerides, cholesterol esters and phospholipids) were measured. After separation of these classes by silicic acid chromatography, their constituent fatty acids were measured by gas-liquid chromatography.

The two atheroma grades showed similar concentrations of lipid classes and fatty acid compositions of triglycerides and cholesterol esters. All tissues of corn oil-fed patients, including atheromata, showed higher contents of linoleic acid (18:2) in all lipid classes than those of the control group; differences were greater the longer the intake. Most marked changes in fatty acid composition occurred in triglycerides (*i.e.,* from 10 to 40 per cent 18:2). Alterations in fatty acids occurred most rapidly in the plasma and liver, while the rate at which atheroma lipids acquired the characteristics of the fed fat was far slower and comparable to adipose tissue.

The findings constitute strong evidence for the existence of a slow flux of atheroma fatty acids and interchange with dietary fat.

The Binding Capacity of Tuberculous and NonTuberculous Human Serum for I^{131}-Labeled Extracts from Tubercle Bacilli. RICHARD S. FARR and HUBERT BLOCH, Pittsburgh, Pa. (introduced by Wallace N. Jensen).

Circulating antibodies against antigens from *Mycobacterium tuberculosis* have been detected in many tuberclous patients. A sensitive quantitative method is needed to evaluate the clinical significance of these antibodies and the nature of the antigens which elicit their production. The present study describes a quantitative system which measures binding between constituents of *M. tuberculosis* and antibodies in sera from tuberculous patients.

A cell-free aqueous extract composed of multiple antigens of *M. tuberculosis* was labeled with I^{131} and incubated with 0.0125 ml human serum at 4° C for 24 hours. Soluble antigen-globulin complexes were then precipitated with rabbit antihuman globulin. The I^{131} activity of the precipitate was used to measure the binding capacity of patient sera for the bacterial extract. As a result of specific bonds between antibodies and I^{131}-labeled antigens in the extract, the average binding capacity in sera from tuberculous patients was higher than in sera of normal individuals or patients with a variety of nontuberculous diseases. In a series of sera from 88 tuberculous patients, 37 had a higher binding capacity than was found in any of the 63 nontuberculous control patients. Enhanced binding capacity was found in a tuberculous patient with negative tuberculin tests (10 mg Old Tuberculin) and was absent in normal subjects with positive tuberculin tests. Specificity was established by demonstrating that antigenic material from sources other than tubercle bacilli (analogous extracts from *E. coli,* heterologous red cells and bovine serum albumin) failed to interfere with the reaction, whereas 1 μg of the unlabeled test antigen greatly reduced precipitation of the I^{131}-labeled antigen.

These results suggest that the humoral immune response in tuberculosis can be quantitated more precisely than previous methods have permitted.

The Effect of Hypercalcemia on Renal Tubular Function. THOMAS F. FERRIS, HOWARD LEVITIN and FRANKLIN H. EPSTEIN,* New Haven, Conn.

A 6 year old boy with congenital hypoparathyroidism had been treated for two years with vitamin D. He had several confirmed episodes of hypercalcemia during this period and finally entered the hospital with a two month history of polyuria and anorexia. Laboratory tests on admission showed: CO_2 7.4 mm per L, K 1.9 mEq per L, Cl 111 mEq per L, BUN 10 mg per 100 ml. The urine was alkaline and hypotonic to plasma. Polyuria and hyposthenuria were resistant to vasopressin. At autopsy he was found to have nephrocalcinosis.

Prompted by this clinical history, a study was undertaken to evaluate the renal effects of hypercalcemia induced in rats by vitamin D; 200,000 units of vitamin D were given intraperitoneally for 4 consecutive days. This dose maintained an elevated serum calcium for at least 12 days without an elevation in BUN. Rats so treated were found to have a diminished ability to concentrate the urine but minimum urinary osmolality attained during water diuresis was unimpaired. Rats put on a low K diet for 6 days and then rendered hypercalcemic were found to have a diminished ability to conserve potassium when compared to pair-fed controls. This potassium wasting continued up to 7 days after the cessation of vitamin D, when the animals were sacrificed. The ability of hypercalcemic rats to excrete an acid load was essentially unchanged when compared to controls, until the dosage of 1.75 mmoles NH_4CL per 100 g rat was attained. At this level the hypercalcemic animals displayed a diminished ability to excrete acid, accompanied, however, by an elevated BUN.

It appears that experimentally induced hypercalcemia produces a renal tubular lesion characterized by loss of concentrating ability and impairment of potassium conservation. Repeated episodes of vitamin D intoxication may have produced analogous pathological and functional changes in the case described.

In Vitro Stimulation of Glucose Oxidation in Thyroid Slices by Thyroid Stimulating Hormone. JAMES B. FIELD, IRA PASTAN, PHYLLIS JOHNSON and BETTY HERRING, Bethesda, Md. (introduced by J. Edward Rall).

In vitro effects of thyroid stimulating hormone (TSH) on thyroidal iodide and phospholipid metabolism have been previously demonstrated. Since these effects are delayed they are not felt to represent the primary action of TSH. Thyroid slices incubated with radioactive glucose showed a ratio greater than unity when $C^{14}O_2$ derived from glucose-1-C^{14} is compared to $C^{14}O_2$ obtained from glucose-6-C^{14}. This indicates the existence of the hexose monophosphate pathway. Addition of TSH to thyroid slices produced a two- to eightfold increase in glucose-1-C^{14} oxidation to $C^{14}O_2$ while the increase in glucose-6-C^{14} oxidation was less marked. This effect of TSH was manifest within 5 minutes after the start of the incubation and suggests that the primary action of TSH might be on glucose metabolism in the thyroid. Adrenocorticotropin, prolactin, growth hormone or follicle-stimulating hormone were ineffective. TSH had no effect on glucose oxidation in liver or testis slices. Although TSH increased glucose uptake by thyroid slices this does not appear to be its mode of action, since insulin also increased glucose uptake but did not stimulate glucose oxidation. The activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in thyroid slices was not increased by TSH. Gluconate-1-C^{14} oxidation was not stimulated by TSH. None of the antithyroid drugs interfered with glucose oxidation to $C^{14}O_2$ although there was some inhibition of the TSH stimulatory effect when propylthiouracil was present. Acetylated TSH itself was inactive and when present in 5 times the concentration of TSH there appeared to be some inhibition of the TSH effect. Serotonin, epinephrine and norepinephrine also stimulated glucose oxidation to $C^{14}O_2$ while histamine, acetylcholine, tryptophan and 5-hydroxyindoleacetic acid were inactive.

The Krebs Cycle and Diabetic Ketosis. DANIEL W. **FOSTER** and MARVIN D. SIPERSTEIN,* Dallas, Tex.

It is generally held that the overproduction of ketone bodies which accompanies diabetes is due primarily to an inability of diabetic liver to maintain normal levels of Krebs cycle carboxylic acids, in particular of oxalacetic acid. With deficiencies of these acids, oxidation of acetyl-CoA to CO_2 should proceed at a decreased rate, resulting in accumulation of acetyl CoA, which must in turn condense to form excessive ketone bodies. Despite wide acceptance of this theory of ketogenesis, there is little evidence that the Krebs cycle in diabetic liver is, in fact, deranged.

As part of an investigation of the problem of ketogenesis, a sensitive gas chromatographic method of measuring the acids of the citric acid cycle has been developed. By use of ether extraction of NaCl-saturated homogenates and subsequent fractionation on glutarate polyester columns, 0.01 μg of each Krebs intermediate can be recovered and quantitated. Livers from normal and severely ketotic (total blood ketones 25 to 194 mg per 100 ml) alloxan diabetic rats were studied. Levels of oxalacetate in the normal rat liver varied from 39 to 151 μg per g and in the diabetic, from 34 to 309 μg per g. This does not represent a significant difference. Fumarate in the normal liver ranged from 2.5 to 6.2 μg per g and in the diabetic, from 7.2 to 19.8 μg per g. Succinate concentration in the normal was 107 to 177 μg per g; in the diabetic 120 to 179 μg per g. Assay of isocitrate, citrate and *cis*-aconitic acids likewise failed to show any differences between the two groups.

These studies indicate that intermediates of the tricarboxylic acid cycle are not decreased in diabetic liver and that contrary to present concepts, the ketosis of diabetes must be due to factors other than deficiencies of the Krebs cycle substrates.

Some Properties of the Breakdown Products of Human 7S Gamma Globulin and Antibodies. E. C. FRANKLIN, New York, N. Y. (introduced by H. S. Lawrence).

Treatment of human 7S gamma globulin with cysteine and papain by the method of Porter yielded 75 to 90 per cent nondialyzable fragments. Ultracentrifugal examination revealed a major peak with an s rate of 3.4 to 3.5S and small amounts of more rapidly sedimenting material. The fragments can be fractionated into two major components (I and II) by gradient elution chromatography on a carboxymethylcellulose column, pH 7.6 (0.01 to 0.4 M). Each can be further separated into two peaks (IA and B), (IIC and D) on a diethylaminoethylcellulose column, pH 8 (0.01 to 0.3 M). Fractions A, B, and C each had s rates of 3.4 to 3.5S and molecular weights of 40,000 to 55,000. Fraction IID comprised less than 5 per cent of the total and was heterogeneous. Each fraction retained some antigenic properties of gamma globulin when tested with a rabbit antiserum.

Agar double diffusion studies and immunoelectrophoresis demonstrated close antigenic relationship of Fractions IA and IIC while IB contained an antigenically distinct component. More than three-fourths of the carbohydrate was associated with Fraction I (hexose approximately 3 per cent; hexosamine approximately 2 per cent) while Fraction II contained less than 0.4 per cent of each. Fragments prepared from antibody to streptokinase and diphtheria toxin retained the ability to neutralize the antigen. More than three-fourths of this antibody neutralizing activity was associated with Fraction IIC. The remainder was present in Fraction I. Fragments of precipitating antibody against horse serum from a patient with serum sickness no longer

precipitated. The whole digest and Fraction IIC inhibited precipitation by the original serum almost completely while Fraction IA was much less active. When tested by reverse passive cutaneous anaphylaxis in the guinea pig none of the fractions fixed to the skin, but the active fraction, IIC, greatly diminished the passive cutaneous anaphylactic reaction of the original serum with horse serum.

The Effect of Insulin upon the Peripheral Uptake of Plasma Free Fatty Acids in Man. SAMUEL J. FRIEDBERG, ROBERT F. KLEIN, MORTON D. BOGDONOFF, E. HARVEY ESTES, JR. and DAVID L. TROUT, Durham, N. C. (introduced by William M. Nicholson).

In the dog, insulin has been shown to cause a prompt reduction in the release of plasma free fatty acids (FFA) from peripheral tissues into the blood, with no change in the rate of disappearance of FFA from the blood. The present study, in man, shows that an increased removal of FFA does occur following insulin administration.

Ten male subjects were studied in the resting state following an overnight (12 to 15 hour) fast. Palmitic-acid-1-C^{14} (0.004 to 0.012 μc) was given by constant infusion through a forearm vein. Simultaneous arteriovenous samples were drawn at intervals from the opposite brachial artery and antecubital vein or from a femoral artery and vein. Following the attainment of a constant level of radioactive FFA in the arterial blood, 5 subjects received 2 units of HGF-free insulin injected into the sampled artery. In all 5 subjects, there was a marked fall in both arterial and venous FFA, a rise in specific activity, and in addition, a consistent fall in the level of labeled FFA in the arterial blood. The overall effect was a reduction in FFA turnover. In 5 control subjects there was no change in the levels of FFA or of labeled FFA. As long as the infusion was maintained there was a positive arteriovenous difference of labeled material.

These data suggest that in man insulin not only inhibits the release of FFA into the blood but also increases the rate of disappearance of FFA from the blood.

Conjugated Amino Acids in Deproteinized Plasma of Patients with Renal Failure. GEORGE W. FRIMPTER and DAVID D. THOMPSON,* New York, N. Y.

Free alpha amino nitrogen in deproteinized plasma from patients with uremia was found to be approximately normal. When deproteinized plasma was hydrolyzed with 6 N HCl, a mean increase of alpha amino nitrogen amounting to 3.0 mg per 100 ml (a 65 per cent increase) occurred as determined by the manometric ninhydrin method. This is contrasted to a mean increase of 0.6 mg per 100 ml (11 per cent) in normal subjects. The compounds giving rise to increased alpha amino nitrogen dialyzed rapidly through cellophane, suggesting that the conjugated compounds were of relatively small molecular weight.

Chromatographic separation of these compounds was performed employing a preparative ion-exchange column, 165 x 1.8 cm, of Amberlite IR-120. Two hundred 3 ml fractions were collected, and aliquots were analyzed for ninhydrin color before and after hydrolysis. Three peaks appeared after hydrolysis where little or no color was detected prior to hydrolysis. These fractions were examined further by paper chromatography, analytical column chromatography employing an automatic recording apparatus, solvent extraction, and other appropriate tests. One peak was shown to contain phenylacetylglutamine, a substance present in normal urine but not previously identified in blood. Another peak, not retained on the acidic resin, was eluted near column volume with the blood glucose. This peak could not be resolved further by chromatography on a basic resin or on paper. After hydrolysis the amino acids identified were: aspartic acid, glutamic acid, threonine, serine, glycine, alanine. The third peak displayed properties similar to those of the second.

These studies identify some of the increases in previously "unidentified nitrogen," or the nonprotein, nonurea, nitrogenous substances in the plasma of patients with uremia.

The Occurrence of Rheumatoid Factor and other Gamma Globulin Abnormalities in the Families of Patients with Agammaglobulinemia. HUGH FUDENBERG, JAMES L. GERMAN, III and HENRY G. KUNKEL,* New York, N. Y.

The sera of relatives of 13 individuals with congenital agammaglobulinemia and of 10 with idiopathic "acquired agammaglobulinemia" have been examined for quantitative aberrations in gamma globulins and for certain qualitative abnormalities, particularly the rheumatoid and lupus factors. The gamma globulin levels in the probands of the congenital group were all less than 100 mg per 100 ml and in the acquired group the values ranged from 50 to 250 mg per 100 ml as determined by zone electrophoresis. Three of the patients in the congenital group had arthritis, and three in the acquired group developed various atypical manifestations of "connective tissue disease." Hypergammaglobulinemia was found frequently in family members of both groups. In addition, marked diminution of the β_{2A} or β_{2M} immunoglobulins was present in some of the families. In parents and siblings of probands with "acquired agammaglobulinemia," the incidence of "rheumatoid factor," as evaluated by four different serologic parameters, was II of 24, or 46 per cent. This was significantly different from a control group of 23 families with an incidence of 4 out of 88 positive reactions or 5 per cent ($p. < 0.001$). Nine of the ten probands with the "acquired" form of the disorder had at least one parent or sibling with rheumatoid factor. This incidence of positive families was also significantly different from the incidence in normal families. In addition, antinuclear (lupus) factors were present in relatives in two of the "acquired agammaglobulinemia" families. In congenital agammaglobulinemia the familial incidence of rheumatoid factor was not significantly increased (4 of 38 relatives).

These data suggest a familial predisposition to aber-

ration in immunologic response, sometimes resulting in agammaglobulinemia, sometimes in hypergammaglobulinemia, and often in serologic abnormalities of the "rheumatoid factor" type. The studies perhaps aid in connecting on a genetic basis the various diseases associated with these gamma globulin aberrations.

Effect of In Vitro Sonic Oscillation on Distribution of Serum Lipoproteins in Idiopathic "Essential" Hyperlipemia and Other Hyperlipidemic States. ROBERT H. FURMAN, K. LAKSHMI, LEONARD N. NORCIA and R. PALMER HOWARD, Oklahoma City, Okla. (introduced by Stewart Wolf).

Ultracentrifugal partition of the serum lipids in a variety of hyperlipidemic disorders has been carried out at the following solvent densities: 1.006, 1.019, 1.063 and 1.21 g per ml. In idiopathic hyperlipemia, 40 to 90 per cent of the serum cholesterol and phospholipid may be found in the chylomicron-rich $d < 1.006$ g per ml fraction, while less than normal amounts are found in the $d > 1.063$ g per ml (α) and $d > 1.019 < 1.063$ g per ml (β) fractions. In "normal" sera only 10 to 14 per cent of the serum cholesterol and phospholipid is found in the very low density fraction.

When normal sera are subjected to sonic oscillation (10 kc per second, 250 W, 2 hours) the lipoprotein distribution remains essentially unaltered. However, when sera from hyperlipemic patients are so treated, ultracentrifugal partition of the lipids reveals marked decrease in the amount of cholesterol and phospholipid found in the low density fraction, while there is a quantitatively similar increase in the lipid content of the α lipoprotein fraction. For example, in two hyperlipemic patients with serum cholesterol levels of 458 and 350 mg per 100 ml, respectively, only 32 and 11 mg were present as α lipoproteins, while 390 and 278 mg were present as low density lipoproteins. After sonic oscillation, α cholesterol increased to 216 and 101 mg, respectively, with equivalent losses from the low density fractions.

These and similar data from other patients indicate that in "essential" hyperlipemia significant amounts of high density α lipoproteins are "bound" to low density lipoproteins and/or chylomicrons. In primary "essential" hypercholesterolemia and in hypercholesterolemia secondary to hypothyroidism or nephrosis, no redistribution of ultracentrifugally-defined lipids is noted following sonic oscillation. In sera from patients with hypercholesterolemia secondary to biliary obstruction, redistribution of lipids following sonic oscillation indicates marked binding of high density lipoproteins to "beta" lipoproteins.

Studies of Gastrointestinal Pathophysiology in Acute Asiatic Cholera. EUGENE J. GANGAROSA, WILLIAM R. BEISEL, CHANYO BENYAJATI and HELMUTH SPRINZ, Washington, D. C. and Bangkok, Thailand (introduced by John B. Youmans).

The diarrhea of Asiatic cholera has been ascribed to massive transudation of fluid across a bowel wall denuded by epithelial desquamation. Intestinal biopsy specimens and physiological studies obtained in bacteriologically proven cases of cholera during the 1959 Thailand epidemic failed to substantiate this classic concept.

Serial biopsy specimens obtained with the "Crosby Capsule" at several intestinal levels during active diarrhea showed acute enteritis with intact mucosal epithelium. Cessation of the clinical diarrhea could not be correlated with any specific change in pathology. An additional microscopic finding in all cases consisted of atrophic thinning of the mucosal layer with blunting and fusion of villi. These chronic changes were comparable to those seen in dietary deficiency states such as sprue and remained after the enteritis had cleared. The possibility that areas of desquamation were missed by the biopsy technique seemed unlikely, since intravenously injected inulin or Evans blue dye failed to leak into the bowel lumen. Reports that the stool in cholera contains insignificant amounts of protein, and demonstrations by Gordon that intravenous radioiodinated PVP does not appear in abnormal amounts in the cholera stool lend additional support to the presence of an intact epithelium. Stool potassium, pH, and bicarbonate were shown by Watten, Phillips and colleagues to be higher than simultaneous plasma values implying that cellular "work" was involved in the elaboration of the cholera stool. Prompt and complete absorption of tritiated water and iodine^{131} molecules by the upper intestine was shown in acute cholera, during periods when transit time through the bowel was less than 2 hours.

The stimuli initiating the unique clinical and biochemical characteristics of cholera diarrhea remain unknown. However, it seems possible that atrophic changes in the intestinal mucosa associated with nutritional deficiencies may predispose an individual to clinical cholera.

An Evaluation of the Urine Hydrolysis Test for Primitive White Blood Cell Differentiation. EDWARD GARDNER, JR., CLAUDE-STARR WRIGHT* and BETTIE Z. WILLIAMS, Augusta, Ga.

Accurate identification of primitive leukemic white blood cells in peripheral blood and bone marrow is desirable for most efficient therapy. Even with a variety of technical aids it is often difficult to classify the cell type.

A means of differentiating cells of the myelocytic series from other leukocytes by subjecting them to urine was first proposed by Brachet. The test involves exposure of bone marrow or peripheral blood films, previously fixed in methyl alcohol, to the activity of fresh urine. The nuclei of myelocytic cells are "lysed" while lymphocytic and monocytic nuclei remain relatively intact.

The present study is concerned with three phases of the urine hydrolysis test: *1*) conditions influencing the test; *2*) the nature of the substances in urine responsible for the "lytic" effect; and *3*) application of the test to clinical material.

It was found that three factors, time and temperature of incubation and pH of urine, are important. Optimum conditions for cell differentiation were incubation of blood films in neutral or slightly alkaline urine at a temperature of 60° C for 15 minutes. Most urine specimens had to be adjusted from a slightly acid pH.

The effect of purified ribonuclease and desoxyribonuclease and various concentrations of sodium chloride solutions on blood films was determined. Only a solution of desoxyribonuclease in buffered saline produced results comparable to the effect of urine.

Application of the urine test to blood and/or bone marrow films from 18 cases of leukemia substantiates the validity of the test in 17 cases.

Pregnanetriol Excretion and Urinary Pregnanetriol/Aldosterone Ratio in Normal Subjects and Patients with Arterial Hypertension. JACQUES GENEST, WOJCIECH NOWACZYNSKI, ERICH KOIW and THOMAS SANDOR, Montreal, Canada (introduced by J. S. L. Browne).

Extension of our study on the role of adrenal cortical hormones in human hypertension has shown a significant decrease in mean urinary pregnane-3α, 17α, 20α-triol excretion in groups of patients with essential, renal and malignant hypertension as compared to that of normal subjects. Sixty-eight determinations in 56 normal subjects and patients with essential, renal and malignant hypertension were made. Normal females used for the study collected their urine only during the first part of the menstrual cycle in order to avoid the rise in urinary pregnanetriol during midcycle. Urine collections from women with hypertension were made without regard to the menstrual cycle. All patients and normal subjects were on unrestricted diets.

Mean urinary pregnanetriol excretion determined according to Bongiovanni's procedure is significantly decreased in groups of patients with essential, renal and malignant hypertension as compared with that of normal subjects ($p < 0.01$). During this study, Bongiovanni's procedure was found to be inaccurate in many cases because of the presence of two substances interfering with pregnanetriol determination. One of these has already been identified as Compound III, previously described, and the second was identified by various procedures, including infrared spectrometry, as the 5-pregnene-3β, 17α, 20α-triol. But the validity of results obtained with Bongiovanni's procedure was confirmed by the use of a specific method recently developed in which pregnanetriol is determined after isolation in a high degree of purity. The ratio of two urinary steroids varying in opposite directions, pregnanetriol/aldosterone, is significantly decreased in all three groups of hypertensive patients and is below lower limits of normal range in 92 per cent of patients with various types of arterial hypertension.

These findings have great interest because of recent observation of the hypotensive action of progesterone in experimental and human arterial hypertension by Armstrong. This adds further evidence for a disturbance in the adrenocortical function of hypertensive patients.

Presence of an Altered Amino Acid Sequence in Hgb M (Boston Type). PARK S. GERALD, MARY L. EFRON and MARGARET J. PEASE, Boston, Mass. (introduced by Louis K. Diamond).

The group of abnormal hemoglobins categorically known as the Hgb M's are associated with dominantly-transmitted cyanosis which simulates congenital methemoglobinemia. Both the Hgb M and normal adult hemoglobin may be demonstrated by electrophoresis of hemolysates from cyanotic members of such families. In addition to being electrophoretically abnormal, the Hgb M pigments are spectroscopically anomalous when in the methemoglobin state. This latter unusual property has been thought to be due to the formation of an *internal* complex between the ferric iron and a new active group in the globin portion of the molecule.

Chemical studies of the protein portion of Hbg M (Boston type) have now been accomplished by the "fingerprint" technique developed by Ingram. Comparison of the tryptic digest fragments from Hgb M (Boston type) with those from the Hgb A_1 fraction of the same patient, as well as from normal adults, has revealed the presence of an amino acid alteration in at least one such fragment. This is evidenced by the absence from the Hgb M (Boston type) fingerprint of a spot constantly present in the Hgb A_1 fingerprint, and in addition, the appearance in the former of a "new" spot which reacts with staining reagents for tyrosine. From these data, it seems reasonable to postulate that the mutation represented by Hgb M (Boston type) results in the substitution of a tyrosyl residue for the amino acid which occurs at this point in the sequence of Hgb A_1.

This is the first demonstration that abnormal spectroscopic properties of the hemoglobin molecule need not result solely from changes in the heme group, but may be attributable to alterations in the amino acid sequence of the globin.

Family Studies on the Chromosomal Complement of Mongols. JAMES L. GERMAN, III and ALEXANDER G. BEARN,* New York, N. Y.

The karyotypes of two female mongols and their parents were studied by means of short-term bone marrow cultures. The first patient, aged 18 months and the third of three siblings, showed the classical mongoloid appearance, congenital cardiopathy, and mental deficiency. The mother and father, aged 36 and 35 years, respectively, when the child was born, showed no mongoloid characteristics. The mongol showed 47 chromosomes, the small acrocentric number 21 being trisomic. Sex chromosomes present were (XX). The mother had 46 chromosomes (XX), the father 46 (XY). In the second patient, aged two months and the fourth of four siblings, pathognomonic clinical features of mongolism were notably absent, although the facies was suggestive of mongolism. The mother was 26 when the child was born, the father 30. Despite the difference in the clinical findings in the two cases, the karyotypes were grossly identical. Abnormalities of chromosome number 21 were not detected in the parents.

Disturbances in chromosomal mechanics giving rise to a trisomic complement could arise in several ways, including nondisjunction during miosis, somatic nondisjunction during early embryonic cell division, or the presence of an additional chromosome in the parental gonial cells. However, in view of the well recognized maternal age effect

it seems probable that the majority of mongols are the result of maternal nondisjunction. No evidence for somatic mosaicism was found in either parent examined in the study. However, the possibility that parental germinal mosaicism might account for a proportion of cases, particularly in those born to young mothers in whom there is more than one affected member in the sibship, is not excluded by the present findings.

Effect of Acute Uremia on Arterial Blood Pressure in Rabbits. CARMELO GIORDANO, HERMAN WEINSTOK and JOHN P. MERRILL,* Boston, Mass.

Neither ureteral ligation nor bilateral nephrectomy causes hypertension in the rabbit. Present experiments were designed to study the effect of the addition of urea loading to ureteral ligation on arterial blood pressure. In previous investigations we have shown both *in vitro* and *in vivo* that elevated concentrations of urea (0.05 M) will inhibit monoamine oxidase activity of mytochondria from rabbit kidney or liver. Twenty animals were divided into 3 groups. In Group 1 bilateral ureteral ligation was done and 10 to 20 minutes afterward 2 g of urea per kg body weight was administered by stomach tube. Such a load increased the blood urea nitrogen concentration (BUN) to 150 ± 10 mg per 100 ml. Group 2 served as control, having only ureteral ligation. Group 3 received only the urea load. Blood pressures were taken at 5-minute intervals for 20 minutes before and 4 hours following the urea load using an ear manometric capsule. A significant rise in blood pressure appeared in Groups 1 and 3 during the first 2 hours after urea loading. No significant change in blood pressure occurred in Group 2. Death occurred in Group 2 in 3 to 4 days, whereas death occurred in Group 1 in 10 to 12 hours following the urea load. The curves of serum osmolality, hematocrit and BUN indicated that urea had completely equilibrated in total body water in 10 to 15 minutes and could not therefore account for blood pressure rise at 2 hours on the basis of expansion of extracellular fluid.

The possibility that the relation of urea loading to increased arterial pressure may be related to inhibition of monoamine oxidase activity is considered.

A Rapid Urine Test for Pheochromocytoma. STANLEY E. GITLOW and ELIZABETH KRUK, New York, N. Y. (introduced by Milton Mendlowitz).

Analysis of urine for vanillylmandelic acid (VMA) has proven to be a reliable diagnostic technique for pheochromocytoma, but the procedures heretofore used for VMA measurement have been either complex or prolonged and therefore unsuitable for use by a routine clinical laboratory.

A rapid urine test for the presence of excessive quantities of VMA was reported in 1959, but it required that the patient avoid certain foods and drugs prior to sample collection. Failure to do so resulted in a false-positive test, necessitating the use of the time-consuming confirmatory tests. A modification of this simple screening test has permitted the use of random urine specimens collected from patients following no specific diet. As with the original test, no false-negative results were obtained. A series of 31 cases of pheochromocytoma gave uniformly positive test results. Ten urine samples, which had previously given false-positive results due to inadequate dietary control, were negative when tested in the following manner. A urine aliquot equivalent to 1.0 mg creatinine was hydrolyzed at pH 2 and 100° C for 10 minutes, after which the pH was buffered at 4.0 and the urine extracted with ethyl acetate. The aqueous layer was acidified with HCl to a pH of 0.5 to 1.0, extracted with ethyl acetate, and the organic layer blown to dryness and dissolved in dilute K_2CO_3. Diazotized *p*-nitroaniline was added and the purple azo-VMA compound extracted into *n*-amyl alcohol: ethanolamine (100:1) for reading on a Beckman DU spectrophotometer at 450 and 550 mμ (vs. H_2O blank). The ratio $\left[(R)=\frac{\text{density } 450 \text{ m}\mu}{\text{density } 550 \text{ m}\mu}\right]$ normally exceeded 1.25 (mean = 1.63), whereas no test of urine from a patient with a pheochromocytoma exceeded 1.20 (mean = 0.70). This simple and rapid test should enable the physician to screen large numbers of hypertensive patients for the presence of pheochromocytoma.

Blocking Antibodies after Brucella Hyperimmunization and Infection in Rabbits and Mice. HARRY GLENCHUR, HORACE H. ZINNEMAN and WENDELL H. HALL,* Minneapolis, Minn.

Continued injection of heat-killed *Br. abortus* resulted in persisting agglutinating antibody in rabbits. Weeks of massive injections were required to produce blocking antibody (slow-moving gamma globulins). With further injections, the blocking activity moved into the β-globulins. This migratory phenomenon was also observed in a man with acute brucellosis. Hyperimmunized rabbits also had a marked rise in serum gamma globulins, the majority of which were absorbable by homologous antigen. Brucella-infected rabbits, too, demonstrated increased serum gamma globulins which, however, were mostly not absorbable. Hyperimmunization did not impair the rate at which the animal controlled a brucella bacteremia.

Serum precipitins appeared in hyperimmunized rabbits after 2 to 15 weeks. The time of appearance of precipitins was inversely correlated with the weekly dosage of antigen. As immunization continued, more precipitin lines appeared, until there were 3 to 4. Absorption of the hyperimmune sera with whole brucella cells removed some but not all the precipitins. The precipitins disappeared 14 weeks after cessation of immunization (the absorbable one first) although the agglutinins persisted in low titers. In brucella-infected mice, the blocking antibody appeared early, associated with an increase of both β- and gamma globulins. Mice infected with brucella showed only a single serum precipitin after 80 days of infection.

Since the antigen for the precipitin test was from

disrupted brucella, this work suggests that antibodies form to internal antigens as degradation of the bacterial cell occurs *in vivo*. An outpouring of nonspecific globulin also occurs in active infection. Immunization with intact, killed brucella cells produces antibody globulin but no nonspecific gamma globulins. In response to infection, the mouse produces more β-globulins and early blocking antibodies. Thus species differences in the serological response to brucella infection are emphasized.

Electron Microscopy of the Human Glomerulus in Early Diabetes. F. C. GOETZ, J. F. HARTMANN and A. LAZAROW, Minneapolis, Minn. (introduced by C. J. Watson).

Needle or surgical biopsies of the kidney have been obtained in 23 patients with diabetes mellitus, the duration of the disease varying from 5 months to 28 years. They have been studied by accepted light and electron microscopic techniques, with special attention to the width and appearance of the glomerular basement membrane. Control material was provided by nondiabetic human biopsy and autopsy material (Bloom, Vernier and Hartmann).

The diabetic glomerulus characteristically showed irregular thickening of basement membrane. In two 19 year old patients with duration of diabetes well documented at 6 and 22 months, respectively, basement membrane measurements showed that significant thickening was already present (2,385 to 5,565 and 2,772 to 5,568 Å units, in comparison with the normal value of 3,000 $\pm$ 550 Å, [SD]). Neither of these patients showed clinical or laboratory evidence of renal or other vascular disease, and by light microscopy the glomeruli appeared normal. Nearly all patients with 5 years or more of diabetes showed some degree of change, whether or not clinical evidence of renal disease was present. Where changes were extreme, basement membrane reached 13,000 Å in thickness, with complex folding and formation of anastomosing trabeculae of basement-membrane-like material in nodular masses. These severe changes were accompanied by marked broadening of some epithelial foot-processes and by thickening and vacuolation of endothelial cytoplasm.

It is concluded that a characteristic glomerular change may begin within one to two years of the apparent onset of diabetes, and that this change progresses in a more or less smooth continuum as the duration of diabetes increases, finally leading to extreme changes with the formation of nodular masses. Involvement of glomerular capillaries in diabetes may occur much earlier than had heretofore been suspected. This finding has implications important to understanding of the pathogenesis of diabetic kidney disease.

Meralluride-Induced Solute and Water Diuresis in Hydrated Man. MARVIN GOLDSTEIN, A. DANIEL HAUSER and MARVIN F. LEVITT,* New York, N. Y.

In 7 maximally hydrated subjects meralluride produced a prompt but transient increase in C_{H_2O} and C_{osm}, averaging 4 and 1.5 cc per minute, respectively. Within one hour these values had returned to control levels. Thereafter a more sustained and progressive increase in C_{osm} developed which reached an average peak increment of 12 cc per minute two hours after the administration of meralluride. Coincidentally, urine flow (V) progressively increased to the same extent so that C_{H_2O} remained constant throughout this phase of the solute diuresis. At the time of maximum mercurial response an infusion of calcium or sodium sulfate caused a prompt further increase in C_{osm} and C_{H_2O}, averaging 6 and 3 cc per minute.

In 4 comparably hydrated subjects infused with a hypotonic mannitol load, meralluride likewise produced a two-phase response, the first phase corresponding to that described above. The second more sustained response produced maximum increases in C_{osm} and V, averaging 24 cc per minute or twice that observed without the solute load. As above, this solute diuresis occurred without a measurable change in C_{H_2O}. The administration of dimercaprol during the second phase of meralluride diuresis in hydrated subjects eliminated the similar increments in C_{osm} and V so that C_{H_2O} remained unchanged.

The transient initial phase of mercurial diuresis seems explicable by the inhibition of proximal salt absorption. The persistently isosmotic character of the tubular rejectate during the sustained phase of diuresis, despite the capacity for C_{H_2O} to be independently increased before and during this phase, is difficult to explain by either a proximal or early distal effect. Unless meralluride inhibits both proximal and distal salt absorption with a unique balance of differential effects so as to maintain C_{H_2O} constant, these data are consistent with the hypothesis that mercurials prevent a late distal isosmotic salt reabsorption in hydrated man.

The "Diabetic" Pituitary: Observations on the Carbohydrate Metabolism of Pituitary Tissue. CHARLES J. GOODNER and NORBERT FREINKEL,* Boston, Mass.

Despite the classical evidence that hypophyseal hormones may influence diabetes mellitus, the responsiveness of normal pituitary tissue to insulin and the carbohydrate metabolism of the pituitary in diabetes mellitus have never been examined. Since these factors may condition pituitary function, the following studies were undertaken.

Isolated portions of anterior (AP) and posterior (PP) pituitary from normal rats and calves were incubated separately with differentially-labeled glucose-C^{14}. Appreciable hexose-monophosphate shunt activity was demonstrated in AP and PP. In addition, both structures exhibited rapid glycogen turnover, net glycogenesis *in vitro*, and appreciable levels of phosphorylase activity. AP and PP of both species were responsive to insulin *in vitro*. Addition of insulin to the suspending medium significantly ($p < 0.01$) enhanced glucose assimilation and the disposition of radioactivity into glycogen-C^{14}, lipid-C^{14} and $C^{14}O_2$. To assess the effects of acute insulin deprivation, pituitaries were excised from rats 20

hours following total pancreatectomy. During incubation with glucose-U-C^{14} *in vitro,* AP from these animals formed 40 per cent less $C^{14}O_2$ ($p < 0.001$) and 10 per cent less lipid-C^{14} ($p < 0.05$) than AP from sham-operated controls. Administration of insulin during the postoperative period prevented this deterioration of AP carbohydrate metabolism. No abnormalities in the disposition of labeled glucose were demonstrable during incubation of PP from depancreatized rats.

Conclusions: The presence within the pituitary of pathways for *1*) the storage of glucose, *2*) the rapid mobilization of glycogen, and *3*) the generation of reduced pyridine nucleotides during oxidation of glucose-6-phosphate suggests mechanisms whereby glandular carbohydrate economy may influence hormonogenesis. Potential derangements of pituitary glucose metabolism in diabetes mellitus are suggested by the responsiveness of hypophyseal structures to insulin *in vitro.* That this is indeed the case, at least for AP, is suggested by the finding in rats rendered acutely aninsulinemic by total pancreatectomy.

Studies on the Origin of the Fecal Fat. HYMIE GORDON, Cape Town, South Africa (introduced by Victor A. McKusick).

Fifteen normal subjects were maintained on constant very low fat diets (6.1 to 8.6 g of fat daily) for periods varying from 6 to 24 days. While on this diet, each subject's stools were collected in 3 or 5 day batches and their fat content was determined. The mean daily fecal fat content was 2.4 g (SD 1.2).

Various dietary manipulations were then carried out on groups of these subjects. When the dietary calories were progressively increased without altering the fat intake, there was no significant change in the fecal fat content. Increasing the dietary crude fiber intake increased the fecal fat only when very large artificial fiber loads were given.

The addition of either saturated or unsaturated fat to the diet increased the fecal fat content; in general, the greater the dietary load of fat, the greater was the fecal fat content. To determine whether this increase in fecal fat was due to unabsorbed dietary fat or to the increased excretion of fat from the body, a series of experiments was carried out in 5 subjects using intravenous cottonseed oil ("Lipomul I.V."; Upjohn). When the intravenous fat was administered instead of dietary fat, the fecal fat content fell toward the basal (very low fat diet) level; when it was administered in addition to dietary fat, there was no further increase in the fecal fat content. These observations support the hypothesis that in subjects consuming diets containing ordinary amounts of fat, much of the fecal fat is derived from unabsorbed dietary fat.

Iron Absorption and Turnover in Hypoxia. MORTIMER S. GREENBERG, HELENA WONG, STEPHEN A. MILLER, ROBERT W. SCARLATA and THOMAS C. CHALMERS,* Boston, Mass.

Enhancement of iron absorption during experimental hypoxia has been attributed to a fall in serum iron (SI) concentration. Increased absorption of iron in rats, as measured by total body counting, was found to begin after 6 to 8 hours of hypoxia and was preceded by an elevation rather than a fall in SI.

The temporal relationship of SI turnover to absorption was studied in 48 rats whose iron stores had been labeled by the intravenous injection of 20 μc of $Fe^{59}Cl_3$ one week previously. In a 3×2 factorial design, 3 groups of 16 rats each received hypoxia ($Po_2 = 76$ mm Hg) for 0, 2 and 8 hours, and one-half of each group received 1 mg of ferrous sulfate intragastrically 2 hours before sacrifice for determination of SI and serum Fe^{59}. The fasted rats had SI concentrations of 170, 211 and 156 μg per 100 ml for the 0, 2 and 8 hour periods. The corresponding values for the iron-fed rats were 234, 299 and 310 μg per 100 ml. Serum Fe^{59} levels, corrected for hemoglobin radioactivity, were 346, 982 and 727 cpm per 2 ml for the fasted rats and 374, 721 and 958 counts for the others. By analysis of variance the elevations in SI for hypoxia and for iron feeding are significant at the 0.01 and 0.001 levels, respectively, and the elevation of Fe^{59} with hypoxia is also highly significant ($p < 0.001$).

Thus mobilization of storage iron, apparently not blocked by an oral iron load, is responsible for the rise in serum iron after 2 hours of hypoxia. After 8 hours, when enhanced absorption can be demonstrated, the SI in the fasted rats is falling, suggesting utilization of iron by the bone marrow. In the iron-fed rats the SI and Fe^{59} remain high, reflecting both movement out of storage and enhanced absorption. These data suggest a primary effect of hypoxia on serum iron turnover; the latter may be the factor directly related to enhanced absorption.

Mechanism of Neuromuscular Block in Myasthenia Gravis. DAVID GROB* and RICHARD J. JOHNS, Brooklyn, N. Y. and Baltimore, Md.

Essential differences have been found in the effect of transmitter substance (acetylcholine, ACh) on neuromuscular transmission of normal subjects and myasthenic patients. Intra-arterial administration of ACh produces, in both, block of neuromuscular transmission, which is more marked and prolonged in myasthenic patients. The nature of the block may be characterized by its effect on the subsequent injection of ACh. Only in myasthenic patients does this ACh-induced block inhibit the depolarizing action of subsequently injected ACh. The block may be further characterized by observing whether or not it is reversed by ACh. In normal subjects it is not. In myasthenic patients who respond well to anticholinesterase medication, the ACh-induced block is reversible by ACh, but in the minority who respond poorly it is not. The latter ACh-inhibitory, non-ACh-reversible block may be termed "ACh-insensitive." Patients may become ACh-insensitive during exacerbation of their disease, or rendered locally ACh-insensitive by repeated intra-arterial administration of ACh or choline, or by prolonged repetitive nerve stimulation, which causes local accumulation of endogenous transmitter. Patients in the ACh-insensitive state are often treated with excessive doses of anticholinesterase compounds which render them

even more insensitive. Cessation of anticholinesterase medication, or potassium administration or hypothermia usually increases responsiveness to ACh and may be followed by clinical improvement.

These observations indicate that myasthenia gravis is due to an ACh-inhibitory neuromuscular block produced by endogenous transmitter. It is likely that ACh combines with several forms of receptor substance in the motor end-plate to produce different types of block: non-ACh-inhibitory in normal subjects and ACh-inhibitory in myasthenic patients, in most of whom the block is ACh-reversible, but in some is non-ACh-reversible. In myasthenic patients there may be one or more abnormal forms of receptor substance, and the predominant form may determine the type of block, clinical state, and response to anticholinesterase medication.

Cardiac and Renal Responses to Acute Volume Loading in Patients with Heart Disease. JACOB GROSSMAN, ROBERT ROSENBLUM, SUN HING LAU and MORRIS WOLFMAN, New York, N. Y. (introduced by Louis Leiter).

In normal subjects and 14 patients with mitral stenosis of minimal to moderate severity, the effects of acute increase in body fluid volume on cardiac and renal functions were determined. Cardiac catheterization was performed with control measurements of pressures in the pulmonary circulation, cardiac output, renal hemodynamics and electrolyte excretion. Two L of a hypotonic solution of glucose in water containing sufficient vasopressin (0.25 mU per kg per hour) to maintain low urine flow (1 ml per minute) were infused in 2 hours. Renal hemodynamics and electrolyte excretion were measured continuously. Repeat determinations were performed twice at the end of volume loading, and again 1 hour later.

Changes in hematocrit and plasma sodium concentration reflected rapid equilibration. In normal subjects, this relatively small (5 per cent) expansion in fluid volume was associated with increase in cardiac output, renal hemodynamics and sodium excretion. A delayed rise in urine flow despite vasopressin resulted from mild osmotic diuresis. Of 5 patients with normal responses, 1 with normal cardiac output and 2 of 4 with slightly reduced outputs (cardiac index 2.4 to 3.0) presented no history of failure. In 5 additional patients (cardiac index 1.9 to 2.7), renal hemodynamics rose significantly in the absence of or preceding the increase in cardiac output. Fluid loading failed to increase pulmonary pressures. Correlation between normal pulmonary pressure and cardiac response to volume was not observed. Five of 7 patients whose cardiac output rose had elevated pulmonary pressures; conversely, of 5 patients whose cardiac output remained unchanged, 4 exhibited normal initial pressures. Electrolyte and water excretion generally paralleled renal hemodynamics rather than cardiac output.

Under volume loading, cardiac patients, prior to failure, behave as normal subjects. After failure, renal response may vary independently of cardiac output. Moderate elevation of pulmonary arterial pressure may facilitate cardiac output response to volume loads in less severely ill patients.

Amino Acid and α-Keto Acid-Induced Hyperinsulinism in the Leucine-Sensitive Type of Idiopathic Hypoglycemia. MELVIN M. GRUMBACH * and SELNA L. KAPLAN, New York, N. Y.

A group of infants and children with "idiopathic" hypoglycemia has been shown to exhibit a sharp fall in the concentration of blood sugar following the administration of L-leucine. In the present studies, data obtained in two children with leucine-induced hypoglycemia (L.-I.H.) indicate that certain amino acids and keto acids promote an increase in circulating insulin.

When L- or DL-leucine, or L-isoleucine was fed (150 mg per kg), a rapid and striking fall in blood glucose concentration without glycosuria was obtained. D-leucine, L-alanine, L-valine, DL-threonine, and L-glycine did not have a significant effect. The α-keto acid analog of leucine, α-ketoisocaproic acid (α-KIC) induced hypoglycemia when administered intravenously, but its keto acid metabolites did not. Examination of the plasma amino acids and urinary amino and α-keto acids before and after the administration of leucine indicated *1*) a normal pattern and *2*) a rise in serum leucine consistent with that obtained in normal subjects. Thus, evidence of a defect in amino acid or keto metabolism was not found. In incubation experiments a direct effect of leucine on the rate of oxidation of labeled glucose by whole blood was not demonstrated. Epinephrine masked the hypoglycemic effect of leucine. It was shown that leucine caused a rise in plasma lactate and a fall in plasma phosphate and potassium, findings consistent with an insulin-like action, although a significant increase in peripheral glucose uptake was not detected. Plasma insulin determined directly by immunoassay by Yalow and Berson after feeding leucine rose sharply to levels 14 to 19 times the fasting concentration in 15 to 45 minutes. The mother and sister of one patient showed a moderate response to leucine, whereas none was found in normal subjects nor in one patient with an islet-cell carcinoma.

It is concluded that leucine, isoleucine, and their keto acid analog induce an increase in the concentration of plasma insulin in L.-I.H. irrespective of the blood glucose level, an effect which may be attributed to their action on the insulin-secreting mechanism.

Intensification of Rabbit Atherosclerosis by Chronic Hypothalamic Stimulation. C. G. GUNN, Oklahoma City, Okla. (introduced by Meyer Friedman).

The following experiments were performed to test the hypothesis that central nervous system mechanisms may influence experimental atherosclerosis. Chronic stimulating or mock electrodes were implanted in diencephalic areas of male rabbits of comparable age, size and serum lipid level; 14 experimental and 15 control rabbits were then fed a cholesterol-added diet for 3 months. The experimental group differed by receiving electrical stimulation via hypothalamic electrodes intermittently 5 days

each week at parameters slightly above behavioral and autonomic thresholds. A third group of 4 stimulated animals received a noncholesterol diet. Blood pressure, cholesterol, phospholipid, total lipid and 17-OH-corticosteroid concentrations were measured each month.

After 3 months, postmortem examination included gross and microscopic grading of aortic and coronary atherosclerosis and actual analysis of aortic cholesterol content. All determinations were performed and recorded without knowledge of the source of the material. All groups ate a comparable amount and showed similar weight gains. The stimulated animals on a noncholesterol diet showed neither artherosclerosis nor lipidemia. On the other hand, the stimulated cholesterol-fed rabbits demonstrated more than a twofold increase over the control rabbits' aortic and coronary atherosclerosis and aortic cholesterol content (4.63 vs. 2.0 g per 100 g dry weight; $t = 3.5$, $p < 0.01$). These stimulated animals also showed significantly higher serum cholesterols (1,587 vs. 1,215 mg), phospholipids (609 vs. 435 mg) and total lipids (2,785 vs. 2,117 mg) ($t = 3.4$, 3.6, and 3.4, respectively, $p < 0.01$ throughout). The concentration of corticosteroids was not significantly different among the three groups.

Hypothalamic stimulation thus significantly intensified the atherosclerosis and increased the circulating blood lipids in cholesterol-fed rabbits. This response seemed most marked when electrodes were in or near the ventromedial nucleus and less pronounced when anterior hypothalamic areas were stimulated.

The Kinetics of Erythropoiesis. CLIFFORD W. GURNEY and EDWARD FILMANOWICZ, Chicago, Ill. (introduced by Leon O. Jacobson).

Erythropoiesis was investigated in the hypertransfused mouse in which erythrocyte production is eliminated without known alteration of metabolism or marrow damage. Following one injection of erythropoietin, a highly purified α_2-glycoprotein extracted from plasma of anemic sheep, a wave of erythropoiesis swept through the marrow, culminating in a reticulocytosis in the peripheral blood, maximal at 72 hours and returning to zero by 144 hours.

With increasing doses of erythropoietin, the peak reticulocytosis increased until a maximum response of 1.4 per cent was reached (12 ml sheep plasma equivalent). Since mice have the capacity to produce erythrocytes far in excess of this rate, the effect of multiple doses was investigated. The reticulocytosis obtained clearly exceeded that predicted from the projected summation of individual doses. For example, from the time and magnitude of response to a single submaximal dose, a peak reticulocyte response of 2.1 ± 0.6 per cent was expected following 5 daily injections; however an observed response of 5.2 ± 1.3 per cent was found on the sixth day. A similar result was obtained when Fe^{59} uptake by newly formed erythrocytes was employed as a measure of erythropoiesis.

These studies suggest a more complex mechanism of action of erythropoiesis than simply the initiation of differentiation from stem cells. It would appear that this hormone also enhances proliferation of erythroblasts or activates reticulum so successive periods of stimulation induce progressively the differentiation of unexpectedly large numbers of stem cells. A single erythropoietin is sufficient to induce both erythroblast proliferation, as measured by reticulocyte production, and hemoglobin formation, as measured by Fe^{59} incorporation. Finally, these simple experiments, in which consistent results are obtained following administration of erythropoietin to animals whose erythropoiesis is suppressed without apparent damage, suggest that this system provides a useful model for the investigation of many facets of cell growth.

Significance of the Distribution and Cytology of Iron in Primary Hemochromatosis During Treatment with Phlebotomies. GABRIEL HAIBY, PRAWASE WASI and MATTHEW H. BLOCK,* Denver, Colo.

The distribution and cytologic characteristics of storage iron in patients with primary hemochromatosis were studied before, during and while completing treatment with phlebotomies. Before treatment there was moderate cirrhosis with iron present in Kupffer cells, hepatic cells, and portal connective tissue. Most of the iron, seen as coarse, brownish crystals measuring 2 to 5 μ, was in the hepatic cells at the periphery of the lobule and in the portal connective tissue. Less iron, in amorphous form or in small yellowish-green crystals measuring 0.5 to 2 μ, was demonstrable in the hepatic cells at the center of the lobule and in Kupffer cells.

After 60 to 80 phlebotomies the iron in the central hepatic cells and in Kupffer cells was markedly decreased. There was little change in the amount of iron in other areas. In contrast, after about 100 phlebotomies, the patients with continued phlebotomies developed an anemia with cytologic and biochemical characteristics of iron deficiency. There was little iron left in the peripheral hepatic cells. Practically all the iron, in the form of large yellow crystals, was in the portal tissue.

The iron of the central hepatic cells and Kupffer cells was probably the most recently deposited because, as described elsewhere, the most recently deposited iron is the first used for erythropoiesis and is present in amorphous form or as small green crystals. We postulate that the residual large brown crystals of iron, found in the portal tissue after treatment, were originally laid down in peripheral hepatic cells and later incorporated into the proliferating connective tissue which had replaced these cells after they had degenerated. This iron is mobilized for erythropoiesis with difficulty, thus explaining the paradox of an iron deficiency anemia in patients with considerable tissue iron.

Maximum Rates of Excretion of Titratable Acid. JOSEPH S. HANDLER, ANDRZEJ WOJTCZAK and MARTIN GOLDBERG, Philadelphia, Pa. (introduced by J. Russell Elkinton).

Under pentobarbital anesthesia, dogs were given 0.1 N hydrochloric acid and sodium phosphate (pH 7.3) at

varying rates intravenously. When phosphate was administered so that serum phosphate concentration rose slowly to a level of 10 to 20 mmoles per L, the excretion of titratable acid reached and remained at an apparent maximal rate (range 20 to 30 μEq per minute per kg body weight). During this plateau in titratable acid excretion the rate of phosphate excretion continued to increase, urinary hydrogen ion concentration was considerably lower than it was earlier in the same animal, and in some dogs the rate of potassium excretion also rose. The plateau was unaffected by increasing serum P_{CO_2}. Whenever serum phosphate rose rapidly due to accelerated administration of phosphate, urinary concentration of hydrogen ion rose and the rate of excretion of titratable acid increased considerably (to more than 40 μEq per minute per kg body weight) even in situations where previously a plateau had been obtained in titratable acid excretion. The highest rates of titratable acid excretion did not necessarily occur in those dogs who manifested the highest rate of phosphate excretion or the most severe acidosis as measured by whole blood pH. Lower rates of titratable acid excretion were obtained in dogs that did not receive an acid load in addition to the phosphate load. Occasionally, but not reproducibly, the clearance of phosphate exceeded the simultaneous clearance of creatinine.

The data suggest two mechanisms for titratable acid excretion under these circumstances. The first is hydrogen ion secretion into tubular fluid rich in phosphate buffer wherein the rate of titratable acid excretion is limited by a maximum rate of hydrogen ion secretion. The second mechanism, apparently related to serum phosphate concentration and its rate of increase, may involve tubular secretion of an acid phosphate ion as postulated in "phosphate aciduria."

Penicillin Antibody in Disease. JEAN HARRIS and JOHN VAUGHAN,* Rochester, N. Y.

The hemagglutination test for the detection of penicillin antibody (Ley) has been applied to a study of patients with *1*) recent penicillin reactions, *2*) recent penicillin therapy without reactions, *3*) autoimmune hemolytic disease, *4*) systemic lupus erythematosus, and *5*) miscellaneous medical illnesses.

Six of 16 patients diagnosed clinically to have developed allergic reactions to penicillin had positive hemagglutination reactions. Thirteen patients were also skin tested; 6 had positive immediate reactions and 4 had delayed reactions. Only 3 patients had both hemagglutinating antibody and positive skin tests.

Sera from 167 patients, obtained at random from the hospital chemistry laboratory, were all negative for hemagglutination; 31 sera selectively obtained from the medical ward services from patients 3 to 7 days after completing a course of penicillin therapy also were negative. Three of 307 sera from patients receiving oral prophylaxis for rheumatic fever were positive. Four of 12 patients with lupus erythematosus and 4 of 21 patients with acquired hemolytic disease were positive.

The characteristics of penicillin antibody were examined. Enhancement of agglutination of penicillinized cells coated with antibody by an anti-γ_2-globulin rabbit serum was taken to indicate in three instances that the patient's antibody was a γ-globulin. Enhancement by an anti-non-γ_2-globulin rabbit serum in three instances, and dependency of agglutination upon heat-labile normal serum component (s), was considered to be consistent with complement fixation. Zone electrophoresis in one instance resulted in the separation of the hemagglutinating antibody among the fast moving gamma globulins.

Hemagglutinating antibody to penicillin develops infrequently, except in patients developing allergic reactions to penicillin, and in systemic lupus erythematosus and acquired hemolytic disease. Its development in the latter diseases may be a reflection of an enhanced capacity for patients with these two diseases to make antibody.

Inadequacy of the "Pore Theory" to Account for the Action of Neurohypophyseal Hormones on Water and Solute Movement Across a Living Membrane. RICHARD M. HAYS and ALEXANDER LEAF,* Boston, Mass.

It is customary to regard movement of water across living membranes as occurring by diffusion or by bulk flow (Poiseuille flow). *In vitro* measurements of net (Δ_w) and unidirectional (ϕ_w) fluxes of water across the toad bladder were made gravimetrically and isotopically, respectively. Without vasopressin, Δ_w is very small (2.1 μl per cm^2 per hour) even with large osmotic gradients across the membrane and large ϕ_w (334 μl per cm^2 per hour). These findings define an average pore radius of some 6 Å and are consistent with transport by diffusion. With vasopressin added to serosal medium without an osmotic gradient, Δ_w remains essentially zero (no active transport of water) but ϕ_w nearly doubles (584 μl per cm^2 per hour). However, in presence of osmotic gradients, Δ_w increases strikingly with hormone without further change in ϕ_w. For gradient of 163 mOsm per kg water Δ_w averages 225 μl per cm^2 per hour, a very large value comparable to the hydraulic flow through porous artificial membranes. This response to hormone is independent of sodium or potassium in the bathing medium and is shown to result, largely if not entirely, from an action of the hormone on the mucosal surface of the membrane. Diffusion could account for less than 1 per cent of such net movement of water and conventional calculations of mean pore radius, assuming Poiseuille flow, yield values as high as 40 Å. Such large pores are inconsistent with the low permeability of the membrane to most small molecules and ions, *i.e.*, reflection coefficients for Cl and thiourea (radii < 3 Å) of 0.9995 and 1.00, respectively, in spite of very large net transfers of water. Movement of water through "pores" likewise would fail to explain the very striking and specific increases in permeability of the membrane following vasopressin to urea, acetamide, proprionamide, butyramide, cyanamide and dimethylformamide while permeability to many molecules and ions of similar and smaller radii is unaffected by hormone.

An alternative hypothesis will be presented.

Supportive Effect of Adrenal Steroids on Acetate Metabolism. ALLEN R. HENNES and TOMMY W. REDDING, Oklahoma City, Okla. (introduced by R. H. Bayley).

We have studied the effect of maintenance doses of cortisone on utilization of acetate–1–C^{14} in two patients with adrenal insufficiency. Each patient served as his own control. Prior to one of their two studies, each patient was taken off maintenance cortisone for two days. At the start of all experiments, patients were normotensive, and glucose, sodium, potassium, and chloride concentrations were normal. However, both subjects developed definite symptoms of adrenal insufficiency 6 to 8 hours after start of experiment when cortisone had been omitted. Plasma lipids were fractionated on silicic acid columns. $C^{14}O_2$ was determined by the method of Frederickson and Ono.

When cortisone was omitted, the following defects in synthesis of plasma lipids were found in both patients. *1*) Peak specific activity in plasma triglycerides was one-eighth to one-tenth of control. *2*) Disappearance of triglycerides from plasma was definitely slower than in the control state. *3*) Appearance of C^{14} in plasma free cholesterol was one-half and three-fourths of control. *4*) Esterification of cholesterol was significantly slower than in the control state. $C^{14}O_2$ specific activity curves were different in adrenal insufficiency from control curves, in one case strikingly so, indicating an effect of adrenal steroids on this extremely stable parameter of metabolism.

It is concluded that a maintenance level of 17-hydroxycorticoids is required for normal synthesis and disappearance of plasma triglycerides. Synthesis of free and esterified cholesterol is definitely increased by maintenance cortisone, but effects are less striking than on triglyceride synthesis. Defects in synthesis and removal of triglycerides and abnormal utilization of acetate appear to be major and early abnormalities in adrenal insufficiency.

Serum Folic Acid Activity: Assay and Nature. VICTOR HERBERT, Boston, Mass. (introduced by W. B. Castle).

Prior attempts to assess folic acid deficiency by microbiologic assay of human serum have met with scant success. The present studies were undertaken in part to determine why a more recent attempt was successful, and in part to determine the nature of the folic acid activity in serum. It was found that most of the serum folic acid activity is highly labile unless protected (presumably against oxidative destruction) by ascorbic acid or d-iso-ascorbic acid. When adequately protected, serum may be assayed directly, without autoclaving to precipitate protein or "release" folic acid activity. Activity may be converted from labile to stable form by autoclaving in the presence of adequate ascorbic acid. Serum folic acid activity, either protected by ascorbic acid or converted to stable form, allows only slight growth of *S. faecalis* and *L. citrovorum*. The lability of this material, as well as its relative unavailability for *S. faecalis* and *L. citrovorum,* may explain the failure of earlier attempts to assess microbiologically the folic acid nutritional status of man. These results suggest the serum folic acid activity may be due largely to one or more polyglutamates (possibly diglutamyl derivatives) of one or more of the folic acid coenzymes. Should such prove to be the case, the serum folic acid activity may represent primarily folic acid converted in the liver to metabolically active triglutamyl pteridine forms, rather than unchanged ingested folic acid-active materials. Parenthetically, it was observed that the *L. casei* assay medium used sufficed as a basal medium for *S. faecalis* and *L. citrovorum,* thereby eliminating the need for different media for the three microorganisms.

Reaction of Rheumatoid Leukocytes with Fluorescent Aggregated Gamma Globulin. EVELYN HESS and MORRIS ZIFF,* Dallas, Tex.

In a study of the binding of rheumatoid factor to cellular elements, it was observed that washed leukocytes obtained from the blood of rheumatoid arthritis patients with high titers of rheumatoid factor were agglutinated by aggregated human gamma globulin (Cohn Fraction II). Leukocytes from nonrheumatoid individuals were not agglutinated under the same circumstances. They were rendered agglutinable, however, by incubation with rheumatoid serum. These observations suggested that the leukocytes of rheumatoid blood are coated with rheumatoid factor.

In order to further establish this fact, buffy coats were separated by a phytohemagglutin sedimentation method and stained with a fluorescein isothiocyanate labeled and heated sample of FII. The presence or absence of fluorescent staining of the leukocytes was observed under a long wave ultraviolet lamp. Fluorescence, which could be blocked by prior treatment with unlabeled FII, was observed in the case of the rheumatoid leukocytes even when the titer of rheumatoid factor in the serum was low or negative. This occurred with 39 of 42 rheumatoid preparations and 8 of 13 from children with rheumatoid arthritis. Sera from 7 of the 8 positively reacting juvenile preparations gave negative serological tests for the rheumatoid factor. Among 37 nonrheumatoid buffy coats, one from a patient with erythema nodosum and a second from a patient with lues fluoresced. Both of these also gave positive serological tests by the latex fixation method. Thus, fluorescent staining of buffy coat appears to provide a sensitive test for the "rheumatoid factors."

In addition to providing the basis for a test, the phenomenon described indicates that the rheumatoid factors are adherent to cells which are in contact with serum. Fluorescent staining of blood smears has shown that both granulocytes and nongranulocytes are coated. The mechanism of the binding of the rheumatoid factor by leukocytes and its possible significance will be discussed.

Factors Influencing Deoxyribonucleic Acid Synthesis In Vivo. HOWARD H. HIATT* and TADEUSZ B. BOJARSKI, Boston, Mass.

Thymidylate (TMP) kinase is an essential enzyme in the reactions leading to deoxyribonucleic acid (DNA)

synthesis. We have found kinase specific activity to be low in adult human and rat tissues known to synthesize little DNA (liver, kidney, lung), and high in proliferating tissues (fetal and neonatal liver and kidney, gastrointestinal mucosa, bone marrow, tumors). Activity in human liver is threefold and in dog liver eightfold that present in rat liver. Orotic acid-C^{14} incorporation studies indicate a correlation between the level of kinase activity and the amount of DNA synthesis. Kinase activity in regenerating rat liver increases up to 30-fold beginning 18 hours following partial hepatectomy, and returns to resting levels within 120 hours. A less marked increase follows CCl_4 administration. The increase in regenerating liver can be prevented by 5-fluorouracil (FU), which is known to interfere with TMP synthesis. Up to a tenfold increase in activity in several tissues is observed following prolonged administration to normal rats of thymidine, which is presumably converted to TMP within the cell. The effects of FU and thymidine are probably explained by our *in vitro* observations that kinase activity is labile in the absence of TMP, but stable in its presence. Thus, enzyme activity *in vivo* probably changes with variations in the level of substrate, which acts as protector. Our data also suggest a regulatory mechanism for DNA synthesis in regenerating liver at a reaction earlier than that involving TMP kinase.

Hyperlipogenesis in Adipose Tissue of Rats Limited to a Daily Two Hour Feeding Period. GUY HOLLIFIELD, Charlottesville, Va. (introduced by William Parson).

Glucose loads, fed to rats after a fast, cause a prompt increase in the amount of C^{14}-labeled acetate incorporated into lipids by liver slices. This increase in lipogenesis is accompanied by a rise in the activity of pentose phosphate enzymes. Liver slices from rats trained to eat their day's ration in one hour also show increased lipogenesis. While adipose tissue is known to be the major site of lipid synthesis and has high pentose phosphate enzyme activity, little is known of its adaptive response to manipulation of food intake.

In this study rats were allowed ground chow only 2 out of each 24 hours; 5 to 6 rats were sacrificed at the end of the feeding period after 1 to 7 days on this program. The *in vitro* incorporation of C^{14}-labeled acetate into lipids by epididymal fat, liver glycogen, and free fatty acid content (FFA) of adipose tissue was measured. The incorporation of C^{14}-labeled acetate into lipids by adipose tissue increased over 30 times by the fifth day of this regimen. This remarkable increase in the rate of lipogenesis was accompanied by a gradual fall in the FFA content of adipose tissue while liver glycogen rose for the first 3 days. Adipose tissue from animals trained for 5 days had 300 to 400 per cent more glucose-6-phosphate and 6 phosphogluconate dehydrogenase activity than did animals fed in this way for 1 day. The activity of these enzymes in livers from these animals was unchanged. The glycogen content of livers from animals fasted for 24 hours after 4 or 5 days of training was very much greater than that in untrained animals fasted for 24 hours.

These studies demonstrate the remarkable capacity of adipose tissue to adapt to manipulation in the time of feeding and have interesting implications in the overall scheme of intermediary metabolism.

The Effects of Mephentermine on Cardiac Output in Normotensive and Hypotensive Dogs. A. W. HORSLEY and JOHN W. ECKSTEIN, Iowa City, Ia. (introduced by E. L. DeGowin).

There are conflicting reports concerning the hemodynamic responses to mephentermine (Wyamine) in intact dogs. These studies were done to determine the effects of the drug on cardiac output. Right atrial and femoral arterial pressures and cardiac output were measured in Nembutal-anesthetized and also in gallamine-paralyzed dogs before and after intravenous administration of mephentermine sulfate (0.3 to 0.6 mg per kg). Observations were made in normotensive dogs and in those made hypotensive by hexamethonium (100 mg i.v.). Indocyanine green dye was injected into the right atrium and continuously recorded time-concentration curves were obtained from the carotid artery. Control values were obtained during a 5 minute period before mephentermine injection. Experimental observations were made when arterial pressure became stable after having risen to a maximum level.

Cardiac output increased in each of 22 experiments when mephentermine was given. It increased from an average of 2,421 ml per minute during control periods to an average of 2898 ml after the drug in 6 paralyzed normotensive dogs ($p < 0.01$); from 1,799 to 2,465 in 6 paralyzed hypotensive dogs ($p < 0.01$); from 2,457 to 3,280 in 5 anesthetized normotensive animals ($p < 0.01$); and from 2,754 to 3,394 in 5 anesthetized hypotensive dogs ($p < 0.02$). Significant increases in stroke volume were noted only in the normotensive dogs. Stroke volume did not increase in the dogs with ganglionic blockade because of marked increases in heart rate with mephentermine. Heart rate tended to decrease in the paralyzed normotensive group and increase in the anesthetized normotensive group. Atrial pressure tended to increase in each group but the changes were small and insignificant. Calculated "central blood volume" increased in 21 of the 22 experiments.

The increased cardiac output and central blood volume may be associated with systemic venous constriction. Venous constriction has been observed in man following mephentermine administration.

Lipid, Carbohydrate and Purine Abnormalities in Von Gierke's Disease. R. RODNEY HOWELL, DORIS ASHTON and JAMES B. WYNGAARDEN,* Durham, N. C.

Three siblings with glycogen storage disease of liver showed fasting abnormalities of serum FFA (1,100 to 2,500 μmoles per L), triglycerides (570 to 1,145 mg per 100 ml), phospholipids (370 to 500 mg per 100 ml), cholesterol (287 to 485 mg per 100 ml), total lipids

(1,535 to 2,735 mg per 100 ml), ketones (12 to 29 mg per 100 ml), lactate (47 to 98 mg per 100 ml), pyruvate (1.8 to 1.9 mg per 100 ml), glucose (G) (18 to 59 mg per 100 ml), and urate (9.5 to 11.7 mg per 100 ml). Glucagon infusions led to gradual rise of blood glucose (max. 100 to 140 mg per 100 ml) and fall of FFA (1,569 to 360 μmoles per L), prompt rise of lactate (198 mg per 100 ml), and pyruvate (3.8 mg per 100 ml), and fall of serum CO_2 (11 mEq per L). Liver obtained at operation from one sibling (7 year old male) showed <10 per cent of normal G-6-phosphatase activity, but normal phosphorylase, G-6-P dehydrogenase, 6-P-gluconate dehydrogenase, phosphoglucomutase and fructose diphosphatase activities. Liver fat was 15.3 per cent, glycogen 9.3 per cent, lactate 438 mg per 100 ml, nitrogen 1.4 per cent (of wet weight of liver). Glucose-1-C^{14} and -6-C^{14} were utilized by liver slices in normal proportions ($C^{14}O_2$ from C-1/C-6 = 2.3 at 45 minutes). However, total utilization of glucose per gram of liver N was decreased, possibly explaining diabetic type glucose tolerance observed. Liver slices incubated with epinephrine or glucagon showed appreciable glycogen breakdown and lactate production. A postulated dynamic sequence is: phosphatase deficiency → increased G-6-P disposition into glycogen *and* via oxidative and glycolytic pathways → increased production of TPNH, DPNH, acetyl CoA, and lactate → increased synthesis of cholesterol, fatty acid, triglyceride → fatty liver and hyperlipidemias. The hyperlactic acidemia → increased muscle glycogen (1.7 per cent; forearm A/V lactate = 47.7/38.3 mg per 100 ml, decreased urate clearance (2.8 ml per minute per m^2) and hyperuricemia. Hypoglycemia → epinephrine and/or glucagon release → glycogen breakdown and accentuation of all of above.

Serological Reactions with a Newly Isolated Myxovirus. G. D. HSIUNG, PETER ISACSON and ROBERT W. MCCOLLUM, New Haven, Conn. (introduced by John R. Paul).

Using human or rhesus monkey kidney cell cultures, a virus was isolated from the blood of a fatal case of infectious hepatitis which occurred during an epidemic. This agent, designated as the DA virus, grew in primate kidney cells but without cytopathic effect, its presence being detected by the formation of plaques under agar overlay. A hemagglutinin was present, the characteristics of which served to classify this agent as a myxovirus closely related to mumps-Newcastle disease viruses. Serological studies have also indicated antigenic crossing with mumps and certain simian myxoviruses. Human sera have been tested for antibodies to this virus using techniques of plaque neutralization, hemagglutination-inhibition, complement-fixation, and agglutination of chick red cells treated with the DA virus, a phenomenon originally demonstrated by Burnet and Anderson in 1946, in which another myxovirus had been shown to have cell sensitizing properties which give rise to hemagglutination with sera from cases of infectious mononucleosis and hepatitis and some other diseases. Sera from jaundiced cases of infectious hepatitis from the same epidemic showed higher rates of antibodies to the DA virus than were found in sera from persons in the same area but without overt evidence of hepatitis. Nine of 11 serial serum series from experimentally induced hepatitis in humans showed fourfold or greater rises in the DA-treated cell agglutinin. No rises of this agglutinin were noted in serial sera from cases of measles or poliomyelitis, but elevated titers were noted with frequency in certain types of hepatic cirrhosis.

The data indicate an association between positive serological tests involving the DA virus and certain forms of liver disease, but do not warrant conclusions as to any specific etiologic role of this virus.

Metabolic Regulation of Cardiac Output. WILLIAM E. HUCKABEE,* Boston, Mass.

In mild exercise, no correlation was found between change in O_2 consumption (ΔV_{O_2}) and change in cardiac output (Δ C.O.) in normal or heart failure patients, nor consistent differences between the groups in the slope of this "response curve." However, the response of C.O. to an O_2-sensing mechanism has not been clearly established; biochemical changes which are only irregularly related to P_{O_2} might be responsible. Therefore, the tissue metabolic changes of hypoxia were induced in dogs in such a way that blood and cellular O_2 levels would remain high, *i.e.,* by inhibition of cytochrome oxidase with cyanide, 0.02 to 0.04 mmoles per kg, intra-arterially. C.O. increased markedly, reaching sustained levels of 700 per cent increase. Despite continuing increase in venous blood and tissue P_{O_2} (80 to 90 mm Hg), the C.O. increase was progressive for 15 to 20 minutes. V_{O_2} and V_{CO_2} were reduced 20 to 50 per cent; blood pH was constant. The cardiac response, of a magnitude previously produced only by muscular exercise, was associated with reduced ventricular diastolic pressure and was not prevented by vagotomy, adrenalectomy (plus phentolamine), ganglionic blocking agents, antihistaminics, or clamping of the carotid arteries. Several metabolic inhibitors blocking at levels lower than cytochrome oxidase had no stimulating effect; the highest block was effected at pyruvic dehydrogenase by oxythiamine. C.O. was diminished by this procedure in dogs and pigs; it remained normally responsive to infusions, epinephrine and cyanide, and always returned to the reduced level again after these stimuli. It was concluded that: *1*) Blood flow is not responsive to changes in molecular O_2, but to metabolic processes in the tissues without regard to P_{O_2} or V_{O_2}. *2*) The effective metabolic change is not all-inclusive, but lies between cytochrome oxidase and pyruvic dehydrogenase. *3*) The metabolic change induces a cardiac response by unknown mechanisms, not involving filling pressure, catecholamines or sympathetic nerves.

High Altitude Pulmonary Edema. HERBERT N. HULTGREN,* WARREN B. SPICKARD and KURT O. HELLRIEGEL, Palto Alto, Calif.

Eighteen patients with a unique variety of acute pulmonary edema, occurring upon exposure to an altitude

of 12,200 to 15,300 feet, were observed at the Chulec General Hospital in La Oroya, Peru from 1950 to 1959. Initial manifestations appeared 12 to 36 hours after arrival from a lower elevation. Symptoms consisted of cough, hemoptyses, dyspnea, and weakness.

Physical examination revealed tachycardia, hypotension, cyanosis, and pulmonary rales. Signs of heart failure were absent. Temperature elevations exceeding 100° were seen in only 3 patients and leukocytosis greater than 12,500 cells was seen in only 5 patients. X-ray study revealed bilateral pulmonary densities which were usually patchy. In 6 patients diffuse pulmonary exudates filled both lung fields. No enlargement of the left ventricle or left atrium was noted, but transient prominence of the pulmonary artery occurred in 7 patients. Electrocardiograms revealed right ventricular "strain" patterns and peaking of the P waves which became more normal on recovery.

Bed rest and oxygen administration resulted in complete clinical recovery and clearing of the pulmonary exudate in 24 to 48 hours. Digitalis was ineffective. No deaths occurred.

Fifteen of the 18 patients had been thoroughly acclimatized, and developed pulmonary edema upon returning to the mountains after a 1 to 3 week stay at sea level; 3 patients had not previously been exposed to high altitude. None gave a history of a preceding respiratory tract infection. Eleven patients were less than 18 years of age and 14 were males. Recurrent episodes in 4 patients and 2 occurrences in siblings suggest an individual and a familial susceptibility. There was no racial predilection.

High altitude pulmonary edema thus appears to be a unique effect of anoxia upon the circulation. The data suggest that factors other than left ventricular failure may be important in its pathogenesis.

Thyroxine Metabolism in the Absence of Thyroxine-binding Globulin and its Implications with Regard to the Physiological Role of Hormonal Binding. SIDNEY H. INGBAR,* Boston, Mass.

A patient with a newly-recognized syndrome, characterized by absence from the plasma of thyroxine-binding globulin (TBG) has afforded a unique opportunity to evaluate the hypothesis that TBG is a regulator of the peripheral turnover of thyroxine. Despite a protein-bound iodine of only 2 μg per 100 ml, there were no clinical stigmata of myxedema; BMR, cholesterol, and thyroid uptake of I^{131} and I^{127} were normal. These discrepant findings prompted electrophoretic studies of the patient's serum, which revealed normal binding of thyroxine by pre-albumin, but no demonstrable binding by TBG. Associated abnormalities included atrial septal defect and testicular atrophy. Androgenic hormones decrease thyroxine-binding by TBG. However, the patient's manifest hypogonadism and failure of prolonged administration of prednisone to restore TBG in the serum excluded gonadal and adrenal androgens as the cause of his binding abnormality.

In earlier studies designed to elucidate the physiologic function of TBG, estrogens increased TBG and concomitantly decreased clearance of thyroxine from the plasma by peripheral tissues. However, changes in hormonal metabolism secondary to alterations in TBG could not be distinguished from possible direct effects of estrogen on hormonal degradation. In the present patient, control studies of thyroxine metabolism revealed that, while PBI was low and total hormonal turnover normal, the rate of clearance of thyroxine was abnormally rapid. Although a six week course of estrogen induced fluid retention and pronounced gynecomastia, it did not increase TBG or change the kinetics of thyroxine metabolism. Direct estrogenic effects on hormonal degradation were thereby excluded.

These findings indicate that TBG regulates the rate of removal of thyroxine from plasma, presumably by limiting its availability to the cell. They further suggest that in man, thyroid activity is regulated by the delivery of hormone to peripheral tissues, rather than by the concentration of hormone in the circulation.

Effects of Alcohol on the Liver: Mechanism of the Impaired Galactose Utilization. KURT J. ISSELBACHER* and ELIZABETH A. MCCARTHY, Boston, Mass.

Previous investigators have observed that small amounts of alcohol ingested by normal individuals produce immediate and striking inhibition of galactose tolerance, reflecting an impairment of hepatic galactose utilization. Since the galactose tolerance test is a very sensitive index of liver function, we have investigated the mechanism whereby alcohol can produce such pronounced alterations in carbohydrate metabolism.

Observations that the administration of alcohol to rats results in a doubling of the hepatic levels of reduced diphosphopyridine nucleotide (DPNH) suggested that the inhibition of galactose metabolism by alcohol might be secondary due to the increased DPNH levels—especially since a key enzyme in galactose metabolism (uridine diphosphogalactose-4-epimerase) is inhibited by DPNH. Consistent with this hypothesis were the findings that the alcohol inhibition of galactose oxidation in rat liver homogenates was enhanced by factors increasing the metabolism of alcohol (resulting in concomitant increases in DPNH) and completely reversed by metabolites reducing the DPNH concentration. In erythrocytes, which readily metabolize galactose but have no alcohol dehydrogenase (ADH), there was no inhibition of galactose oxidation by alcohol. However, when exogenous ADH was added, alcohol produced a marked inhibition of galactose oxidation by red blood cells. Examination of all the soluble enzymes involved in the conversion of galactose to CO_2 revealed no inhibition of enzyme activity by alcohol. In the presence of ADH however, even very low concentrations of alcohol produced an 80 to 90 per cent inhibition of one enzyme, namely UDP-galactose-4-epimerase.

These studies indicate that alcohol inhibits galactose utilization in the liver by producing increased cellular

levels of DPNH. The increased DPNH effectively inhibits a key enzyme in galactose metabolism. The results serve to emphasize the importance of cellular concentrations of pyridine nucleotides, as well as their oxidation-reduction state, in the overall regulation of carbohydrate metabolism.

Oxidative Hemolysis and Precipitation of Hemoglobin: Heinz Body Anemias as an Accelerated Form of Red Cell Aging. JAMES H. JANDL* and DAVID W. ALLEN, Boston, Mass.

Although the basis for hypersusceptibility to the hemolytic effect of certain aromatic amines has been well characterized recently, the actual mechanism of the hemolytic process is poorly understood. Comparisons were made of the effects *in vitro* of drugs such as phenylhydrazine upon red cells and upon solutions of crystallized hemoglobin. In either state hemoglobin was initially transformed reversibly to methemoglobin and then irreversibly to "sulfhemoglobin" and a fast moving component on electrophoresis and column chromatography. Eventually hemoglobin in solution was precipitated into small, blue-staining coccoid bodies identical in appearance and manner of evolution to Heinz bodies. These denaturative changes in hemoglobin apparently involved oxidation of the thiol groups of hemoglobin, since they were slowed by reduced glutathione (GSH) and partially reversed by various thiols. GSH in the presence of phenylhydrazine and hemoglobin became bound to hemoglobin by forming mixed disulfides; in so doing GSH increased the size, while diminishing the number, of "Heinz bodies" formed. These interactions may explain the disappearance of red cell glutathione and certain differences in Heinz body size previously observed by others during drug-induced anemias.

Since these drugs appeared to catalyze the oxidative denaturation of hemoglobin by oxygen, the effects of prolonged exposure of red cells and hemoglobin alone to atmospheric oxygen *in vitro* were investigated. Under O_2, but not under CO, the same sequence of oxidative injury slowly evolved: methemoglobin, sulfhemoglobin, a fast moving hemoglobin on electrophoresis, and, finally, the formation of typical Heinz bodies.

It is concluded that: *1*) Hemolytic aromatic amines cause oxidative denaturation of hemoglobin and its precipitation as Heinz bodies. *2*) These drugs are redox intermediates between oxygen and hemoglobin because of their capacity to react well either with paired or with single electrons. *3*) Heinz body anemias represent an acceleration of normal mechanisms of red cell aging.

A "Precursor Solution" of Pancreatic Juice: Evidence for an Exchange Mechanism. H. D. JANOWITZ* and D. A. DREILING, New York, N. Y.

Any hypothesis of pancreatic electrolyte secretion must at least account for *1*) the apparent direct relationship between HCO_3 concentration (range 25 to 150 mEq per L) and rate of flow; *2*) the reciprocal relation between Cl and HCO_3 in an isosmotic fluid; and *3*) dependence of secretion on carbonic anhydrase activity.

Pancreatic juice in man was obtained by duodenal intubation with a collecting system which obviated gastric contamination and minimized intestinal loss. The rate of flow and composition of secretin stimulated secretion (1.0 μ per kg) was studied in 27 subjects before and after cholinergic blockade (Lakeside, compound No. JB 323, 340). Although the rate of flow was markedly reduced (mean depression 65 per cent, range 38 to 89 per cent) there was no significant alteration in HCO_3 concentration (mean change +3 per cent, range 0 to 8 per cent). Similar dissociation of HCO_3 and flow rates has been reported by us after carbonic anhydrase inhibition with Diamox (J. clin. Invest. 1957, **36**, 904). This is in marked contrast to the reciprocal rise in Cl and fall in HCO_3 concentrations when rate of flow is reduced by adrenal cortical hormones (cortisol, prednisone) in normal subjects or that which occurs spontaneously in pancreatitis. Na and K concentrations, which approximate plasma levels, remain independent of rate of flow in all circumstances.

These results suggest that the pancreas elaborates a "precursor solution" of isotonic Na and K HCO_3 which is then modified by a process in which Cl is exchanged for HCO_3 within the gland, and requires carbonic anhydrase activity. It is presumed that this exchange occurs in the intercalated duct recently shown by electron-microscopy to bear microvilli.

"Trachoma Viruses" Isolated in the United States. ERNEST JAWETZ,* PHILLIPS THYGESON, LAVELLE HANNA, CHANDLER DAWSON and YUKIHIKO MITSUI, San Francisco, Calif. and Tokushima, Japan

Trachoma is an eye disease affecting 400 million persons in the world, often causing blindness. It is very prevalent in Asia and Africa but rare in the United States. Recently strains of "trachoma virus" have been isolated in Africa and Asia. We have succeeded in growing for the first time "trachoma viruses" from patients in the United States.

The viruses were isolated by injecting conjunctival scrapings in broth-saline containing 4 mg per ml streptomycin into the yolk sacs of 7-day embryonated eggs, incubated at 35° C. After 1 to 4 blind passages embryos died and elementary bodies were seen in stained yolk sac smears. After additional passages egg LD_{50} titers reached 10^7 per ml. The agents fixed complement with specific antisera to psittacosis virus, but were not infectious for small laboratory animals or tissue cultures by usual technics.

One strain (Bour) was isolated from acute trachoma in a white California resident, with a serum complement-fixing (CF) titer of 1:64 to psittacosis-LGV group antigens. This virus diluted 10^{-3} produces intense follicular conjunctivitis with abundant inclusions in *M. cynomolgus*. Instilled into the eyes of human volunteers it produces typical acute trachoma with inclusions. A second strain (Asgh) was isolated from a trachomatous relapse in a Pakistani who had acquired the infection

more than 20 years ago. During the past 5 years, living in Britain and the United States, he had been free from ocular symptoms. His serum CF titer to the group antigen was 1:4. That virus diluted 10^{-1} produces only minimal follicular conjunctivitis with rare inclusions in monkeys. Thus it appears that either the geographic origin or the persistence in an infected host may influence pathogenetic properties of "trachoma viruses." Three additional strains were isolated from active trachoma in Apache Indian children at the San Carlos Reservation. The pathogenetic position of these strains is under study.

Characterization and Significance of Inhibitors Controlling the Activation of Fibrinolysis in Man. ALAN J. JOHNSON,* New York, N. Y.

The level of circulating inhibitors in the plasma of normal individuals is immediately concerned with the control and regulation of fibrinolysis in man. The mechanism of fibrinolysis by streptokinase (SK) may be divided into 3 stages: *1*) activator formation by streptokinase and proactivator; *2*) plasmin formation by activator and plasminogen; and *3*) fibrinolysis production by plasmin and fibrin. Urokinase, an activator from human urine, reacts with plasminogen directly as in Stage 2 above.

An inhibitor of streptokinase, Stage 1, and an inhibitor of activator, Stage 2, will be described in the present studies. Both occur in the α-globulin fraction and appear to be nonenzymatic proteins since they are nondialyzable, digested by trypsin and pepsin, fast-acting and are relatively temperature independent. They are labile at 4° C and are readily denatured by organic solvents. The streptokinase inhibitor is measured specifically by SK inhibition and is not affected by urokinase *in vitro* or by spontaneous activator *in vivo*. It resembles SK antibody which also acts at Stage 1, neutralizing SK, because it usually increases *in vivo* 5 to 10 days after parenteral SK injection or following natural infection with the streptococcus. The increase was not proportionate, however, with respect to SK antibody. The streptokinase inhibitor is different from SK antibody since it is: *1*) ether labile, *2*) forms no precipitate with SK in gel-diffusion studies, and *3*) shows no reaction on passive cutaneous anaphylaxis. The activator inhibitor is measured specifically, by urokinase inhibition. It differs from the streptokinase inhibitor in its pH stability and does not give a delayed response to the parenteral injection of SK.

These (two) inhibitors have been found to determine, in part, the thrombolytic effect of intravenously administered SK, to control physiological spontaneous fibrinolysis, and to be deficient, if not pathogenetic, in pathological spontaneous fibrinolysis.

Fatty Acid Patterns of Human Lymph and Serum after Corn Oil or Coconut Oil Feeding. HERBERT J. KAYDEN, ARTHUR KARMEN, ALLAN E. DUMONT and JOSEPH BRAGDON, New York, N. Y. and Bethesda, Md. (introduced by Charles E. Kossmann).

The effect of different dietary fats upon the fatty acid composition of the lipoproteins of human lymph and serum was studied. The thoracic duct was cannulated in the neck in 2 subjects and samples of lymph were collected in the fasting state and after feedings of 100 g of corn or coconut oil. Serum samples were obtained after comparable corn and coconut oil feedings.

Chylomicrons were isolated from all samples of serum and lymph by centrifugation at $26{,}000 \times G$ (sp. gr. 1.005) for 30 minutes and then purified by saline washes and repeated centrifugations in the same gravitational field. After removing the chylomicrons, lipoproteins of the various density classes were separated from the infranates by successive ultracentrifugations at specific gravities of 1.019, 1.063 and 1.21. The fatty acid composition of each fraction was determined by gas chromatography.

The fatty acid composition of the lymph chylomicrons 8 hours after ingestion of fat became virtually identical with that of the corn oil or the coconut oil in the test meal. The fatty acid composition of the serum chylomicrons showed similar changes to a lesser degree.

Comparisons were made of the changes in the fatty acid composition of the other lipoprotein fractions of the serum and lymph. Little change was produced in the low density fraction 1.019 to 1.063 by either the corn or coconut oil. The density classes 1.005 to 1.019 and 1.053 to 1.21, however, showed definite enrichment with the predominant components of the dietary fat.

The prompt appearance of the fed fatty acids in the high density lipoprotein fraction is in accord with the hypothesis, based on analysis of the peptide composition, of a close association between chylomicrons and high density lipoproteins.

A Comparative Study of Pulmonary Circulatory Effects of Intravenous Isoproterenol in Pulmonary Emphysema, Heart Failure and Mitral Stenosis. KAYE H. KILBURN and HERBERT O. SIEKER,* Durham, N. C.

The cardiopulmonary circulatory effects of intravenous isoproterenol were determined in various types of cor pulmonale in order to separate causes of pulmonary hypertension which often coexist, e.g., chronic pulmonary insufficiency and heart failure. The results were compared with those obtained during exercise, oxygen breathing, acetylcholine infusion, acetyl strophanthidin administration, and unilateral occlusion of the pulmonary artery. Studies were done on 37 patients with pulmonary emphysema with and without heart failure, 8 with heart failure alone, 8 with mitral stenosis, and 7 normal controls. Cardiac output and pressures in the right heart, pulmonary circulation and arterial system were measured. Pulmonary vascular resistance was calculated.

Exercise increased cardiac output and pulmonary artery pressure in the 3 groups of patients. In heart failure, isoproterenol increased the cardiac index (1.9 to 3.0 L per minute per m^2), lowered pulmonary artery pressure (41 to 35 mm Hg), and lowered pulmonary vascular resistance (466 to 297 dynes-sec-cm^5). In pulmonary emphysema, it increased the cardiac index and pulmonary artery pressure (2.9 to 3.9 L per minute per m^2; 30 to 35 mm Hg). Patients with mitral stenosis showed a similar response. In emphysema, oxygen breathing and

acetylcholine lowered pulmonary artery pressure without altering cardiac output, and acetyl strophanthidin mimicked the effect of isoproterenol. Unilateral pulmonary artery obstruction increased pulmonary artery pressure in emphysema but not in heart failure. Patients with emphysema and heart failure combined showed the heart failure type of response to isoproterenol.

It is suggested that isoproterenol is useful in separating pulmonary hypertension related to left ventricular failure from that associated with structural changes in the lungs or mitral valve. These studies also imply that left ventricular failure in patients with pulmonary emphysema will be manifest primarily as right ventricular failure. Finally, it is suggested that isoproterenol may be useful in treatment of heart failure either with or without pulmonary emphysema.

The Effect of Cell Structure and Growth Hormone on Protein Synthesis in Striated Muscle. DAVID M. KIPNIS and ERIC REISS, St. Louis, Mo. (introduced by Carl V. Moore).

The effects of cell structure and growth hormone on the metabolic events regulating protein synthesis in muscle form the basis of the present report. Use of the "intact" *in vitro* rat diaphragm permitted investigation, under steady state conditions, of *1*) intracellular transport of amino acids as measured with the nonutilizable amino acid α-aminoisobutyric acid-1-C^{14} (AIB); *2*) intracellular amino acid concentration (pool size) as reflected by lysine and proline determinations; and *3*) protein synthesis as determined by incorporation of labeled amino acids into tissue protein.

Amino acid transport decreased 50 per cent following hypophysectomy and was restored to normal by *in vivo* administration of growth hormone. This effect appeared to be partially mediated by insulin since it was greatly reduced in alloxan-diabetic hypophysectomized animals. Furthermore, *in vitro* addition of bovine or human growth hormone produced small and, at times, equivocal results.

Intracellular pools of lysine and proline were not appreciably changed by hypophysectomy or growth hormone administration; similar results were obtained with AIB *in vivo*. Amino acid incorporation into protein decreased 50 per cent following hypophysectomy and was restored to normal by bovine growth hormone administered *in vivo* but not *in vitro*. Despite this hormonal effect, the specific activity of the intracellular amino acid pool was similar under all conditions, suggesting direct shunting of amino acids entering cells to sites of protein synthesis or a very small "effective" pool. This conclusion was strengthened by the observation that label appeared in protein as a linear function of time whereas specific activity of the amino acid pool approached equilibrium exponentially.

Growth hormone stimulates both amino acid transport and protein synthesis. These processes are apparently so intimately coordinated that changes in one are accompanied by similar changes in the other. The "total" intracellular amino acid pool does not appear to be of functional significance in protein synthesis.

Observations on the Mechanism of Tolerance to the Local Shwartzman Phenomenon Using Isotopically Labeled Endotoxin. DIETER KOCH-WESER* and JOHN M. MOSES, Cleveland, Ohio.

Tolerance to endotoxins has been studied using the local Shwartzman phenomenon as a measure of host reactivity. When appropriate doses of lipo-polysaccharide from *E. coli* (LPS-C) are used, this is a reliable index of tolerance. Tolerance was induced in two different ways, either by 7 or more daily injections of the *same* LPS-C, or by 7 daily injections of a sonic cell wall preparation of Paracolon, containing another lipo-polysaccharide (LPS-P). The mechanism of tolerance was investigated by using LPS-C labeled either with $carbon^{14}$ or with $chromium^{51}$. Continuous blood clearance, the role of "trapping" in the lung and of removal by the RES were determined by cannulating one carotid artery and leading the cannula through a scintillation counter into the opposite jugular vein, as well as by assaying the radioactivity in several organs after an intravenous injection of labeled LPS-C.

Approximately 96 hours after the first injection, the serum of rabbits made tolerant with LPS-C contains precipitins which combine *in vitro* with added labeled LPS-C. The precipitate, which by counting can be quantitated in terms of LPS-C content, has no endotoxin activity as measured by its ability to prepare for the local Shwartzman phenomenon. When serum from an animal made tolerant by injections of LPS-C is given to a normal rabbit, the recipient becomes tolerant to LPS-C for at least 48 hours. If labeled LPS-C is given intravenously to rabbits made tolerant by repeated injections of LPS-C, the radioactive material is rapidly trapped in the lungs, presumably as an endotoxin-precipitin complex. The serum of rabbits made tolerant to LPS-C by repeated injections of LPS-P does not contain precipitins to LPS-C and, injected into normal animals, does not produce tolerance to LPS-C.

It would appear, therefore, that there are at least two mechanisms involved in tolerance, one associated with antibody formation, which can be passively transferred, the other nonspecific, which cannot be passively transferred and seems to be related to an activated RE system.

Modification by Diet of Urinary Amino Acid Pattern in Cystinuria. FELIX O. KOLB and HAROLD A. HARPER, San Francisco, Calif. (introduced by Peter H. Forsham).

The urinary amino acid pattern of patients with cystinuria and cystine stones shows a specific hyperexcretion of lysine, cystine, arginine, and ornithine in the presence of normal plasma concentrations on the basis of a renal tubular reabsorptive defect, virtually complete for lysine and cystine and partial for arginine and ornithine. The oral administration of methionine and *cys-*

teine markedly enhanced the cystine excretion in cystinurics, while minor increases were observed in normals. In contrast, oral administration of *cystine* produced no increase in urinary cystine even in cystinurics. This is readily explained by differences in the solubility of individual amino acids affecting absorbability from the intestine. After methionine and cysteine feeding, the plasma levels increased significantly while no appreciable increase was noted after cystine. Drastic restriction of dietary methionine- and cysteine-containing proteins has been studied over a period of 4 years in 14 human subjects and in 1 cystinuric dog. By dietary means urinary cystine was reduced without consistently altering urinary lysine or arginine. Under different dietary conditions various excretion patterns of the 4 affected amino acids have been produced without any fixed ratios. On only intravenous glucose as a source of calories a human cystinuric showed the same urinary excretion of cystine and lysine as when on marked dietary restriction, suggesting that this is a minimum of cystine derived from amino acids of endogenous protein. Dietary restriction has been of little value in reducing cystinuria in 3 cystinuric children whose greater need for protein anabolic processes might not have been met by the diet. Two of the three children continued to form stones. In contrast, the reduction of urinary cystine by diet in 11 adults and 1 dog correlated with decreased stone formation and even resolution of stones.

Studies With a Cyclic Phosphamide Ester of Nitrogen Mustard: Cyclophosphamide. D. R. KORST, F. D. JOHNSON, C. D. COBAU, M. H. RENNIE and E. P. FRENKEL, Ann Arbor, Mich. (introduced by Paul S. Barker).

Cyclophosphamide (Cytoxan), an ester of nitrogen mustard, is activated by intracellular enzymes to the active *bis,* β-chlorethyl group. We have performed studies in animal and man to determine tolerance, bone marrow effect, and plasma protein changes.

The turnover of radioiron (Fe^{59}) and tritiated thymidine (H^3T) has been used in rats and in rat bone marrow 24 hour culture; 22 mg per kg of drug in 6 rats results in a 24 hour RBC uptake of 5.3 ± 2.3 per cent. Normal rat Fe^{59} turnover is 18.2 ± 2.5 per cent and after 0.25 mg per kg of HN_2 is 10.3 ± 3.7 per cent. Mice with myeloma tumor (X5563) and gamma globulin peaks show the same degree of suppression of iron turnover after the drug without significant alteration in protein pattern. P^{32} localizes in highest concentration in marrow, intestine and tumor. The change in H^3T turnover is not as significant as Fe^{59}. The *in vivo* changes are not seen in the marrow culture experiments where cyclophosphamide is added, while depression is obtained with agents like NaF. This indicates lack of cyclophosphamide activation *in vitro*. Erythropoietic stimulation is possible *in vitro* in presence of the drug.

Patients receiving up to a total of 100 mg per kg total dose show almost complete failure of plasma clearance and turnover of Fe^{59} in the immediate post-treatment period. Doses in the 30 mg per kg range do not produce this effect. Three patients with multiple myeloma improved; two symptomatically without alteration in abnormal proteins, and a third patient has had two remissions with positive decrease in abnormal protein only after the initial large dose of the drug.

Cyclophosphamide induces the same bone marrow depressant effects *in vivo* as other alkylating agents. It is not active in bone marrow *in vitro*. It may be useful in treatment of multiple myeloma.

Acute Adult Glomerulonephritis: Studies of Healing and Progression to Chronicity. DANIEL S. KUSHNER, PAUL B. SZANTO and ALVIN DUBIN, Chicago, Ill. (introduced by S. Howard Armstrong, Jr.).

Most adults (analogously to children) have been presumed to recover completely from β-streptococcal acute glomerulonephritis; some hold nonstreptococcal pathogenesis for ominous adult chronic glomerulonephritis. Correlation of serial renal biopsies with recurrent streptococcal infections, quantitated incidental pyelonephritis, electrophoretically quantitated proteinuria, steptococcal antibodies, and crude glomerular filtration rates, in all adults since 1956 at Cook County Hospital with presenting diagnosis of acute glomerulonephritis was a first step in evaluation of these presumptions. Analysis of 45 patients leads us to this breakdown:

Histologic healing: 10; streptococcal relationship initially: all; subsequently: 2; associated nephritic exacerbations: 1; incidental pyelonephritis: 1; average follow-up only 8 months.

Histologic chronicity, clinical healing: 7; streptococcal relationship initially: 6; subsequently: 4; associated nephritic exacerbations: 3; incidental pyelonephritis: 3; average follow-up 19 months.

Histologic and clinical chronicity: 4; streptococcal relationship initially: all; subsequently: 1; no nephritic exacerbation or pyelonephritis; average follow-up 21 months.

Uncertain initial classification: acute vs. chronic with hematuric exacerbation: 16; streptococcal relationship initially: only 8; subsequently: 1 (no nephritic exacerbation); incidental pyelonephritis: 7; average follow-up 16 months.

Declined biopsy: 8; streptococcal relationship initially: all; subsequently: 1; no nephritic exacerbation or pyelonephritis; GFR's <70 ml per minute: 4; average follow-up 18 months.

Carbohydrate-rich α_1-globulin was prominent in initial proteinuria (maximum 10 g per 24 hours) of which gamma globulin never exceeded 12 per cent.

When present, histopathologic stigmata of chronicity comprised: *1)* Residuals of glomerular inflammation qualitatively approaching, while quantitatively not equaling, those considered characteristic of chronic glomerulonephritis. *2)* Persistent focal interstitial lymphocytic infiltrates. *3)* Focal tubular atrophy; interstitial fibrosis. The persistence of these changes in patients apparently fully recovered raises the question as to whether existing

presumption of the long-term innocuous character of streptococcal acute glomerulonephritis in the adult may require revision.

Rapid Induction of Isolated Riboflavin Deficiency in Man. MONTAGUE LANE, CHARLES E. MENGEL and DOROTHY J. DOHERTY, Bethesda, Md. (introduced by Stanley M. Levenson).

Evidences of a deficiency state have been observed occasionally in human riboflavin deprivation studies, but only after months of dietary restriction. Previous efforts to induce clinically recognizable deficiency rapidly with riboflavin antagonists have been unsuccessful. Pharmacological studies have shown that galactoflavin is a riboflavin antagonist and antitumor agent in rodents. It is absorbed following ingestion by man and is not degraded to inactive metabolites. This agent was therefore selected, in conjunction with a riboflavin restricted diet, for trial in the present investigations.

Three adult males with metastatic neoplasms were placed on a semisynthetic diet restricted to a maximum of 0.5 mg of riboflavin per day, adequate in calories, protein, fat, and minerals, and supplemented with other vitamins. Galactoflavin was given orally in doses of 1.5 to 3.0 g per day. Urinary riboflavin excretion exceeded intake following the administration of galactoflavin, indicating that it displaced body riboflavin. Signs of riboflavin deficiency were evident 21 to 30 days after the start of galactoflavin administration. All patients developed seborrheic dermatitis of the face and ears, angular stomatitis, and cheilosis. One patient demonstrated extensive loss of filiform papillae and "mushrooming" of fungiform papillae of the tongue, burning, and loss of taste. Seborrheic dermatitis of the scrotum was also noted. The described signs were rapidly and completely reversed with riboflavin therapy. A striking finding, not previously reported in human deprivation studies, was the development of reticulocytopenia and severe anemia in the three patients. Blood loss or hemolysis was not evident. The anemia did not respond to parenteral administration of iron and vitamin B_{12}. Reticulocytosis occurred promptly upon supplementation with riboflavin.

In the patients studied clinical riboflavin deficiency was induced rapidly and riboflavin appeared to be essential for normal erythropoiesis.

Micropuncture Study of Net Transtubular Movement of Water and Urea in the Rat Kidney. WILLIAM E. LASSITER, CARL W. GOTTSCHALK* and MARGARET MYLLE, Chapel Hill, N. C.

Anesthetized, nondiuretic male rats were infused with C^{14}-labeled inulin carboxylic acid or urea, and fluid was subsequently collected by micropuncture from proximal and distal convolutions on the surface of the exposed kidney. Radioactivity of tubular fluid, ureteral urine, and plasma was determined in a windowless flow counter. Puncture sites were localized by microdissection.

Although there was considerable scatter among individual samples, the average fluid/plasma inulin ratio increased progressively along the proximal convolution and averaged 2.8 in the final 30 per cent of the convolution. The fluid/plasma urea ratio, on the other hand, quickly reached a value of approximately 1.5, but showed little further increase along the length of the convolution. Hypo-osmotic fluid from the first portion of the distal convolution had an average fluid/plasma inulin ratio of 7.9. The urea ratio in early distal samples was similar to that of inulin. Urea concentration decreased along the distal convolution, while the inulin concentration tended to rise. Ureteral urine/plasma urea ratios in these animals were low, averaging one-tenth that of inulin.

These results suggest that, in the presence of antidiuretic hormone, the entire cortical nephron is permeable to urea, except for the ascending limb of the loop of Henle, which is also believed to be relatively impermeable to water. Concentration gradients presumably favor diffusion of urea out of all segments of the nephron except the loop of Henle, where net diffusion appears to occur into the thin descending limb from the medullary interstitium. The high medullary interstitial concentration of urea is thought to result from diffusion of urea out of the collecting duct and the action of the countercurrent system.

Renal Tubular Secretion of Uric Acid in the Mongrel Dog. WILLOUGHBY LATHEM,* BERNARD B. DAVIS and GERALD P. RODNAN, Pittsburgh, Pa.

The prevailing view of the mechanism of uric acid excretion in the mongrel dog is that a filtration-reabsorption mechanism is involved. This hypothesis was examined in the present study by increasing the rate of urate excretion by two means: mannitol-induced osmotic diuresis and intravenous sodium urate loading. Anesthetized dogs were used and urine was collected from the ureter or the bladder. Sodium urate was given intravenously at a rate of 30 mg per minute and mannitol (20 per cent) was administered simultaneously at a rate of 9 ml per minute. Under these conditions the plasma urate concentration increased from an average of 0.30 mg per 100 ml to levels of 5 to 25 mg per 100 ml and the rate of urate excretion increased strikingly. In 11 of 18 animals the rate of urate excretion exceeded the rate of filtration (P urate $\times$ creatinine clearance) by 5 to 35 per cent. This high rate of excretion could not be accounted for by non-ionic diffusion; there was no significant relationship demonstrable between urinary pH and urate excretion.

These results are therefore indicative of tubular urate secretion. An attempt to demonstrate the site of secretion by stop flow analysis was not successful. Reabsorption in a proximal site could be demonstrated, but secretion could not.

The demonstration of tubular urate secretion in the mongrel and Dalmatian dogs and in man and the rabbit suggests that this is a general or common mammalian transport mechanism.

Antibody Production in Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA). S. L. LEE, L. E. MEISELAS, S. B. ZINGALE and R. RICHMAN, Brooklyn, N. Y. (introduced by Robert Austrian).

To determine whether or not hetero-, iso-, and auto-antibodies vary in similar fashion in patients with SLE and RA, antibodies in each category were followed in a series of patients after their exposure to bacterial and rickettsial antigens. Five patients with clinical and laboratory evidence of SLE and 15 patients with typical RA were inoculated with vaccines of brucellae and rickettsiae. Antibodies to *Br. abortus, Br. melitensis* and *R. ricketsii* were assayed by appropriate serologic reactions. The titers of isoagglutinins and of the "abnormal antibodies" of SLE and RA were measured simultaneously in all patients.

Antibacterial and antirickettsial antibodies appeared in the patients with SLE at comparable times and in titers similar to those observed in a control group of patients; in the patients with RA the titers were somewhat higher. When titers of antibacterial antibodies rose, corresponding increases in isoagglutinins were observed regularly. Antinuclear "antibodies" of SLE were observed, however, to vary independently of heterologous and isologous antibodies. On the other hand, variations in the titer of the latex fixation reaction in patients with RA corresponded with the observed changes in heterologous and isologous antibodies. The lack of correlation between variations in the titer of "auto-antibody" and of other antibodies in patients with SLE suggests that, in this disease, auto-antibodies may be relatively uninfluenced by factors which affect the behavior of other antibodies.

Pantothenic Acid, Fatty Liver and Alcoholism. CARROLL M. LEEVY, WILLIAM S. GEORGE, HERMAN ZIFFER and HERMAN BAKER, Jersey City, N. J. and New York, N. Y. (introduced by Harold Jeghers).

Circulating levels of pantothenic acid, thiamine, nicotinamide, folic acid, pyridoxine and cyanocobalamin were determined by microbiological techniques in 20 normal subjects and 36 patients hospitalized with acute alcoholism without clinical stigmata of vitamin deficiency. Liver biopsy in the latter group revealed normal liver in 12, fatty liver in 10 and cirrhosis in 14. Results were correlated with histopathology and tissue vitamin levels. A consistent finding was a two- to threefold increase in circulating pantothenic acid with a decrease in hepatic tissue levels in patients with fatty liver and cirrhosis with fatty metamorphosis and necrosis. This was accompanied by a decrease in circulating thiamine, nicotinamide and folic acid; pyridoxine levels were unchanged. Cyanocobalamin, unlike pantothenic acid, was normal in patients with fatty metamorphosis and increased with liver cell necrosis. Vitamin levels were within normal range in both alcoholic subjects without hepatic lesions and in patients with inactive cirrhosis.

Mobilization of liver fat by diet was associated with a concomitant decrease in pantothenic acid levels and an increase in thiamine, nicotinamide and folic acid; vitamin therapy produced unsustained alterations in circulating levels in this group. Infusion of 15 per cent ethanol for 60 minutes (maximum blood levels 100 to 125 mg per 100 ml) under conditions of hepatic vein catheterization produced an increased hepatic output of pantothenic acid.

Circulating pantothenic acid appears to provide a highly sensitive index to liver injury. Decreased ability of the liver to bind pantothenic acid causes increased circulating levels and depletes stores of this vitamin. This results in decreased coenzyme A activity and abnormal fat metabolism.

The Relationship Between Cationic Amino Acids and Potassium in Rat Muscle In Vitro. NORMAN G. LEVINSKY, IAN TYSON and ARNOLD S. RELMAN,* Boston, Mass.

An intact rat diaphragm technique which permits prolonged observation of steady state relationships has been used to study cation equilibrium. Unlike the *in vivo* situation, intracellular K *in vitro* is unchanged (about 150 mEq per L) as external K is reduced from 5 to 1 mEq per L. Since it has been reported that cationic amino acids (CAA), primarily lysine, accumulate in K-depleted muscle, we have studied the *in vitro* interactions between CAA and K.

At bath K's from 5 to 1 mEq per L, addition of lysine approximately at *in vivo* CAA plasma concentration (0.8 mEq per L) lowered muscle K by 5 per cent or less. Further increases in bath lysine to 4 mEq per L progressively reduced muscle K, but there was no more change at 8 or 16 mEq per L of lysine; up to 30 to 40 per cent of muscle K was lost. This loss was always approximately equal to the lysine accumulated, and Na did not increase. At any external lysine concentration, replacement of muscle K by lysine was usually increased by a decrease in bath K. Other CAA, including nonmetabolizable 2,4-diaminobutyric acid, also produced significant losses of muscle K. By contrast, neutral amino acids caused little or no loss of K, and anionic amino acids caused none. A high external lysine concentration did not slow the rapid accumulation of K when diaphragms previously leached of K were incubated in a bath containing K.

It is concluded that CAA compete with K for intracellular position as cations in muscle, but probably not by competing for transport at the cell surface. At the expected plasma levels in K-depletion *in vivo*, this competition is apparently not sufficient to produce the observed muscle K loss or Na accumulation. Factors other than CAA and lowered external K must play a role.

A Study of Tuberculin Reactions in an Indian Hospital Population. RICHARD A. LEVINSON and MARTIN M. CUMMINGS,* Oklahoma City, Okla.

In order to determine the prevalence of infections due to human, avian and atypical mycobacteria, tuberculin skin tests were performed on 179 patients and employees of the U.S.P.H.S. Indian Hospital (Shawnee, Okla.).

This Indian group was chosen for study because of their well defined demographic characteristics and their unusually high degree of tuberculin reactivity. In addition 19 Caucasian employees of the hospital were tested. The subjects were tested intradermally with 0.1 ml (0.0001 mg) PPD-S, PPD-Avian and PPD-Battey. All reactions were observed and measured at 48 hours. A reaction was considered "positive" when more than 5 mm of induration was observed. Seventy-four per cent of the Indians reacted to PPD-S, 34 per cent to PPD-B and 46 per cent to PPD-Avian. The relationships between the number and size of reactions to the different tuberculins were analyzed using the Chi-square test. Thirty per cent reacted both to PPD-S and PPD-B, 43 per cent to PPD-S and PPD-Avian and 26 per cent to PPD-B and PPD-Avian. The degree of cross-reactivity between avian, Battey and human tuberculins was significantly high. None of the Indians reacted to PPD-Avian alone and only 2 reacted to PPD-B alone. However, 4 of 19 white employees reacted to avian tuberculin alone and 2 others reacted to avian and Battey tuberculin. These findings suggest that infections with avian tubercle bacilli as well as atypical mycobacteria may be responsible for the unusual tuberculin hypersensitivity patterns being observed in certain geographic areas.

The Effect of Pulmonary Air Cysts on Respiratory Mechanics and Pulmonary Diffusing Capacity. BENJAMIN M. LEWIS, L. C. REED, JR., AKIO FURUSHO and THOMAS H. SNIDER, Detroit, Mich. (introduced by Gordon B. Myers).

Resistance to airflow, compliance, diffusing capacity and residual volume was measured in 9 patients with large pulmonary air cysts. All 9 had decreased diffusing capacity. Four patients had normal respiratory mechanics, and abnormalities in the other 5 ranged from a marked elevation of inspiratory resistance to moderate increases of expiratory resistance without relation to the helium dilution residual volume. Specifically, the residual volume was low in 4 patients, indicating poor bronchial communication of their cysts. One patient had a marked elevation of inspiratory resistance, probably due to expansion of the cysts as intrathoracic pressure fell, together with moderate elevation of expiratory resistance and low compliance. Two, one with histologically proved sarcoid, had moderate elevations of expiratory and inspiratory resistances and low compliance. The final patient had normal respiratory mechanics. Three patients had normal residual volumes, indicating that the cysts communicated with the bronchi. Two of these had normal values for compliance and resistance, while the third had moderate increases of expiratory and inspiratory resistance and a lowered compliance. The last 2 patients had an elevated residual volume, supposedly indicating a communicating cyst plus emphysema of the surrounding lung, yet resistance and compliance were normal in one and inspiratory and expiratory resistance moderately increased in the other. Thus, the mechanical effects of large air cysts can not be related to the patency of bronchial communication. Other factors, the etiology, extent and location of the cysts, are probably more important. Pulmonary air cysts uniformly displace or destroy normal pulmonary tissue as shown by the lowered diffusing capacity, but this decrease in diffusing capacity, also, could not be related to the patency of bronchial communication.

The Relation of Erythropoiesis to Iron Absorption. ALLYN B. LEY, New York, N. Y. (introduced by David A. Karnofsky).

The endogenous factors which have principally been related to the control of absorption of iron from the gut have been *1*) the amount of the body stores of iron, *2*) the degree of anemia, and *3*) the rate of erythropoiesis.

We have observed the development of hemochromatosis in 3 patients with refractory anemia but with erythroid hyperplasia of the marrow. In none could the anemia be ascribed to hemolysis. In none could the iron overload reasonably be attributed to transfusion. In the one patient so studied, plasma iron turnover was markedly increased. Hence, in this patient, and by inference in the other two, while "effective" erythropoiesis was markedly diminished, "ineffective" erythropoiesis was markedly increased.

Measurements of the gastrointestinal absorption of a 1 mg dose of radioiron showed values of 55 to 86 per cent while the patients were anemic. When the same test dose was used after the anemias had been corrected by transfusion, the per cent absorption fell to 11 to 25 per cent.

Similar studies were done in 3 patients with thymomas who were equally anemic, but whose marrows showed practically no erythroid cells and whose plasma iron turnover rates were very low. In this group, the absorption, while the patients were anemic, varied from 25 to 33 per cent. After transfusion to normal or near normal levels of hematocrit, the per cent absorption fell to 10 to 21 per cent.

These results are interpreted to indicate that iron absorption is predominantly controlled by the rate of erythropoiesis, whether erythropoiesis is effective or ineffective. It appears likely, however, that anemia itself, regardless of erythropoiesis, also promotes the absorption of iron.

The Cytological Localization of Human Growth Hormone with Fluorescent Antibody. A. LEZNOFF, J. FISHMAN, L. GOODFRIEND, E. E. MCGARRY, B. ROSE and J. C. BECK,* Montreal, Canada.

Previous attempts to demonstrate the cytological localization of anterior pituitary hormones with fluorescent antibody have been inconclusive, probably because of the lack of purity of the hormones used as antigens. Using gel diffusion and hemagglutination techniques, our laboratory and others have shown that antisera to Raben's more highly purified preparation of human growth hormone are species specific and hormone specific.

Antisera to human growth hormone were conjugated to

fluorescein isothiocyanate and applied to sections of normal human pituitaries, eosinophilic adenomata from patients with acromegaly, chromophobe adenomata, and an unclassified pituitary adenoma from a patient with severe Cushing's disease. The fluorescent antisera could be seen to localize almost exclusively in pituitary eosinophilic cells and in eosinophilic adenomata. There was no staining of other organs or adenomata. The fluorescent staining could be inhibited with nonfluorescent antihuman growth hormone and absorbed out by prior incubation with human growth hormone. Unstained sections and sections stained with heterologous fluorescent conjugates showed no fluorescent localization. Fluorescent antihuman gamma globulin localized in blood vessels and fibrous septa of the pituitary.

These studies contribute additional evidence for the specificity of our antisera to human growth hormone, and constitute direct evidence that the pituitary eosinophil is the cellular site of production or storage of growth hormone in the human. Using similar techniques further studies on the cellular site of corticotropin are in progress.

Stimulation of Hepatic Fatty Acid Synthesis by Ethanol in Vivo and in Vitro. CHARLES S. LIEBER, LEONORE M. DECARLI, and RUDI SCHMID,* Boston, Mass.

To investigate the mechanism by which ethanol produces a fatty liver, 14 male rats were given a tracer dose of acetate-1-C^{14} intraperitoneally, and 8 g per kg ethanol or isocaloric amounts of glucose by gastric tube. They were sacrificed 16 hours later. In all animals, specific activity of fatty acids in the liver was much greater than in adipose tissue. Compared to litter mates fed glucose, in ethanol-treated rats, the concentration of total fatty acids in the liver was increased; C^{14} incorporation into fatty acids was enhanced by 40 per cent in the liver and decreased by 50 per cent in adipose tissue, suggesting that ethanol stimulates hepatic fatty acid synthesis. This was confirmed in 14 other rats given labeled acetate 5 days *prior* to a 4 day course of ethanol or glucose administration. In contrast to the previous experiment, the specific activity of fatty acids in liver was less than in adipose tissue. In the ethanol-treated animals, hepatic fatty acid concentration was increased and specific activity reduced, as compared to animals pair-fed with glucose.

In comparison to glucose or acetate, incubation of rat liver slices with ethanol demonstrated both a three- to eight fold increase in incorporation of acetate-1-C^{14} into fatty acids ($p < 0.001$) and increased *net* synthesis of fatty acids ($p < 0.02$). In adipose tissue, which lacks alcohol dehydrogenase, ethanol had no effect on fatty acid synthesis.

In the liver, oxidation of ethanol is coupled with reduction of disphosphopyridine nucleotide (DPN); *in vitro,* another DPNH generating system (sorbitol-fructose) reproduces the effect of ethanol on fatty acid synthesis. Furthermore, a hydrogen acceptor such as methylene blue abolished this effect. This indicates that the stimulation of hepatic fatty acid synthesis by ethanol results from increased formation of DPNH produced on ethanol oxidation.

Stability of Protein in Intestinal Epithelial Cells. MARTIN LIPKIN, THOMAS P. ALMY* and HENRY QUASTLER, New York, N. Y. and Upton, N. Y.

Previous studies by others and ourselves have demonstrated rapid incorporation of amino acids into intestinal mucosal proteins, and rapid removal or "turnover" of protein in the entire intestinal mucosa. The stability of synthesized protein within individual cells, however, has not been studied. For this purpose, rates of leucine incorporation into various cell types and intracellular locations in jejunal mucosa of mice were measured by quantitative microautoradiography, following injection of leucine-H^3.

Leucine incorporation is most rapid in epithelial cell cytoplasm adjacent to growing microvilli. Incorporation is successively decreased in epithelial cell cytoplasm adjacent to nuclei, in nuclei, and in connective tissue. Incorporation is rapid in crypt epithelial cells which are undergoing division and differentiation, and is successively decreased in cells at the base, center and tip of the villi. As epithelial cells migrate to tip of villus, label is retained at original sites within individual cells. In cortisone-treated animals, identical results are seen. At comparable intervals after leucine injection, label is found to be rapidly removed from Paneth's cells and pancreatic acinar cells.

The results indicate a high degree of stability of synthesized protein in intestinal epithelial cells, rather than rapid intracellular protein turnover. The stability of synthesized protein in these mammalian cells *in vivo* is similar to that found in growing cultures of *Escherichia coli,* and recently in mammalian cells in tissue culture.

Bleeding Diathesis in Children with Liver Glycogen Disease and in Their Parents. CHARLES U. LOWE,* JULIAN L. AMBRUS, CLARA M. AMBRUS, LUIS L. MOSOVICH, IRVING B. MINK and JOSEPH E. SOKAL, Buffalo, N. Y.

Detailed examination of hemostatic factors revealed abnormalities in each of 6 children with liver glycogen disease (LGD) and also in at least one parent of each. Hemorrhagic episodes were encountered in 4 of the patients. Both the hepatic enzyme abnormalities and the hemostatic defects varied. Children with different hepatic defects had different clotting defects. Hemostatic abnormalities could not always be demonstrated, but when present, the pattern was consistent for a given patient. Two of the subjects were siblings; their abnormalities were similar: prolonged clotting and bleeding time, defects in PTA, PTC, and possibly in Stuart-Prower Factor, and increased Factor V. In one family in which both parents had abnormalities, they resembled those of the child—definitely prolonged recalcified plasma clotting time, slight decrease in prothrombin, and increase in Factor V. All of the children and some of the parents

had thrombocytosis on one or more occasions, reaching values as high as 1,200,000 per mm^3. No defects were found in members of the fibrinolysin and retractozyme systems, in platelet factors, or in capillary morphology. Fresh serum was more effective than whole blood in controlling bleeding. In one instance, blood from a patient's mother failed to control hemorrhage; however, blood from a normal donor proved effective. The mother was found to have a clotting deficiency.

This occurrence of hemostatic defects seems too regular to be coincidental and suggests that such abnormalities may constitute a basic feature of LGD. Studies of carbohydrate metabolism have proved unsuccessful in identifying the possible heterozygous state of this familial disease; it is intriguing that a study of clotting factors has revealed abnormalities among parents without history of bleeding. It would appear that the manifestations of LGD, as well as the genetic mechanisms involved, are more complex than has been generally appreciated.

Inhibition of Serotonin Production by Isonicotinic Acid Hydrazide with Control of Symptoms in the Malignant Carcinoid Syndrome. GEORGE D. LUDWIG, Philadelphia, Pa. (introduced by Francis C. Wood).

The author has previously shown that isonicotinic acid hydrazide (INH) inhibits growth of higher plants by blocking conversion of tryptophan to indole-3-acetic acid, the main plant growth hormone. It was shown that INH forms a Schiff-base complex with pyridoxal phosphate, thus depriving a decarboxylase or transaminase of required cofactor. Anticipating that INH might inhibit 5-hydroxytryptophan decarboxylase, and thus 5-hydroxytryptamine (serotonin) production, 2 patients with proven metastatic carcinoid were given 400 mg of the drug daily. Serotonin concentrations in whole blood and platelet fractions were measured spectrofluorometrically before and during INH administration. Daily urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) was measured with the nitroso-naphthol and Ehrlich reagents, and individual urinary indoles by two-dimensional paper chromatography, for 5 control days and 10 days during INH administration. In both patients general clinical improvement and decrease of diarrhea occurred. In one, roentgenograms showed reversal to normal of a previously disordered motor pattern of the small bowel. Coincident with the symptomatic improvement, concentrations of serotonin in the whole blood and the platelet fraction were decreased 30 and 50 per cent, respectively; average daily excretion of 5-HIAA, as measured with Ehrlich reagent, was decreased 30 to 40 per cent, and paper chromatograms showed a marked decrease in 5-HIAA, 5-HIAA-sulfate, and serotonin.

In contrast, iproniazid (Marsilid), which differs from INH in being a potent monamine oxidase inhibitor, failed to inhibit plant growth or conversion of tryptophan to indole-3-acetic acid, and other authors have shown that iproniazid aggravates symptoms of metastatic carcinoid, presumably by increasing the circulating serotonin concentration as a result of inhibiting its oxidation to 5-HIAA by monamine oxidase.

Formiminoglutamic Acid versus Serum "Folic Acid" as an Index of Folic Acid Deficiency. A. LEONARD LUHBY and JACK M. COOPERMAN, New York, N. Y. (introduced by Alvin F. Coburn).

We have shown that measurement of urinary formiminoglutamic acid (FIGlu) after a standard histidine metabolic load is an early and sensitive index of folic acid deficiency. This has been confirmed by Spray and Witts. Recently, Baker and Herbert and others have proposed a test for folic acid deficiency based on determination of serum "folic acid" activity by *Lactobacillus casei.*

Urinary excretion of FIGlu after a 15 g L-histidine monohydrochloride metabolic load for two to three days was compared with serum "folic acid" by *L. casei* assay in patients with megaloblastic anemias of various origins. A new, modified *L. casei* assay for serum "folic acid" activity was employed which increases the accuracy and sensitivity of previous procedures.

Urinary FIGlu of over 30 μg per ml or over 35 mg per 24 hours by the end of the loading period was found indicative of folic acid deficiency as established by invariable, dramatic response to minute doses of folic acid but not to vitamin B_{12}.

Serum "folic acid" was found in normal subjects to vary between 2 and 30 mμg per ml. Initial serum "folic acid" in patients found folic acid-deficient by urinary FIGlu after standard histidine loading and by therapeutic trial with very small doses of folic acid ranged from 2 to 15 mμg per ml.

Thus, serum *L. casei* "folic acid" activity, known not to be specific for folic acid, was not a good measure of folic acid deficiency since values found in folic acid-deficient patients fell within range of normals. Urinary FIGlu determination after histidine loading, however, based upon a specific biochemical defect in folic acid deficiency, was invariably found an early and accurate index of folic acid deficiency.

In Vitro Stimulation of Hexokinase by Insulin. ROBERT M. MACLEOD, ROSE BROWN and WILLIAM S. LYNN,* Durham, N. C.

Previous studies performed in this laboratory indicate that incubation of rat epididymal adipose tissue with insulin stimulates the conversion of glucose-1-C^{14} and -6-C^{14} to glycogen, CO_2, glyceride-fatty acid, lactate and the oxidation of glucose via the hexosemonophosphate shunt, when incubated in Krebs phosphate buffer with air as the gas phase. Since insulin stimulates glycolysis, glycogenesis and the hexosemonophosphate shunt, it was concluded that the locus of action of insulin must be either to increase tissue permeability to glucose, or to stimulate hexokinase. Measurements of intracellular glucose and glucose-6-phosphate concentrations have indicated that insulin caused a marked decrease in intracellular glucose, with a concomitant increase in glucose-6-phosphate concentration. However, total water, urea, raffinose, inulin, and sodium spaces remained unchanged in the hormone-treated tissue as compared to controls.

The intracellular glucose concentration in control is 45 per cent lower than extracellular glucose; however, this limitation of transport can be overcome by addition of digitonin, i.e., intracellular glucose equals extracellular glucose. Under these conditions the addition of insulin is still capable of lowering intracellular glucose and increasing glucose-6-phosphate concentrations, as measured by enzymatic and radioisotopic methods. Therefore, under these conditions, the action of insulin must be to stimulate the phosphorylation of glucose rather than the transport of glucose into adipose tissue.

The Capacity of Tubercle Bacilli to Assume a Latent State In Vivo. ROBERT M. MCCUNE, New York, N. Y. (introduced by Walsh McDermott).

An increasing proportion of the disease load in hospitals today is being produced by microbial infections that have been resurrected from a dormant or latent state. The term *latent* is used in the strict sense to describe an infection whose presence in the tissues cannot be demonstrated by any known technique, yet the fact that infection *is* present is established by its subsequent reappearance. The capability of viruses to assume the latent state has long been recognized, but the extent to which bacteria possess this same capability has only recently been extensively studied. Thus far, the experimental induction of a latent bacterial infection has been accomplished only in the case of tubercle bacilli as previously reported from this laboratory. In the experimental model, large populations of tubercle bacilli completely vanish from the tissues of mice treated with a nicotinamide derivative, pyrazinamide, and a companion drug according to certain established time-dose relationships.

In the present studies it has been found that: *1*) latency has been induced in at least four-fifths of the animals from which the tubercle bacilli have vanished; *2*) time is an important factor—at least 9 weeks are necessary for induction of latency and 6 weeks to many months for the resurrection of the bacilli; *3*) once the latent state is assumed it is remarkably stable and resurrection during the first month after induction cannot be produced even by large doses of hydrocortisone; *4*) the nature of the tissue environment is an essential factor in the assumption of the latent state; *5*) the concurrent adverse influence on the tubercle bacilli, that is a necessary prerequisite for experimentally induced latency, apparently must be specific. Three drugs with widely varied antituberculous effectiveness can exert such an influence, but penicillin cannot. Experiments to determine the role of vaccine-induced immunity as an "adverse influence" are virtually completed.

Insulin's Control of the Role of the Liver in the Disposition of a Glucose Load in Diabetic and Nondiabetic Dogs. LEONARD L. MADISON, BURTON COMBES and REUBEN ADAMS, Dallas, Tex. (introduced by Elias Strauss).

The physiological role of the liver in disposing of a carbohydrate load was examined by measuring the effects of intravenously administered glucose on hepatic glucose balance before and after insulin administration in normal, mildly diabetic and severely diabetic dogs. Hepatic glucose balance was determined in 24 studies from measurements of hepatic blood flow (Bradley's technic) and arteriohepatic venous glucose concentration difference (Somogyi iodometric method) in dogs with portacaval shunts, a preparation permitting measurement of *hepatic* rather than *splanchnic* glucose balance.

In normal dogs after 60 minutes of intravenous glucose, the liver, which initially was contributing 46 mg per minute was now storing (extracting) 24 mg per minute, a net positive balance of 70 mg per minute. Hepatic glucose storage started at a mean arterial glucose concentration of 116 mg per 100 ml. Insulin pretreatment prior to glucose loading lowered this storage threshold to 92 mg per 100 ml. Although glucose infusions in mildly diabetic dogs (mean fasting blood sugar 145 mg per 100 ml) also resulted in hepatic glucose storage, this occurred only when mean arterial glucose concentration reached 200 mg per 100 ml. In contrast, hepatic glucose storage never occurred in the more severely diabetic dogs (mean fasting blood sugar 240 mg per 100 ml) despite elevation of arterial glucose to levels as high as 490 mg per 100 ml. When these more severely diabetic dogs were pretreated with insulin prior to glucose infusion, hepatic glucose storage occurred and at a mean arterial glucose concentration of only 164 mg per 100 ml.

These data indicate that insulin controls the magnitude of the role of the liver in the disposition of a carbohydrate load. Not only is the arterial glucose level at which the liver starts storing glucose progressively elevated with increasing severity of diabetes but this level is promptly lowered toward normal by insulin administration.

The Cleavage of Disulfide Bonds in Thyroid Tissue by Thiourea. F. MALOOF and M. SOODAK, Waltham and Boston, Mass. (introduced by J. Lerman).

We have previously described a thyroidal cytoplasmic particulate system which desulfurates S^{35}-thiourea. Thiocyanate ion and reduced diphosphopyridine nucleotide are required. Liver and kidney tissue are inactive.

The major sulfur product is protein-bound sulfur (PB-S^{35}; 50 per cent). The S^{35} is firmly bound to protein, but about 80 per cent of it can be displaced by nucleophilic anions (0.01 M), as sulfite or cyanide, by 0.1 N NaOH or by thiols (0.01 M) as cysteine. This is evidence that the S^{35} is bound to protein in disulfide linkage (PB-S-S^{35}).

Potent inhibitors of this system are sulfite and thiols which effectively split disulfide (S-S) bonds: $RSSR' + SO_3^{--} \rightleftarrows RSH + R'SSO_3$ (idem thiols). Preincubation of the particulate fraction with sulfite (0.01 M), cysteine (0.01 M) or thioglycollic acid (0.01 M) leads to about 80 per cent inhibition of the subsequent desulfuration of thiourea. Studies with sulfhydryl (SH) group inhibitors reveal that a free SH group is not involved. Incubation with labeled sulfite ($Na_2S^{35}O_3$) yields protein-bound radioactivity (PB-S-$S^{35}O_3$). The $S^{35}O_3$ is firmly bound

to protein but can be displaced by precisely the same reagents that remove the S^{35} from the PB-S-S^{35}. Hence sulfite and the sulfur of thiourea seem to be attached to thyroid protein in a similar manner.

These data suggest that a disulfide bond in thyroid tissue is cleaved by thiourea to form a new-S-S-bond. This formulation is supported by chemical studies in which thiourea was found to cleave the disulfide bond of cystine. Of interest is that this thyroid particulate preparation has 3 and 7 times the cystine content of similar preparations of kidney and liver. Parallel observations on the *in vitro* iodination reaction suggest a sulfenyl iodide (SI^+) intermediate resulting from cleavage of a disulfide bond. Competition for this site may explain the inhibitory action of thiourea in the thyroid.

Thrombolytic Activity of Mold Fibrinolysin (Aspergillin O) in Vivo. HECTOR M. MARIN, MARIO STEFANINI,* FRANCO SOARDI and LISELOTTE MUELLER, Boston, Mass.

Aspergillin O, an agent with strong fibrinolytic activity isolated from filtrates of cultures of *Aspergillus oryzae* B-1273, was injected into 70 dogs at average dose of 10 mg per kg body weight. A venous thrombus was produced in 15 dogs with sodium morrhuate technic preliminary to the injection. Animals showed no changes in temperature or pressure. There was oozing only at site of surgical incisions for about one-half hour after injection of Aspergillin O. Coagulation studies indicated increased fibrinolytic activity without simultaneous consumption of profibrinolysin, depletion of clotting accelerators (labile factor V) and some degree of hypofibrinogenemia for 1 to 4 hours. Generation of thrombin was slow and incomplete; activity of prothrombin and stable factors was slightly affected; the generation of thromboplastin was minimally delayed. Direct inspection and venographic studies indicated recanalization of occluded veins within 3 days of production of thrombi. A strong inhibitor developed temporarily following administration of Aspergillin O. Addition of 2 mg of heparin sodium to Aspergillin O inactivated the inhibitor and enhanced the fibrinolytic and clot-delaying activity of the drug. This occurred occasionally, however, when doses of 5 mg per kg or less were administered; it was prevented by the simultaneous administration of small doses of heparin.

Studies have been conducted in 55 humans, examples of which will be given in detail. They indicated that Aspergillin O is not toxic or antigenic and exhibits lytic effect on venous and arterial thrombi, while favoring establishment of collateral circulation following vascular occlusion. Coagulation studies gave results similar to those obtained in animal experiments.

Endocrine Influences on Serum Free Fatty Acid (FFA) in Man. BERNARD H. MARKS and A. GORMAN HILLS,* Miami, Fla.

Significant deviations from normal serum FFA concentration measured after a 14 hour fast, or of the response of serum FFA to glucose ingestion or to epinephrine administration, have been documented in 3 types of endocrinopathy. *1)* Of 8 hyperthyroid patients, mean fasting serum FFA was 1,183 μEq per L; of 6 controls, 731 μEq per L; $\Delta = 452$, $p < 0.01$. After ingestion of 75 g of glucose the decrease in FFA concentration was greater but briefer than normal, and the rise 2 to 4 hours later was excessive ($p < 0.05$). *2)* In uncontrolled mild diabetes mellitus (9 patients) mean fasting serum FFA was elevated (1,179 μEq per L, $p < 0.05$) and FFA response to glucose ingestion was impaired; on chlorpropamide therapy mean fasting FFA was lowered (to 812 μEq per L, $\Delta = -367$, $p < 0.01$); the mean FFA values after glucose ingestion were also lower in the treated patients, although the decrease from fasting values was less. *3)* In primary and secondary hypoadrenalism the rise of serum FFA which normally is present one-half hour after administration of epinephrine (0.3 mg s.c.) was impaired ($p < 0.05$; mean $\Delta = 173$ μEq per L for 8 patients versus 410 μEq per L for 6 controls).

The data are all compatible with the thesis that serum FFA reflects directly the rate of utilization and mobilization of fat, and indirectly and inversely the rate of glucose utilization. They support also the following interpretations. *1)* In hyperthyroidism the normal metabolic responses to fasting and food-taking occur in accelerated and exaggerated form; the demonstrable abnormalities are presumably largely a consequence of the raised metabolic rate produced by thyroid hormone. *2)* The blood-sugar-lowering effect of chlorpropamide in diabetics reflects amelioration of their impaired rate of utilization of glucose.

Steroid Hormone Inhibition of Mammalian Glucose-6-Phosphate Dehydrogenase. PAUL A. MARKS* and JULIA BANKS, New York, N. Y.

This study demonstrates a highly specific steroid hormone inhibition of glucose-6-phosphate dehydrogenase (G-6-PD), an enzyme catalyzing reduced triphosphopyridine nucleotide (TPNH) formation. Dehydroisoandrosterone (DHA) and pregnenolone (PE) and certain other C_{19} and C_{21} steroids with a ketone group at C-17 or C-20 inhibit G-6-PD. At 10^{-7} M, DHA and PE inhibit G-6-PD activity 20 to 30 per cent, and at 10^{-5} M, 65 to 85 per cent. These steroids inhibit purified G-6-PD from human erythrocytes and G-6-PD in crude preparations of various human and rat tissues including liver and adrenal, but not G-6-PD of yeast or spinach. Isocitric and 6-phosphogluconic dehydrogenases, which also catalyze TPNH formation, are not inhibited by these steroids. Estrogens, testosterone, corticosteroids, progesterone and cholesterol have little, and generally no inhibitory effect on G-6-PD at concentrations as high as 10^{-5} M. The steroids are noncompetitive inhibitors of G-6-PD with regard either to glucose-6-phosphate or TPN. This G-6-PD inhibition does not involve a TPN-linked reduction or oxidation of the steroids.

Steroid inhibition of G-6-PD might be a regulatory mechanism of significance in glucose metabolism. TPNH is required for steroid biosynthesis. DHA and PE are

important intermediates in steroid synthesis. Inhibition of TPNH generation by DHA or PE may function as a control mechanism in the formation of these key intermediates.

In Vitro Transfer of I^{131} Anti-$Rh_o(D)$ from "Sensitized" Red Cells. S. P. MASOUREDIS, San Francisco, Calif. (introduced by R. J. Havel).

The *in vitro* "transfer" of I^{131} anti-$Rh_o(D)$ from "sensitized" red cells to "unsensitized" $Rh_o(D)$ positive red cells has been demonstrated and the conditions which affect this process have been studied.

$Rh_o(D)$ positive red cells "sensitized" with I^{131} anti-$Rh_o(D)$ were incubated with "unsensitized" $Rh_o(D)$ positive red cells of a different ABO phenotype. The recipient cell was isolated from the "sensitized" donor cell after reaction by differential agglutination of the donor cell with the appropriate ABO isoantibody. The effectiveness of this procedure in separating the two cell populations was evaluated for each experiment by using an $Rh_o(D)$ negative cell of the same ABO phenotype as the recipient cell. Under these conditions from 30 to 75 per cent of the "sensitized" donor cells were carried over into the recipient cell suspension as a result of incomplete agglutination of the donor cells.

Both A and B cells participate equally with A, B or O recipient cells in the transfer of I^{131} anti-$Rh_o(D)$. The maximum quantity of antibody was transferred between 37° and 45° C. About 15 per cent of the antibody content of the "sensitized" donor cells was found in the recipient cells after one hour's incubation at 37° C. No "transfer" of antibody occurred at 4° or at 56° C. About 9 per cent of the donor cell antibody content was transferred within 30 minutes and a maximum of about 15 per cent was found between 120 and 180 minutes. No increase in quantity of antibody transferred could be demonstrated by prolonging the time of incubation. Recipient cells containing the rh'(C) antigen accepted less I^{131} anti-$Rh_o(D)$ than did rh'(C) negative cells. The antibody transferred in these experiments was sufficient to saturate from 5 to 20 per cent of the $Rh_o(D)$ sites on the recipient cell.

The Influence of Peripheral and Coronary Hemodynamics Upon Patterns of Human Myocardial Oxygen Supply. J. V. MESSER, R. J. WAGMAN, H. J. LEVINE, W. A. NEILL and R. GORLIN,* Boston, Mass.

Patterns of human myocardial oxygen supply may vary dependent upon the influence of coronary artery disease, extracardiac moderator reflexes, and the application of acute stress. Thirty-two patients were studied by coronary sinus (CS) and pulmonary artery catheterization with measurements of coronary flow (CBF), A-V O_2 differences, and systemic hemodynamics at rest and during exercise. Myocardial oxygen consumption (M QO_2), A-VO_2/AO_2, coronary diastolic vascular resistance (CVR), myocardial efficiency (ME), and cardiac index (CI) were calculated. Fifteen patients had near normal circulations, 13 had left ventricular failure (CHF), and 4 had coronary sclerosis (CAD).

Normal oxygen supply pattern was: resting CBF = 80 cc per 100 g LV per minute increasing 25 per cent during exercise, CVR = 69,400 dynes-sec-cm^{-5}, decreasing 26 per cent during exercise, coronary venous oxygen content (CV O_2) = 4.6 vol per cent increasing 3 per cent during exercise, A-VO_2/AO_2 = 71 per cent remaining constant or decreasing during exercise, and ME = 44 per cent increasing 32 per cent during effort.

In resting CHF patients with a CI above 3.0, CBF = 111, CVR = 46,000, CVO_2 = 5.8, A-VO_2/AO_2 = 69 per cent, and ME = 25 per cent; with a CI below 3.0, CBF = 77, CVR = 61,500, CVO_2 = 3.6, A-VO_2/AO_2 = 79 per cent, and ME = 22 per cent. In all pure CHF patients, A-VO_2/AO_2 and CVO_2, whether normal or abnormal at rest, remained constant during exercise, indicating increased CBF adequate to M QO_2; CBF increased 44 per cent and CVR decreased 32 per cent with exercise.

Coronary artery disease patients had normal resting oxygen supply patterns. During exercise, however, CBF increased only 16 per cent, CVR fell only 13 per cent, CVO_2 fell 19 per cent, and A-VO_2/AO_2 increased 8 per cent, while ME increased normally.

In normals, increased M QO_2 is supplied through increased CBF secondary to coronary vasodilatation, without A-VO_2/AO_2 alterations. In low CI, CHF patients, increased resting A-VO_2/AO_2 together with a normal ability for coronary vasodilatation on effort, suggests a coronary vasoconstrictor reflex acting in the resting state. The persistence of, but not increase in, a widened A-VO_2/AO_2 despite greatly increased CBF on effort suggests incomplete abolition of this constrictor tone by the competing influence of increased M QO_2. In contrast, the CAD group normal resting oxygen supply pattern is replaced on effort by abnormal oxygen extraction.

Erythrocyte Glutathione Synthesis. AARON MILLER and MARTHA HORIUCHI, Boston, Mass. (introduced by Charles P. Emerson).

Glutathione in erythrocytes has an important role in their function and survival. Synthesis of erythrocyte glutathione (glutamylcysteinylglycine) was studied *in vitro* with labeled precursors. A method for isolation of radiochemically pure glutathione was developed. Protein-free filtrates of erythrocytes which had been incubated up to 5 hours in media containing S^{35}-cystine, C^{14}-glycine, C^{14}-glutamic acid or C^{14}-glutamine were chromatographed after elution from an anion-exchange resin (IR45) and specific activity of the separated glutathione determined.

Erythrocytes incorporated labeled cystine and glycine into glutathione progressively without change in the glutathione concentration. Erythrocyte glutathione labeled with C^{14}-glycine did not exchange with added unlabeled glycine, indicating that labeled glycine was incorporated into glutathione by true synthesis. No incorporation of labeled glutamic acid into glutathione was found. Intracellular ratios of 1 molecule of labeled glutamic acid to

115 molecules of glycine were observed using paired samples of blood. Similarly, 1 molecule of labeled glutamic acid to 7 molecules of glutamine (the monoamide of glutamic acid) was found within the cells; however, incorporation of labeled glutamine into the glutamic acid moiety of glutathione was not observed.

In summary, labeled cystine and labeled glycine were used to demonstrate true synthesis of glutathione in red cells. Exchange of the terminal glycine with unlabeled glycine was not noted. Labeled glutamic acid could not be incorporated into glutathione because of erythrocyte impermeability. Labeled glutamine entered the red cells more readily than glutamic acid. However, incorporation of labeled glutamine into the glutamic acid moiety of glutathione could not be demonstrated because of either limited cell entry relative to that of labeled glycine, or dilution by the intracellular pool of unlabeled glutamine, or limited conversion to glutamic acid, or any combination of these factors.

The Role of the Edematous State to Respiratory Function in Patients with Primary Cor Pulmonale. WILLIAM F. MILLER, IVAN E. CUSHING and NANCY WU, Dallas, Tex. (introduced by E. E. Muirhead).

Digitalis and diuretics often are vigorously employed in treatment of primary cor pulmonale on the assumption that the edematous state plays some specific role in respiratory symptoms and altered pulmonary function. Our own observations led us to believe that changes in pulmonary function are related to alterations within the lung per se and bear no direct relationship to the edematous state. The formation of edema is merely a manifestation of disturbed venous hemodynamic function and is secondary to pulmonary parenchymal disease.

In order to examine this contention, patients were carefully selected as examples of chronic cor pulmonale, persistently edematous but without evidence of acute infection or other acute disturbance which would account for changes in respiratory function. Fifteen patients were selected for study. Except for reduced physical activity and improved dietary intake (regular diet), hospital treatment was presumably unchanged from outpatient treatment. Lung volume, compliance, resistance and blood gas measurements were made every other day for a 10 day period then, biweekly thereafter. Nine patients exhibited acute changes in respiratory function, demonstrated to be due, either to clearing of retained bronchial secretions or to clearing of acute infection. The remaining 6 patients suitable for study failed to exhibit significant change in ventilatory function as a result of spontaneous diuresis, presumably due to decreased physical work, or induced mercurial diuresis (average weight loss 6.8 kg). There was no significant change in gas exchange functions except in those patients in whom a reduction of physical activity resulted in decreased CO_2 production. In two patients the edematous state was reinduced by large salt and water loads and in two patients by increased physical exertion. In no instance was functional change apparent.

Thus, the edematous state per se is unrelated causally to ventilatory and gas exchange disturbances of primary cor pulmonale.

Abnormal Insulin-Binding Fraction Demonstrated by The Electrophoresis on Ion Exchange Paper of Sera From Diabetic Patients. MARVIN L. MITCHELL, Boston, Mass. (introduced by J. M. Hayman).

Quantitative differences in I^{131}-insulin-binding by sera from insulin-resistant and insulin-responsive diabetic patients have previously been demonstrated by interaction analysis using anion exchange resins. To determine whether or not the insulin was bound by different protein fractions, an ion exchange paper was introduced as the supporting medium for the electrophoresis of serum containing I^{131}-insulin.

Sera from 12 normal subjects, 35 insulin-responsive and 6 insulin-resistant diabetic patients (300 to 5,000 units daily) were equilibrated for approximately 30 minutes with I^{131}-insulin. Following equilibration, electrophoresis (veronal buffer, pH 8.6) of the sera was carried out on paper which had been prepared from a mixture of α-cellulose pulp and a finely ground cation exchange resin, Amberlite IR-120. The protein patterns were similar to the patterns observed after electrophoresis of serum on filter paper.

I^{131}-insulin applied directly to the resin paper remained at the origin; when the insulin binding sites of the resin paper had been saturated with stable insulin, the radioinsulin migrated with a mobility slightly less than that of albumin. I^{131}-insulin in both normal sera and in sera from insulin-responsive diabetic patients migrated with the α- or β-globulins. In contrast, I^{131}-insulin migrated solely with the γ-globulins in sera from the insulin-resistant diabetic patients.

Serum from the same diabetic patient was studied during periods of insulin resistance and insulin sensitivity. The radioinsulin moved with the gamma globulin fraction during the insulin-resistant phase and with the α-globulins during the insulin-responsive phase. Thus, normal distribution of the I^{131}-insulin serum protein complex is associated with insulin responsiveness as compared with the abnormal pattern seen with true insulin resistance.

The Myocardial Exchange of Potassium and Sodium During Respiratory Acidosis. JOHN C. MITHOEFER and FRED D. HOLFORD, Cooperstown, N. Y. (introduced by James Bordley, III).

Concentrations of K^+ and Na^+ were measured in blood samples from the aorta and coronary sinus of anesthetized dogs during oxygen breathing and respiratory acidosis. Acidosis was produced after a control period of oxygen breathing by inspired mixtures of 8 or 19 per cent CO_2 in oxygen or by the technique of diffusion respiration. K^+ and Na^+ concentrations were measured by flame photometer.

During control periods arterial serum K^+ concentration slightly exceeded that of the coronary sinus blood (mean

0.1 mEq per L). During diffusion respiration as the $P_{A_{CO_2}}$ rose a progressive arterial-coronary venous K^+ difference (arterial higher than venous) developed, reaching a mean value of 0.7 mEq per L when $P_{A_{CO_2}}$ was 100 mm Hg then decreasing to 0.3 mEq per L when $P_{A_{CO_2}}$ was 140 mm. After two minutes' recovery when $P_{A_{CO_2}}$ was 60 mm the relationship was reversed, venous concentration slightly exceeding arterial. Similar results were produced by respiratory acidosis from CO_2 breathing.

During acidosis the venous Na^+ concentrations were higher than the arterial when the reverse relationship existed for K^+. However, concomitant reciprocal changes in the arteriovenous (A-V) difference of these ions could not be demonstrated but may have been masked by the small percentage changes which occurred in Na^+ concentration as compared to K^+.

Respiratory acidosis was associated with progressive rise in arterial K^+ concentration but this was shown not to be the cause of the A-V K^+ difference which developed, nor was the latter the result of shift of K^+ from plasma to RBC. The A-V K^+ difference is interpreted as evidence of a transfer of this ion from blood to myocardium in response to acidosis, a shift opposite to the known loss of K^+ from cells of skeletal muscle.

Destruction of Pathogenic Staphylococci by Human Serum. LEWIS A. MOLOGNE and ABRAHAM I. BRAUDE,* Pittsburgh, Pa.

Pathogenic staphylococci, unlike many pathogenic gram-negative bacteria, are reputedly resistant to the bactericidal power of normal human serum. In order to account for the susceptibility of diabetics to staphylococcal infections, their sera were compared with nondiabetic sera for killing of staphylococci.

Bactericidal activity was measured by inoculating approximately 20,000 to 30,000 coagulase-positive staphylococci per ml of serum and performing serial plate counts for 24 hours. Sera from 18 nondiabetics and 22 diabetics were examined for their ability to kill various coagulase-positive staphylococci isolated from 55 infected patients and nasal carriers. Plasma from all subjects, whose sera were examined for bactericidal power, possessed coagulase factor as measured both by clumping and clotting.

Diabetic and nondiabetic sera killed coagulase-positive staphylococci with equal frequency. Marked killing and sometimes sterilization of staphylococci was noted in 118 of 161 tests, while heavy growth occurred in only 43. Staphylococci isolated from infected materials were as susceptible to killing by serum as those recovered from nasal carriers. Bactericidal activity was resistant to heat and independent of the phage type and antibiotic sensitivity of the staphylococci. Different strains of staphylococci of the same phage type varied greatly in susceptibility to the bactericidal power of a given serum.

These results demonstrate that human sera kill pathogenic staphylococci as well as pathogenic gram-negative bacteria, and suggest that the alleged susceptibility of diabetics to staphylococcal infection cannot be related to deficient serum bactericidal power.

The Biliary Dynamics of the Metabolites of Sulfobromophthalein (BSP) in Man. L. S. MONROE and A. L. KITTINGER, La Jolla, Calif. (introduced by E. L. Keeney).

When sulfobromophthalein (BSP) is excreted into the bile following intravenous injection, the dye maintains the same absorption spectrum and indicator characteristics as the pure compound. For this reason it had been assumed that the dye was unchanged. However, in recent years there has been increasing evidence that BSP undergoes molecular changes in the process of excretion. Column chromatography utilizing cationotrophic alumina and descending paper chromatography with a *t*-butanol: formic acid: water system demonstrates 5 fractions of BSP which appear in the bile following the intravenous injection of the dye. Four of these fractions chromatograph differently from the pure dye.

During an attempt to correlate BSP metabolites with various liver diseases, marked variation in the biliary excretion rates of the metabolites has been found. A series of 18 postoperative patients recovering from choledochotomy were used to study this variation. After intravenous injection of BSP (5 mg per kg), timed bile specimens were subjected to column chromatography and the excretion rates of the metabolites were studied for a minimum of 2 hours. The most marked changes were noted in the percentage excretion rates of Fractions I and IV. Following an average level of 54 per cent at 20 minutes after injection, Fraction I (chromatographically similar to pure BSP) decreased progressively throughout the period of observation. Fraction IV having an average level of 10 per cent at the 20 minute period increased progressively at nearly the same rate. The percentage excretion rates of Fractions III and V increased gradually, while that of Fraction II gradually decreased following injection of the dye. Improvement in hepatic function was accompanied by an increase in the slope of the plotted curves of the percentage excretion rates of Fractions I and IV.

The slope of the curves of the excretion rates, plotted during the first hour period after injection, probably mirrors the functional status of the liver and may represent the maximum possible transfer of metabolites by a rate-limited transfer mechanism. Any attempt to correlate liver disease to BSP metabolites should take into consideration the time elapsed after injection. Preliminary studies indicate that the BSP metabolites are not all ninhydrin-reactive, and the nonreactive fractions may be excreted by a mechanism different from the currently proposed conjugation reactions.

An Evaluation of the Acidifying Capacity of the Chronically Diseased Kidney in the Experimental Animal. PETER A. F. MORRIN, NEAL S. BRICKER* and S. WESLEY KIME, JR., St. Louis, Mo.

Metabolic acidosis is a characteristic feature of chronic renal insufficiency. Whether the acidosis develops solely

because of a loss of functioning nephrons, or whether specific tubular defects in hydrogen ion secretion also exist in ordinary forms of chronic renal disease is unknown.

Hydrogen ion secretion by diseased kidneys has been investigated in acidotic (NH_4Cl-loaded) dogs. Permanent hemibladders permitted separate urine collections from both kidneys. Data include: 6 studies on 5 dogs with unilateral pyelonephritis or aminonucleoside-nephritis, and 4 studies on 1 dog with bilateral pyelonephritis. Urinary pH, tritratable acid (TA), ammonia and bicarbonate excretion were measured bilaterally during infusion of mannitol and phosphate. Urine and arterial blood samples were collected anaerobically.

The absolute excretion rates (UV) of ammonia and TA were less for diseased (experimental) than for contralateral control kidneys; and in the animal with bilateral disease, excretion rates were lower for the more severely diseased (i.e., experimental) organ. However glomerular filtration rates (GFR) for the experimental kidneys were decreased proportionately and when excretion rates were calculated per unit of GFR, no differences were observed between experimental and contralateral kidneys. UV_{NH_3}/GFR for experimental kidneys averaged 95 per cent of values for contralateral kidneys [in 9 experiments ratios averaged 0.99 (range, 0.84 to 1.14); and in 1 experiment, 0.55]. UV_{TA}/GFR for experimental kidneys averaged 99 per cent of contralateral values (range, 0.93 to 1.08). Essentially no bicarbonate was excreted and bicarbonate reabsorption/GFR therefore was always equal bilaterally. If total hydrogen ion excretion was expressed as $UV_{NH_3}^{+}$ UV_{TA}^{+} bicarbonate reabsorption, values per unit of GFR remained equal bilaterally.

These results suggest that residual nephrons of the diseased kidneys secreted hydrogen ion in an orderly and efficient manner. The data provide confirmatory evidence that the surviving nephrons of diseased kidneys in the dog retain a high degree of functional integrity.

The Reabsorption of Phosphate by the Kidney in Potassium-Deficient Dogs. ASHTON B. MORRISON, VARDAMAN M. BUCKALEW, JR., RAY MILLER and JOHN D. LEWIS, Philadelphia, Pa. (introduced by Archer P. Crosley, Jr.).

Low serum phosphate levels are often found in patients with potassium deficiency and have also been reported in potassium-deficient rats. To find whether or not the hypophosphatemia results from interference with phosphate reabsorption in the kidney by the lesions caused by potassium deficiency in the tubular cells, the renal tubular maximum for phosphate reabsorption ($TmPO_4$) was measured in 2 dogs before and during potassium deficiency.

Two trained female dogs were fed a control diet adequate in all nutrients. On 7 separate occasions, over a period of 3 months, 4 determinations of $TmPO_4$ were made in each animal by standard methods during phosphate loading. The animals were then fed a diet containing less than 8 mEq of potassium per kg for 4 months; they became potassium-deficient as measured by serum and muscle potassium levels, and the $TmPO_4$ was measured as before. During the last 12 days of the potassium-deficient diet, 25 mg of desoxycorticosterone acetate (DCA) in oil was injected daily into each animal and the $TmPO_4$ measured again.

The mean tubular maximum for phosphate reabsorption in each animal during each of the periods of the experiment, respectively, was as follows in mmoles per minute: in the control period 0.092 ($SE \pm 0.008$) and 0.084 ($SE \pm 0.005$); in the period of potassium deficiency 0.081 ($SE \pm 0.007$) and 0.093 ($SE \pm 0.006$); in the period of potassium deficiency and DCA administration 0.082 ($SE \pm 0.005$) and 0.085 ($SE \pm 0.006$). No significant difference was observed between the values for either animal under the different conditions.

It was concluded that the renal tubular lesions of potassium deficiency did not interfere significantly with phosphate reabsorption by the kidney.

Ethyl Ether Elimination by the Lungs, a New Measure of Effective Alveolar Ventilation. THEODORE H. NOEHREN and DOUGLAS S. RIGGS, Buffalo, N. Y. (introduced by John H. Talbott).

Theoretical considerations of the exchange of inert gases in the lungs have indicated that pulmonary ventilation, cardiac output, and the solubility of the individual gas in blood are major factors controlling the pulmonary elimination of the gas. The relative importance of cardiac output and pulmonary ventilation is determined by the solubility. The highly soluble gases, such as ethyl ether, should be eliminated at a rate directly proportional to the volume of alveolar ventilation, with very little influence by alterations in blood flow unless alveolar ventilation is considerably larger than blood flow.

The present study was designed to test the accuracy of this hypothesis. Ethyl ether solution was administered intravenously at a constant rate to dogs anesthetized with pentobarbital. Ventilation was altered by electrical stimulation of the phrenic nerves through implanted electrodes. Intravenous isoproterenol (Isuprel) was used to vary cardiac output. The procedure was safe and did not necessitate achieving anesthetic concentrations of ether in these animals.

The results indicate a linear relationship between ether clearance by the lungs and alveolar ventilation within the ranges studied. Alterations in cardiac output had very little influence on the amount of ether excreted. These results are in good accord with theory.

This technique offers a new way of measuring effective alveolar ventilation without significant disturbance by circulatory changes. The one-way pulmonary gas exchange achieved by administering the gas intravenously offers distinct advantages in the study of the cardiopulmonary system. It is applicable in a variety of physiological and pathophysiological problems in experimental animals, and should be safe for clinical trial.

Inhibition of Aromatic Amino Acid Decarboxylation in Man and Associated Pharmacological Effects. JOHN A. OATES, JR., LOUIS GILLESPIE, JR., J. RICHARD CROUT and ALBERT SJOERDSMA,* Bethesda, Md.

Decarboxylation is a requisite reaction in the biosynthesis of aromatic amines. The present studies demonstrate inhibition of this process by α-methyl-3,4-dihydroxy-DL-phenylalanine (α-methyl-dopa). Decarboxylation of 5-hydroxytryptophan (5HTP) was measured in four hypertensives by determining urinary serotonin following infusion of 30 mg DL-5HTP. Pretreatment with 2.0 g α-methyl-dopa orally produced 60 per cent inhibition of of serotonin formation. A similar decrease in production of dopamine from 3,4-dihydroxy-L-phenylalanine was demonstrated as well as 80 per cent inhibition of formation of tyramine from tyrosine (125 mg per kg orally) and 50 per cent inhibition of tryptamine formation from tryptophan (50 mg per kg orally). In collaboration with S. Udenfriend, inhibition of 5HTP decarboxylation was confirmed in two carcinoid patients in whom daily administration of 5.0 to 6.0 g α-methyl-dopa decreased urinary 5-hydroxyindole acetic acid 70 per cent and increased 5HTP excretion from <1 and 10 mg to 60 mg per day.

During these experiments a hypotensive effect was noted, and observations were extended to 10 hypertensive patients. Treatment with 0.75 to 4.0 g of α-methyl-dopa daily for 7 to 28 days reduced average standing blood pressures in all cases (range, –22/–16 to –53/–36 mm Hg) and lowered recumbent pressures in 5 patients (–24/–12 to –37/–29 mm Hg). A central effect, manifested as drowsiness, was limited to the first 3 days of treatment.

In addition to offering a basic approach to investigation of blood pressure regulation in man, α-methyl-dopa warrants therapeutic evaluation in diseases characterized by a relative or absolute excess of amines.

Potassium Concentration in the Proximal Tubule of Necturus Kidney. DONALD E. OKEN and A. K. SOLOMON, Boston, Mass. (introduced by Kendall Emerson, Jr.).

A method has been developed to measure the potassium concentration in fluid collected from the proximal tubule of the Necturus kidney. Samples were obtained by a modification of the micropuncture technique of Richards and Walker. Fluid was collected only from the most distal segments which had been identified visually and blocked with injection of Sudan black-stained mineral oil. Since tubular fluid was collected at such a rate that the position of the distal oil droplet remained almost static, the rate of collection approximated normal tubular flow. After each experiment, the distal location of the collection site was checked by further oil injection. Potassium concentrations in tubular fluid, glomerular fluid and serum were determined in the flame photometer of Solomon and Caton on samples measuring 0.3 to 0.4 μl. In 12 experiments, the ratio of tubular potassium concentration to serum potassium concentration was 1.51 ± 0.10, corresponding to a tubular potassium concentration of 5.4 ± 0.1 mEq per L. In 5 of these experiments, the potassium concentration in glomerular fluid was compared with that in serum, giving a mean ratio of 1.05 ± 0.04. Water absorption, measured with C^{14}-inulin in a second series of 12 animals was 33 per cent, a value corresponding to a C^{14}-inulin urine/serum ratio of 1.48 ± 0.08.

In these experiments, the mean concentration ratio of potassium in fluid collected from the distal end of the proximal tubule is essentially equal to that of inulin. Consequently, no potassium absorption from the proximal tubule of the Necturus kidney was observed under our experimental conditions.

Elevated Factors VII and X in Pregnancy, A "Hypercoagulable" State. LIBERTO PECHET, Boston, Mass. (introduced by Benjamin Alexander).

Factor VII has been found elevated in pregnancy. When first observed, Factor X (Stuart) was unknown. Owren's assay, used then, is now known to measure *both* VII (proconvertin) and X. It remained to be determined whether the observed elevation was in VII, X, or in both.

Factor VII was specifically determined by the clot corrective effects on congenital VII-deficient plasma; X, by the "Stypven" method. Also assayed were "Factor VII" by the Owren method, and two-stage prothrombin. Normal values are 70 to 120 per cent for VII, 70 to 110 per cent for X, and 220 to 300 units prothrombin per ml.

In 51 pregnant subjects Factor X varied from 72 to 200 per cent, average 130 per cent. In 33 it exceeded 110 per cent; in 14, 150 per cent. VII varied between 70 and 250 per cent, mean 162. Thirty-six individuals exceeded 120 per cent; 31, over 150 per cent, and 8 exceeded 200 per cent. Owren "Factor VII" varied between 86 and 248 per cent, average 170 per cent. Fifty exceeded 110 per cent; 39, 150 per cent or more; 8 exceeded 200 per cent. Prothrombin was essentially normal.

In some subjects VII was elevated and X normal; in others X was increased with VII only slightly. In most, both were elevated, with Owren values falling in between. In some, however, Owren "VII" was much higher than both VII and X.

The occasional increased Owren "VII" where VII and X were normal, suggests that the assay may reflect entity(s) besides VII and X. The disparity in VII and X, and prothrombin, indicates their distinctness as clotting entities, and makes it doubtful that VII and/or X are prothrombin derivatives. More significant, these high levels in the gravid reflect a truly "hypercoagulable" state, ready to be triggered by the intrinsic or extrinsic thromboplastin mechanisms, in a condition so frequently complicated by thromboembolism.

Studies of the Metabolism of Calcium, Phosphorus and Magnesium in Subjects with Pseudo-Hypoparathyroidism. MAURICE M. PECHET* and EVELYN L. CARROLL, Boston, Mass.

In the preparation of vitamin D_2 by the ultraviolet irradiation of ergosterol a number of isomeric compounds are formed. Complete metabolic studies were carried out in 3 subjects with pseudo-hypoparathyroidism, with 2 of these isomeric compounds, carefully purified, so as to represent by chemical determinations single entities.

Crystalline dihydrotachysterol II, when administered orally in doses of 6 mg daily, produced a marked decrease in fecal calcium, a modest increase in urinary calcium and a pronounced increase in serum calcium. Fecal phosphorus decreased and urinary phosphorus increased; these changes occurred without changes in serum phosphorus levels. Fecal magnesium decreased and urinary magnesium increased while the serum values remained unaltered. Changes in fecal and urinary nitrogen were minimal. The effect of administration of dihydrotachysterol II on urinary phosphorus excretion manifested itself promptly and prior to the effect on urinary calcium excretion; in addition, the magnitude of the changes was greater for phosphorus than for calcium. The effects on phosphorus excretion are probably not secondary to the changes in calcium excretion.

The daily oral administration of 100 mg of suprasterol I produced a marked increase in fecal calcium and no change in urinary or serum calcium. Fecal phosphorus increased to a lesser extent than did the fecal calcium, and the effect wore off much more rapidly for phosphorus following the withdrawal of the suprasterol. Urinary phosphorus increased slightly, and serum phosphorus remained unaltered. Fecal and urinary excretion of magnesium increased and then decreased promptly following the withdrawal of the compound. Whereas vitamin D_2 is known to induce a decrease in fecal excretion of calcium, the isomeric suprasterol induced an increase in fecal excretion of calcium.

Duration of Coagulation Abnormalities Following In Vivo Neutralization of Heparin by Protamine and Polybrene. HERBERT A. PERKINS, D. J. ACRA and MARY R. ROLFS, San Francisco, Calif. (introduced by Stacy R. Mettier).

Following open heart surgery, complete neutralization of heparin is necessary to avoid excessive blood loss. The neutralizing agents by themselves have an anticoagulant effect *in vitro,* but there has been disagreement as to whether excessive amounts *in vivo* are likely to induce a hemorrhagic state.

Protamine was injected intravenously into dogs, and blood levels were estimated by the ability of the blood to neutralize the anticoagulant effect of various concentrations of heparin. Protamine was detectable for periods up to 10 minutes with doses of 5 mg per kg; with 3 mg per kg for less than 5 minutes. However, coagulation abnormalities were evident considerably longer than protamine could be detected by our relatively crude technique. Platelets dropped sharply, the Lee-White clotting time and cephalin time increased, and prothrombin consumption was greatly impaired. Prothrombin times, fibrinogen levels and thromboplastin generation were affected to a minimal degree. With prior heparinization, the effect of protamine on most of the tests corresponded only to the portion unneutralized by heparin. Interaction between heparin and protamine was completed within one minute. Platelets dropped whether or not heparin had been given. Results with polybrene were identical except that larger amounts of heparin were neutralized and coagulation abnormalities were more severe and persistent with the same doses.

It is concluded that excessive amounts of protamine or polybrene can produce a coagulation defect *in vivo.* Significantly increased blood loss following open heart surgery may thus occur, particularly if repeated injections are given at intervals of less than 20 to 30 minutes. In no case was the disappearance of protamine or polybrene followed by the reappearance of heparin in the circulating blood.

Relationship of Phagocytosis to the Fall in Spinal Fluid Glucose in Experimental Meningitis. ROBERT G. PETERSDORF, Baltimore, Md. and Seattle, Wash. (introduced by W. M. M. Kirby).

The mechanism of hypoglycorrhachia in bacterial meningitis is poorly understood, and the fall in GSF glucose observed in these infections has been attributed to cells, bacteria, consumption of glucose by neural tissue and changes in the blood-CSF barrier. Previous experiments have demonstrated that a mixture of leukocytes and pneumococci incubated in CSF *in vitro* act synergistically to depress CSF glucose, suggesting that a combination of cells and bacteria is necessary for hypoglycorrhachia.

In the present experiments, aseptic meningitis was produced in dogs by the administration of saline into the cisterna magna. Four hours later these animals regularly had an exudate containing 3 to 8,000 WBC per mm^3 of CSF, predominantly polymorphonuclear leukocytes. At this time pneumococci were instilled intrathecally and cisternal puncture was performed 3 hours later. In all animals with bacterial meningitis superimposed upon aseptic meningitis, a profound drop in CSF glucose occurred. Control animals given pneumococci without antecedent production of aseptic meningitis, or animals with aseptic meningitis without superimposed bacterial infection, did not experience a fall in glucose. These results provide evidence for synergistic action between bacteria and leukocytes in depressing CSF glucose *in vivo.* In order to clarify the mechanism of the synergistic effect, heat-killed pneumococci were injected intrathecally into animals with aseptic meningitis. Hypoglycorrhachia occurred promptly indicating that bacterial multiplication was not responsible for the fall in glucose. Intrathecal administration of India ink particles to dogs with aseptic meningitis was also associated with a decrease in CSF glucose and a large number of the particles were visible within leukocytes. These findings suggest that leukocytes in the CSF engaged in active phagocytosis rapidly consume glucose, while resting white cells exhibit little glycolytic activity.

On the Mechanism of Carotid Sinus Function. LYSLE H. PETERSON,* ERIC O. FEIGL and PETER GOURAS, Philadelphia, Pa.

A classic principle of cardiovascular function holds that arterial blood pressure is regulated via "baroreceptors" such as those in the walls of the carotid sinus. Actually these receptors respond to stretch of the vessel wall rather than to pressure per se. The magnitude of vessel wall stretch (strain) which results from intravascular pressure depends upon the mechanical properties of the vessel wall (elasticity and viscosity). Thus, the relationship between arterial blood pressure and the nervous activity of the receptors depends upon these mechanical properties. Techniques have been developed and used for the accurate, simultaneous measurement of the intracarotid sinus pressure, carotid sinus strain (change in circumference per unit circumference), the electrical activity of sinus receptors and the mechanical properties of the sinus wall. Experiments on anesthetized (pentobarbital and Dial) dogs have demonstrated that: *1*) The mechanical properties of the sinus wall can change by 100 per cent or more following the application of vasoactive materials to the sinus wall; there are, therefore, contractile elements in the wall. *2*) The mechanical properties of the wall can change reflexly as a result of efferent nerve supply to the sinus wall. *3*) Receptor activity changes, with respect to pressure as the wall properties change. From these findings it can be concluded that the nerve impulse traffic arising from these receptors is a function of two independent variables, wall properties and pressure. Thus, the classic concept of blood pressure regulation is an oversimplification which may be misleading. In addition to the acute "physiological" changes in vessel wall properties it is likely that chronic changes due to aging and disease may also lead to alterations in the cardiovascular regulatory mechanisms.

On the Role of Virus Sulfhydryl Groups in the Attachment of Enteroviruses to Cells. LENNART PHILIPSON and PURNELL W. CHOPPIN, New York, N. Y. (introduced by Igor Tamm).

The precise mechanism whereby animal viruses attach to cells is poorly understood. In the experiments to be described, interaction of entero, myxo, and arbor viruses with erythrocytes and host cells was studied. Hemagglutinating activity of ECHO 7, 11, 12, and 22, and Coxsackie B3 virus was eliminated by *p*-chloromercuribenzoate (PCMB), a compound which combines with sulfhydryl groups forming mercaptides. PCMB-treated virus failed to adsorb to erythrocytes. At $10^{-6.2}$ M PCMB eliminated the hemagglutinating activity of ECHO 7 virus; $10^{-6.5}$ M PCMB was sufficient to cause 50 per cent inactivation. The reaction was rapid in that 10^{-6} M PCMB caused 75 per cent inactivation in 2 minutes at 37° C. The effect of PCMB was reversible by sulfhydryl-containing compounds. Cysteine, reduced glutathione, and mercaptoethanol completely restored the hemagglutinating activity of PCMB-treated virus. Mercuric chloride, thimerosal, and iodine also inactivated the hemagglutinating activity of the ECHO and Coxsackie viruses mentioned. The effect of mercuric chloride but not of thimerosal or iodine was reversed by reduced glutathione. However, thimerosal apparently acts at the same site on the virus protein as PCMB because the latter was capable of blocking the action of thimerosal. ECHO 11 and 22 were inactivated also by iodoacetamide, an alkylating reagent which reacts with sulfhydryl groups. Hemagglutinating activity of arbor and myxoviruses was unaffected by PCMB.

PCMB and thimerosal inactivated the infectivity of the following viruses: ECHO 7, 11, 12, and 22 which hemagglutinate; ECHO 6 and 9 which do not hemagglutinate; two strains of Coxsackie B3, one which hemagglutinates and another which does not; and poliovirus 2, MEFl strain. Preliminary evidence indicates that PCMB or thimerosal-treated virus fails to adsorb to monkey kidney cells. The results obtained suggest that sulfhydryl groups of the virus proteins are necessary for attachment of enteroviruses to erythrocytes and host cells.

Electron Microscopic Study of Tissue Mast Cells and Chromaffin Cells in Man. JOHN H. PHILLIPS and RICHARD G. HIBBS, New Orleans, La. (introduced by George E. Burch).

In various species the typical type of mast cell has been shown to contain serotonin, histamine and heparin. Previous studies in this laboratory revealed chromaffin granules in elongated eel-like cells in human skin which superficially are quite similar to tissue mast cell and have probably been so called in the past. Available indirect physiological and pharmacological evidence suggests that these granules are a local tissue source of epinephrine or norepinephrine or both. As so-called tissue mast cells may in reality represent several cellular types, electron microscopic studies were undertaken to clarify possible anatomical differences. Tissues utilized in these studies were obtained from human digital and abdominal skin (20 specimens) by punch biopsy and from human gastric mucosa (8 specimens) by suction biopsy. At least two distinct types of "mast cells" were identified. One is an elongated cell with granules containing a homogenous material enclosed by a membrane, and the other, an oval circumscribed cell with larger granules composed of peculiar subgranular structures in the form of clusters of laminated rolled scrolls, all enclosed by a distinct membrane. Filling the spaces between these scrolls was a finely particulate matter. It is felt that the oval-type cell with its complex granules is the more classic form of tissue mast cell while the elongated type with its rather simple granule is the chromaffin cell identified by light microscopy.

The intimate relationship of mast cells to the dynamics of the ubiquitous connective tissue is well known. Notable in its effects on the connective tissue is the adrenocorticosteroid. Preliminary electron microscopic observations following steroid administration have shown quite different effects on the two cellular types described above.

Thus evidence is presented in which "tissue mast cells" represent at least two distinct cellular types with many differences in structure and function.

Bladder Smooth Muscle Contributions to Micturition. FRED PLUM, Seattle, Wash. (introduced by R. H. Williams).

Two types of contractions occur when fluid fills the normal urinary bladder: low amplitude (2 to 5 cm H_2O), unsustained, rhythmically recurring waves, and high intensity (75 to 150 cm H_2O), intermittent micturition contractions. Previous studies leave unclarified the interrelationships of these two responses. This investigation demonstrates the genesis of the bladder rhythm, indicates its role in stimulating micturition contractions, and shows how purely local bladder changes alter the rhythm to produce abnormal cystometric changes traditionally attributed to neurological injury.

To separate neurogenic from vesicogenic micturitional changes, isometric cystometrograms were recorded in cats before and after spinal cordectomy, sacral rhizotomy and vesical autonomic ganglionectomy. Bladder wall damage was induced by overdistention, which, in innervated bladders, required either obstructing the urethra or temporarily suppressing micturition by ether anesthesia. Afferent stimuli from the bladder were recorded in single pelvic nerve or sacral dorsal root fibers. To rule out species differences, isometric cystometrograms were recorded from healthy humans before and after spinal anesthesia plus ganglionic blockade.

Rhythmic contractions originated directly in bladder smooth muscle and persisted after all denervation procedures. Rhythmic contractions appeared to provide the necessary stimulus to micturition, since bladder filling invariably evoked rhythmic waves preceding micturition contractions, and maximal afferent nerve activity coincided with each rhythmic wave's rising phase. Denervating the bladder abolished micturition contractions but produced little other cystometric change—additional overdistention was required to supress bladder rhythm and produce a flat, atonic cystometrogram. Identical cystometric atony was induced by overdistending neurologically intact bladders: rhythmicity was suppressed, afferent nerve potentials were markedly reduced, and micturition was abolished until vesical decompression restored intrinsic bladder rhythmicity.

Once distention has suppressed intrinsic bladder rhythmicity, obstructive and neurogenic bladder dysfunction cannot be differentiated by cystometrogram. Effective treatment in either case must be directed toward restoring smooth muscle function.

Quantitative Assay of Alkaline Phosphatase and Lactic Dehydrogenase Activities in the Nephron in Glomerulo-Tubular Imbalance. VICTOR E. POLLAK, SJOERD L. BONTING and ROBERT C. MUEHRCKE, Chicago, Ill. (introduced by Robert M. Kark).

Previous studies showed that in lupus nephritis glomeruli were abnormal before tubules were damaged; moreover, glomerular involvement was always more severe than tubular damage. This unusual relative glomerulo-tubular imbalance led us to study quantitatively enzyme activity in the nephron in lupus nephritis, in order to assess the relationship between enzyme activity in individual anatomical subunits of the nephron and glomerulo-tubular imbalance. Alkaline phosphatase activity (APP) was assayed because qualitative data were available from histochemical staining techniques; lactic dehydrogenase activity (LDH) was assayed because LDH is a key enzyme in cellular energy metabolism.

Seven healthy kidneys and 19 renal biopsy specimens from 13 patients with lupus nephritis were assayed quantitatively for enzyme activity by ultramicrochemical techniques. Activity was measured on individual glomeruli, and dissected portions of proximal and distal convolutions, medullary rays, medulla and papilla containing 20 to 200 cells; results were expressed as moles of substrate split per kilogram dry weight per hour at 37° C.

APP activity was decreased in lupus nephritis in proximal ($p = < 0.001$) and distal convolutions ($p = < 0.001$). APP activity was similar in lupus glomerulitis (mild glomerular lesions, normal tubules) and lupus glomerulonephritis (severe glomerular lesions, abnormal tubules and interstitial tissue).

LDH activity was higher in lupus glomerulonephritis than in healthy kidneys in proximal ($p = < 0.001$) and distal convolutions ($p = < 0.05$). In all structures analyzed LDH activity was significantly higher in lupus glomerulonephritis than in lupus glomerulitis. This difference was not related to renal functional impairment or to the dosage of prednisone used therapeutically (confirmed by experiments in rats). Increased LDH activity in convoluted tubules in lupus glomerulonephritis would appear to be an enzymatic expression of the ability of slightly damaged tubules to respond metabolically to increased metabolic demands imposed by severely damaged glomeruli. By contrast, in Fanconi syndrome with tubulo-glomerular imbalance and severe tubular damage, LDH activity in the convoluted tubules was significantly decreased.

Recall of Type Specific Immunity by Vaccination of Human Beings with Group A Streptococcal Cell Wall Vaccines. ELIZABETH V. POTTER, GENE H. STOLLERMAN * and ALAN C. SIEGEL, Chicago, Ill.

Immunity to Group A streptococci appears to be type specific and is related primarily to the development of antibody against the M protein surface antigen. Human vaccination with Group A streptococci has been discouraged by the toxicity of streptococcal products, the relatively low antigenicity of type specific M protein in purified form, and the multiplicity of streptococcal serological types. Primary immunization in man has been unsuccessful so far. The present studies were undertaken to determine whether or not vaccines prepared from cell walls of streptococci (rich in M protein) are at all antigenic in the human being.

Cell wall vaccines of Type 12 streptococci were pre-

pared by Mickle disintegration according to the method of Barkulis. Type 12 anti-M antibodies were produced in rabbits by primary immunization with whole cells and small doses of the cell wall vaccine were shown to recall these antibodies after their disappearance.

Of 57 children with untreated Type 12 streptococcal pharyngitis (who were part of a controlled study in progress), 41 developed type specific antibody following the natural infection. Ten of these patients no longer had anti-M antibody demonstrable by bactericidal and long chain tests after 2 years of follow-up. These children received 3 to 4 small booster subcutaneous or intradermal injections of Type 12 cell wall vaccine (1.7 to 170 μg of protein per dose) which were well tolerated. Recall of anti-M antibody was successful in all 10 patients. The levels of antibody recalled, though low, were at least as high as those observed following the antecedent natural infection.

The results indicate that M protein in cell wall vaccines is antigenic in man in small, tolerated doses and suggest that primary immunization might be achieved if a sufficient amount of vaccine can be administered in multiple small doses.

An Action of Hageman Factor: Evidence that Plasma Thromboplastin Antecedent is Activated by Hageman Factor. OSCAR D. RATNOFF,* DAVID L. MALLETT and EARL W. DAVIE, Cleveland, Ohio.

The earliest steps in the process of blood coagulation are obscure. Previous studies have indicated that contact of plasma with glass converts Hageman factor from an inactive to an active form. Several authors have suggested that this activated Hageman factor then reacts with plasma thromboplastin antecedent to form a clot-promoting agent.

Partially purified preparations of activated Hageman factor, deficient in other known clotting factors, were prepared from plasmas obtained from patients with deficiencies of plasma thromboplastin antecedent. Similarly, partially purified preparations of plasma thromboplastin antecedent were prepared from plasmas lacking Hageman factor, obtained from patients with Hageman trait. When a mixture of Hageman factor and plasma thromboplastin antecedent was incubated in silicone-coated tubes at 37° C, a clot-promoting agent gradually evolved. The evolution of the clot-promoting agent was temperature- and time-dependent. Suggestive evidence was obtained that the formation of the clot-promoting agent was enzymatic. Partially purified, activated Hageman factor was inhibited by diisopropylfluorophosphate, as shown by Becker, but partially purified plasma thromboplastin antecedent was not affected by this substance. Experiments in which the concentrations of plasma thromboplastin antecedent and of Hageman factor were varied independently suggest that the effect of Hageman factor is to transform plasma thromboplastin antecedent into a clot-promoting substance. This substance is neither thrombic nor thromboplastic in activity and appears to act early in the clotting process.

These studies, then, support the view that Hageman factor is an enzyme which initiates coagulation by its effect on plasma thromboplastin antecedent.

Role of Plasma P_{CO_2} and Carbonic Anhydrase Activity in HCO_3^- Reabsorption. FLOYD C. RECTOR, JR., ALBERT D. ROBERTS, JR., JERRY S. SMITH and DONALD W. SELDIN,* Dallas, Tex.

Renal HCO_3^- reabsorption in dogs was examined using three types of experiments. In Group I, the effect of plasma P_{CO_2} on maximum HCO_3^- reabsorptive capacity (HCO_3^- Tm) was assessed before and after carbonic anhydrase (CA) inhibition (acetazolamide 50 mg per kg). HCO_3^- reabsorption increased curvilinearly as P_{CO_2} was elevated. CA inhibition depressed HCO_3^- reabsorption by a constant amount at all P_{CO_2}'s. In Group II, the effects of P_{CO_2} and CA inhibition on the relationship of HCO_3^- excretion to HCO_3^- Tm were studied. As plasma HCO_3^- concentration was progressively increased in the normal state, HCO_3^- excretion began before the Tm was reached. A similar HCO_3^- leak was noted in respiratory acidosis. CA inhibition, however, caused a HCO_3^- leak which was of far greater magnitude and occurred even at very low levels of serum [HCO_3^-]. Increasing H^+ production by markedly elevating plasma P_{CO_2} failed to obliterate the HCO_3^- leak, whereas administration of Na_2SO_4, a salt capable of intensely acidifying the urine in the normal state, partially corrected it. In Group III, CA inhibition during metabolic acidosis resulted in an inability to acidify urine below the pH of blood, despite elevation of plasma P_{CO_2} to levels as high as 200 mm Hg.

It was concluded that HCO_3^- reabsorption is accomplished by two distinct processes. One process is independent of CA, has a HCO_3^- Tm which is dependent on P_{CO_2}, and is incapable of establishing pH gradients between blood and urine. The other process is dependent on CA, independent of P_{CO_2}, and necessary for the establishment of pH gradients between blood and urine.

Abnormalities of In Vitro Behavior of Structural Lipids of Red Blood Cells from Patients with Hereditary Spherocytosis. CLAUDE F. REED and SCOTT N. SWISHER,* Rochester, N. Y.

To explain increased osmotic fragility, increased permeability to K^+, and premature hemolysis of erythrocytes from patients with hereditary spherocytosis (HS), produced by *in vitro* incubation at 37° C, several investigators have postulated an early onset of "degeneration of the membrane," preceding these changes.

In the present study, serial measurements of the rate of exchange of plasma and erythrocyte phospholipids, and of net changes in lipid composition of these erythrocytes, were made during 8 to 24 hour periods of *in vitro* incubation. These parameters were correlated with simultaneous measurements of glucose disappearance, lactic acid production, cellular K^+ content and changes in osmotic fragility. All studies were made before the oc-

currence of detectable hemolysis, in an attempt to define the prehemolytic sequence of changes.

Under all circumstances studied, the rate of exchange between plasma and erythrocyte phospholipids was significantly less in HS erythrocytes than in normal erythrocytes. A progressive net loss of red cell lipid, significantly greater than that observed in normal erythrocytes, occurred during the first 24 hours of *in vitro* incubation. The predominant loss was from those cellular lipids known to exchange with the corresponding plasma compounds, i.e. sphingomyelin, lecithin, and cholesterol. Lipid loss preceded detectable changes in cellular K^+ content, gross changes in osmotic fragility or measurable hemolysis. Supplemental glucose retarded, but did not prevent the progressive loss of cellular lipid, as it does with normal erythrocytes under similar experimental circumstances.

These observations illustrate a relationship between erythrocyte energy production, maintenance of integrity of the structural lipids, and "membrane function."

The HS erythrocyte appears to utilize energy inefficiently in maintaining integrity of its structural lipids; the observed initial loss of cellular lipid may well represent the postulated "membrane degeneration."

The Adaptation of the Circulation to Supine Exercise and Treadmill Walking. JOHN T. REEVES, ROBERT F. GROVER, GILES F. FILLEY and S. GILBERT BLOUNT, JR.,* Denver, Colo.

Walking is the usual form of exercise for man, yet cardiac catheterization of normal man during treadmill walking has not been reported. Although the circulatory behavior during standing differs greatly from that of supine rest, the effect of posture on the circulatory response to exercise has not been documented previously. To investigate these areas of circulatory physiology, 10 normal men each performed several grades of treadmill exercise with a catheter in the pulmonary artery, permitting repeated measurements of cardiac output by the classical Fick method. These and 12 other normal subjects were also evaluated by this technique during supine exercise and during standing. The behavior of the circulation of the leg was investigated for the various conditions of the experiment by sampling blood through a polyethylene catheter introduced into the femoral vein.

The results showed that during exercise a given quantity of oxygen was consistently transported by a smaller cardiac output (10 to 20 per cent smaller) and a greater arteriovenous (A-V) oxygen difference for the upright than for the supine posture. Further, in the supine posture, the A-V oxygen difference for the leg increased markedly for mild exercise, but with heavier exertion there was little further increase. However, the A-V difference for the leg increased nearly to maximum as the subject assumed the standing posture, and severe treadmill exercise was not accompanied by a further increase. These data indicate that the particular central and peripheral mechanisms called upon to meet the oxygen requirements of exercise depend upon the posture of the individual.

The Hemodynamic Determinants of the Rate of Pressure Change of the Left Ventricle During Isometric Contraction. T. J. REEVES, LLOYD L. HEFNER, W. B. JONES, CECIL COGHLAN, GUSTAVO PRIETO and JOHN CARROLL, Birmingham, Ala. (introduced by Tinsley R. Harrison).

The pressure, time derivative of pressure, circumference and contractility (isometric contractile force-Walton-Brodie strain gage arch) of the left ventricle have been simultaneously recorded in a series of thoracotomized dogs. These parameters were recorded at multiple levels of filling pressure, while variations in myocardial function and systemic resistance were induced by the administration of epinephrine and methoxamine.

The maximum rate of pressure rise (MRPR) of the isometric contraction phase of the ventricular pressure pulse ranged from 456 to 6,610 mm Hg per second. MRPR was shown to have a significant correlation with peak ventricular pressure ($R = 0.624$; $p < 0.001$) with ventricular pressure area ($R = 0.287$; $p < 0.01$) ventricular end diastolic stretch ($R = 0.484$; $p < 0.001$) and with ventricular end diastolic pressure ($R = 0.468$; $p < 0.001$). When graphically displayed, all of these relationships showed a strong tendency to form families of curves, with MRPR per unit systolic pressure, stretch, or end diastolic pressure being greater while epinephrine was being administered than in the control period. Conversely, MRPR was less per unit pressure, circumference or end diastolic pressure when methoxamine was administered. Since epinephrine resulted in increased myocardial contractility and methoxamine resulted in a decreased myocardial contractility, it was not surprising that a highly significant correlation between MRPR and contractility was found ($R = 0.451$; $p < 0.001$). A linear relationship was found between MRPR and the *product* of contractility and the end diastolic stretch of the ventricle ($R = 0.790$; $p < 0.001$). This product may be considered as an index to the maximum tension that would be generated during a completely isometric contraction of the entire ventricle.

The quotient MRPR/end diastolic pressure was found to have an excellent correlation with contractility ($R = 0.655$; $p < 0.001$), indicating the possibility of obtaining a quantitative index to myocardial contractility from the ventricular pressure alone.

Influence of Extracellular Calcium Concentration Upon Myocardial Potassium Transfer. TIMOTHY J. REGAN, GERALD C. TIMMIS, MARTIN J. FRANK, JOHN D. MCGINTY and HARPER K. HELLEMS,* Detroit, Mich.

Since calcium and the monovalent cations frequently have opposing effects upon a variety of physiological phenomena, the influence of varied plasma Ca concentrations on myocardial transfer of potassium and sodium has been studied.

The effects of doubling the normal plasma Ca concen-

trations upon net myocardial transfer of K and Na have been assessed in 7 intact vagotomized dogs by serial paired sampling of arterial and coronary sinus blood. Rapid infusion of calcium induced a net myocardial uptake of potassium over a 4 minute period, paralleling the time course of hemodynamic effects. Planimetry of the arteriovenous difference area was +4.1 cm^2 compared to control period of +0.5 cm^2 ($p = < 0.001$). No sodium changes were noted. Sustained infusions produced a similar effect. Increase in heart rate, arterial pressure, stroke work and myocardial oxygen consumption resulted from hypercalcemia. Prior studies indicate that these changes, per se, do not affect ion arteriovenous differences.

As these hemodynamic and ion alterations are similar to sympathomimetic effects, another series of animals was studied. Neither dibenzyline blockade of adrenergic influence nor depletion of myocardial norepinephrine stores with reserpine modified the response to calcium. Preliminary ultracentrifugation studies of heart muscle indicate that hypercalcemia increases mitochondrial calcium concentrations while other fractions are unaltered. Such accumulation implies that calcium's activity is not exclusively dependent upon an extracellular location. The consequences of reduced plasma concentrations of Ca upon the known K egress induced by strophanthidin was evaluated in 5 dogs by pretreatment with Sodium Versenate. A 20 per cent plasma calcium reduction induced during strophanthidin administration was attended by a significantly greater K loss (planimetered area −16.1 cm^2) than in controls (−2.19 cm^2) without hypocalcemia after Calcium Disodium Versenate pretreatment ($p = < 0.05$).

These studies indicate that extracellular calcium exhibits a control over myocardial potassium transfer that is a direct function of its concentration. Thus, maintenance of the transcellular potassium gradient has a close dependence upon the quantitative activity of this divalent cation.

The Effect of Vasopressin on Hepatic Hemodynamics in Patients with Portal Hypertension. TELFER B. REYNOLDS,* HERMAN M. GELLER and ALLAN G. REDEKER, Los Angeles, Calif.

Infusion of vasopressin (20 to 40 IU per hour) resulted in a fall in wedged hepatic vein pressure (WHVP) in 11 of 13 patients with cirrhosis and portal hypertension. The maximum fall in WHVP varied from 19 to 59 per cent of the original value. Although, in general, the decline in WHVP was greater in those with initially higher pressures, the degree of response could not be predicted. In 2 patients there was no change in WHVP despite systemic symptoms indicating activity of the vasopressin.

Hepatic blood flow (HBF) was estimated in 15 patients and fell in 12 during vasopressin administration. The maximum fall varied from 17 to 63 per cent of the baseline value. In 3 subjects, there was no change in HBF. Hepatic oxygen consumption did not change significantly during vasopressin infusion. There was a good correlation between the magnitude of the fall in WHVP and HBF and, in those patients who failed to show any drop in HBF, WHVP either did not decrease or fell minimally. Hemodynamic changes appeared a few minutes after vasopressin was started, fluctuated irregularly throughout the infusion (15 to 80 minutes) and returned toward baseline values soon after the infusion was terminated. Systemic symptoms included nausea, pallor, abdominal cramps and urgency and, in most cases, could be controlled by decreasing the rate of infusion. There was marked individual variation both in hemodynamic and symptomatic response to similar doses of vasopressin.

The best explanation for these effects appears to be splanchnic vasoconstriction with reduced portal inflow. Although the hemodynamic changes with vasopressin are transitory, irregular, and not completely predictable, they may prove to be of clinical value in patients with bleeding esophageal varices.

The Determination of Pulmonary Blood Flow in Man. MARIO RIGATTO, GERARD M. TURINO and ALFRED P. FISHMAN,* New York, N. Y.

In subjects with normal lungs, pulmonary blood flow may be calculated from the rate of absorption of an inspired soluble gas, its mean alveolar concentration, and its coefficient of absorption in blood. This approach has two advantages over indicator-dilution and direct Fick techniques: *1*) simplicity and *2*) validity in the face of left-to-right shunts. The main deficiencies of previous methods using such gases have been: *1*) overestimation of pulmonary recirculation time, *2*) measurement of flow only during expiration, and *3*) failure to collect simultaneously all data used for the calculation.

The present study was designed to circumvent these difficulties. The uptake of nitrous oxide was measured during a vital capacity maneuver of less than 10 seconds. The mean alveolar N_2O concentration during inspiration was calculated from the volume of inspired N_2O and the residual volume; the corresponding concentration during expiration was measured by a continuous infrared analyzer. The optimal time for the vital capacity maneuver was established by 24 separate determinations of the pulmonary recirculation time which involved: *1*) bronchospirometry, for sampling expired gas from one lung after administering N_2O to the other; and *2*) cardiac catheterization, for sampling mixed venous blood for N_2O after a single breath of N_2O. Pulmonary recirculation occurred within 11 ± 3 seconds.

In 11 subjects, with cardiac indices ranging from 1.7 to 5.4 L per minute per m^2, 19 comparisons were made of the pulmonary blood flow by the N_2O and by the Fick methods. The average difference between the two groups of values was 3 per cent. Individual differences ranged from +34 to −13 per cent, with a mean of $\pm$ 12 per cent. In one subject, 13 determinations in 5 days by the N_2O method showed a mean deviation of $\pm$ 11 per cent.

These results indicate that this method provides a reliable measure of pulmonary blood flow in subjects with normal lungs.

The "Internal" pH of Mitochondria with Observations on the Functional Significance of Mitochondrial Membranes. EUGENE D. ROBIN, JOHN W. VESTER, ROBERT J. WILSON and MARGARET H. ANDRUS, Pittsburgh, Pa. (introduced by Jack D. Myers).

Measurements of the internal pH of isolated rat liver mitochondria have been attempted by using two buffer pairs, CO_2-HCO_3^- and NH_3-NH_4^+. Rat liver mitochondria were prepared by differential centrifugation at 4° C. Measurements using the CO_2 system were done on mitochondria equilibrated with known tensions of CO_2 gas. Total CO_2 was determined manometrically and the Henderson-Hasselbalch equation applied (pK'a: 6.27; α: 0.093). Measurements using the NH_3 system were done by equilibration with sucrose solutions of known pH and ammonia concentration.

Among the many assumptions implicit in these studies, the most important is that external membranes of mitochondria function in a manner similar to cellular membranes and that substances being measured are more or less in free solution in intramitochondrial water. This assumption was tested by determining the relationship between supernatant pH and mitochondrial ammonia concentrations. An increase of supernatant pH produced a rise, and a decrease of supernatant pH produced a fall of mitochondrial ammonia. Thus intramitochondrial ammonia transfer appears to depend on non-ionic diffusion suggesting a functional role for mitochondrial membranes. The "internal" pH of mitochondria as determined by CO_2 measurements averaged 6.58 ± 0.17 units and intramitochondrial bicarbonate concentration averaged 8.46 ± 2.94 mmoles per L mitochondrial water. The mean pH as determined by the NH_3 system averaged 6.25 ± 0.24 units. The difference in pH as determined by the two techniques is presumably related to technical factors.

Of interest was the finding that liver mitochondria, *unequilibrated* with NH_3-containing solutions, contained quite large concentrations of NH_3 (1.60 ± 0.51 mmoles L mitochondrial water). If this represents uncombined NH_3, this substance may be a quantitatively important intramitochondrial buffer. Mitochondrial pH values are at least 0.5 unit lower then the generally accepted internal pH values of liver cells. It appears that one mechanism for uneven H^+ distribution within cells is the presence of distinctly different pH values in subcellular structures.

Occupational and Environmental Influences on Serum Cholesterol: Comparative Study of Two Dissimilar Groups of Female Subjects. RAY H. ROSENMAN,* San Francisco, Calif.

Comparative studies of possible stressful occupational influences on serum cholesterol levels often have failed to eliminate certain other stressful environmental factors or have compared groups differing so widely in their diet and in ethnic origin as to make conclusions impossible. Therefore two groups of 30 to 59 year old California women were randomly selected for study on the basis of different occupation and residence. Group A consisted of 69 San Francisco, Bay area women in key executive positions characterized by immersion in competitive, "deadline" and other socioeconomic stresses, and included 19 executive Catholic nuns. Group B consisted of 107 housewives and clerical secretaries and included 20 nuns teaching elementary school. Almost all Group B women lived and worked in central Costra County, an independent urban community of 35,000, notably devoid of the well known attributes characterizing the intense pace of living and working in densely populated urban and suburban communities.

The two groups of women were not found to differ in average age, habitus, catamenia maturity history, parental history of cardiovascular disease, exercise habits, or diet (calculated from individually kept diet diaries). The serum cholesterol (SC) averaged 294 mg per 100 ml (SE $\pm$ 5.6) in Group A and 217 ($\pm$ 3.5) in Group B. The SC averaged 281 mg per 100 ml (SE $\pm$ 5.3) in premenopausal, and 318 ($\pm$ 8.8) in postmenopausal Group A women, compared to 211 ($\pm$ 3.3) and 247 ($\pm$ 10.0) respectively, in Group B women. An average of 289 ($\pm$ 10) was observed in Group A nuns compared to 229 ($\pm$ 9.5) in Group B nuns. These striking differences were highly significant.

Hemodynamics of Systemic Arterial Hypertension. G. G. ROWE, C. A. CASTILLO, G. M. MAXWELL and C. W. CRUMPTON,* Madison, Wis.

In a series of 21 compensated subjects with systemic arterial hypertension, cardiac output was determined by the Fick principle and coronary blood flow by the nitrous oxide saturation method. For purposes of comparison the series was divided into two groups. Group I contained 14 subjects with hypertension of Smithwick grades 1 and 2. Group II contained 7 subjects in Smithwick grades 3 and 4. Statistical comparisons were done by the *t*-test. In Group I, the cardiac index was normal but in Group II it was reduced (-31 per cent, $p < 0.01$). The calculated total peripheral vascular resistance was elevated in Group I as compared to 30 normals (+47 per cent, $p < 0.001$) and in Group II as compared to Group I (+50 per cent, $p < 0.001$). Pulmonary vascular resistance was elevated in Group II as compared to Group I (+32 per cent, $p < 0.05$). The left ventricular work index in Group I was increased as compared to normal and as compared to Group II (+39 per cent, $p < 0.01$), whereas right ventricular work index was normal in Group I and reduced in Group II (-33 per cent, $p < 0.02$). Coronary blood flow and cardiac oxygen usage per 100 g of LV per minute were not significantly different in the two groups nor was coronary vascular resistance. However, as compared to normal, coronary vascular resistance was increased in both groups. The data indicate that in Smithwick grades 1 and 2 the primary abnormality appears to be elevated peripheral vascular resistance. In grades 3 and 4 vascular resistance is still more elevated and cardiac index reduced.

Foci of Polyoma Virus Infection in House Mice. WALLACE P. ROWE* and ROBERT J. HUEBNER, Bethesda, Md.

Infection with mouse polyoma virus is prevalent in certain laboratory mouse colonies, infection being transmitted primarily by urine and saliva of mice infected as sucklings. To further clarify the epidemiology of this tumor virus, serological and virus isolation studies were made on mice trapped in various parts of New York City. Infected house mice were found, and the infections were sharply localized to mice trapped in certain blocks in the Harlem district. There was a distinct tendency for infections to be localized to certain apartment houses, and even to individual floors. In some foci, 40 per cent of mice were serologically positive. Virus was recovered from organs of 25 per cent of antibody-positive mice, and occasionally from excreta and floor sweepings. No virus was recovered from mites or roaches in infected apartments. Humans, cats and rats from the infected areas did not have antibody to polyoma virus. It is hypothesized that the infection is maintained by urinary contamination of communal nesting areas.

The Effect of Wheat Instillation into the Proximal Ileum of Patients with Idiopathic Sprue. CYRUS E. RUBIN, LLOYD L. BRANDBORG, ARNOLD L. FLICK, CHERILL PARMENTIER, PATRICIA PHELPS and SALLY VAN NIEL, Seattle, Wash. (introduced by Clement A. Finch).

The characteristic mucosal abnormality in idiopathic sprue is obvious in the duodenum and proximal jejunum, whereas normal areas are observed distally. Wheat or rye gluten exacerbates this illness, but relationships between gluten and the mucosal lesion are poorly understood. The hypothesis that gluten is digested and absorbed proximally might explain how the distal intestine is spared. This study was undertaken to determine the effect of exposing the normal distal intestine in sprue to wheat gluten.

Two sprue patients in remission continued their gluten-free diets while wheat flour was instilled into their proximal ileum 3 times daily for 9 days. Studies before and after wheat infusion included: clinical observations, chemical fat balance, $B_{12}Co^{60}$ absorption and roentgenographically positioned peroral mucosal biopsies at 18 levels from terminal ileum to pylorus. Suitable control data were obtained. After the first day of ileal gluten, both sprue patients became irritable and anorexic; by the third day they were obviously ill, and during succeeding days experienced flatulence, distention, cramping and diarrhea. Fat absorption decreased from 92 to 65 per cent in the first patient and from 80 to 10 per cent in the second. $B_{12}Co^{60}$ absorption (Schilling) was unchanged in the first, and in the second decreased from normal (30 per cent) to almost nothing (4 per cent). Wheat instillation in two normals did not affect fat or $B_{12}Co^{60}$ absorption; they felt well despite moderate flatulence and diarrhea.

Eight observers coded and blindly evaluated 116 serially sectioned sprue and control biopsies. There was unanimous agreement that the characteristic sprue lesion developed in the vicinity of wheat instillation where the mucosa had been normal previously. Damage produced distal to wheat instillation progressively diminished; the proximal lesion remained essentially unchanged. This demonstration of intestinal mucosal damage by wheat implicates gluten exposure as the precursor to anatomic changes in idiopathic sprue.

Purification and Properties of a Pituitary Component Which Produces Lipemia in the Rabbit. DANIEL RUDMAN, MARIO DI GIROLAMO, FLOYD SEIDMAN and MARIA B. REID, New York, N. Y. (introduced by David Seegal).

Our previous studies showed that a single subcutaneous injection of an aqueous extract of anterior pituitary glands produces lipemia in the rabbit. Comparable lipemia is not produced by purified preparations of any of the recognized anterior pituitary hormones. Fractionation of the crude pituitary extract has now yielded a fraction (labeled "Fraction H") which produces lipemia in the rabbit. Bioassays have shown that Fraction H contains 0.07 per cent thyroid-stimulating hormone, 0.002 per cent oxytocin, and no detectable amounts of the 6 other pituitary hormones.

The injection of as little as 0.25 mg of Fraction H in the rabbit causes an increase in serum free fatty acid (FFA) concentration from 200 to 2,000 μEq per L within 1 hour. This effect is not suppressed by the intravenous infusion of glucose. The period of elevation of serum FFA concentration varies from 2 to 24 hours, depending on the dosage of Fraction H. The rapid elevation of serum FFA level is followed within 12 hours by a two- to fivefold increase in serum total lipid concentration. In a typical experiment, serum cholesterol increased from 42 to 103 mg per 100 ml, serum phospholipid from 65 to 189 mg per 100 ml, and serum triglyceride from 35 to 1,270 mg per 100 ml.

The following properties of Fraction H have been determined: nondialyzability, precipitation by 40 per cent $(NH_4)_2\ SO_4$ or 5 per cent $CHCl_3\ COOH$, solubility in the presence of high concentrations of ethanol or acetone, weak affinity for anion and cation exchange resins, stability of the biological activity to partial hydrolysis by HCl or pepsin, and disappearance of the biological activity following partial hydrolysis by NaOH or trypsin. Inactivation by trypsin proves the presence of peptide bonds in the hormone molecule.

Inferior Vena Cava Blockade: A Method for Studying Circulatory Dynamics. MARVIN A. SACKNER, TRUMAN G. SCHNABEL, JR.,* MARY B. ALLAN and DAVID H. LEWIS, Philadelphia, Pa.

Reduction in venous return by vasodilators, venesection, tilting, or tourniquets has been utilized to investigate cardiovascular function in man. Obstruction of the vena cava (Farber and Eichna) offers certain advantages over

these methods: it is mechanical, its application is not detected by the subject, and it is associated with minimal changes in pulse rate and oxygen consumption. In 7 normals and 13 patients with heart disease, a balloon catheter was inflated in the inferior vena cava just below the hepatic vein. Inflation was done gradually (10 to 20 minutes) and usually until the balloon was not freely movable. Pressure measurements and Fick outputs were done before, during, and after caval obstruction. In normals, maximum inflation produced an average rise of 14 mm Hg in caval pressure. Pulmonary artery pressure (PAP) and cardiac index fell an average of 30 per cent. Right ventricular filling pressure (RVd) fell an average of 3 mm Hg, with a 50 per cent decline in right ventricular stroke work index (RVSWI). Both systemic (SVR) and pulmonary resistance (PVR) rose slightly. Fifteen minutes after complete deflation RVd had risen to 2 mm Hg above control with a return of RVSWI to only 80 per cent of control. In patients with heart disease, inflation and deflation caused similar alterations in these parameters. In 5 of this group, LVSWI and LVd changed in the same manner as RVSWI and RVd. Elevated PVR secondary to mitral stenosis decreased with inflation. In 2 patients with atrial septal defects, RVd and PAP fell as in the others. However, pulmonary blood flow more than doubled and PVR fell considerably. As a result, these patients showed an increased RVSWI in the face of a decreased RVd.

Inferior vena cava blockade offers a safe and promising method for studying the determinants of cardiac performance and the pathophysiology of cardiac shunts and pulmonary vascular reactivity.

Chromosome Constitution in Human Gonadal Disorders. AVERY A. SANDBERG,* THEODORE S. HAUSCHKA, EDWIN GORDY and GEORGE F. KOEPF, Buffalo, N. Y.

The chromosomal patterns (number, morphology and frequency of chromosomal abnormalities) have been examined in tissue obtained from bone marrow aspirations of a large group of patients with gonadal disorders, especially in those in whom the somatic sex chromatin (as determined from buccal mucosa and blood smears) did not coincide with the "apparent" external sexual characteristics of these individuals. The important findings would appear to be:

1) Subjects with a negative sex chromatin pattern in the buccal mucosa smear and with ovarian dysgenesis do not necessarily have 45 chromosomes (XO) as reported by others. We have shown that in two subjects with the classical syndrome of ovarian dysgenesis the chromosomal constitution is about half 46 and half 45, the former having two X-chromosomes. In one case of Turner's syndrome the entire marrow cell population had the usual diploid number of 46, with two X-chromosomes. In two subjects with Klinefelter's syndrome the preponderant number of metaphases contained 46, with only one X-chromosome being present (instead of the expected XXY condition). It should be pointed out, however, that 8 subjects with ovarian dysgenesis and 6 with Klinefelter's syndrome studied by us have had 45 and 47 chromosomes, respectively, as described by other workers.

2) Heteropycnosis and allocycly of the X-chromosome, not previously described, have been shown to exist in human subjects, most clearly in those with Turner's syndrome. In some subjects with ovarian dysgenesis, especially those with a chromosomal population of 46, differences in size, staining intensity and pronounced allocycly in the replication rate of the X-chromosome have become apparent.

3) In one of two subjects with precocious puberty (menses and breast development since birth) an abnormal number of chromosomes (47-48) has been demonstrated. A normal diploid number of 46 with an XY constitution has been demonstrated in a subject with intersex.

Role of Immunity in the Genesis of Experimental Chronic Pyelonephritis. JAY P. SANFORD, BETTY W. HUNTER, ROBERT E. WINDOM and JANET C. HOGAN, Dallas, Tex. (introduced by Ralph Tompsett).

Factors influencing persistence of pyelonephritis have been extensively investigated. However, immune mechanisms which may determine healing have not been defined. Immunity in pyelonephritis is of critical importance, inasmuch as it may be a major determinant of the repetitive attacks which influence clinical progression.

These studies were undertaken to ascertain the role of immunity in establishment of experimental chronic pyelonephritis. Pyelonephritis was produced in rats by intravascular injections of *E. coli* (0-111: B-4) and renal massage. While this procedure uniformly resulted in acute pyelonephritis demonstrable 2 weeks following a single injection ($>$ 100,000 colonies per g of kidney), kidneys of rats subjected to repetitive injections showed no evidence of active infection 2 weeks following the fourth injection ($<$ 1000 colonies per g). Such rats had antibodies against the 0 antigen (1:256 to 1:4096). These observations suggested that immunity resulting from acute pyelonephritis protected against reinfection by the original organism. To test this hypothesis, rats were actively immunized by *1*) producing acute pyelonephritis, *2*) i.v. injection of viable *E. coli* 0-111: B-4 without renal massage, *3*) injection of 0-111 antigen in Freund's adjuvant and were passively immunized with rabbit *E. coli* 0-111 antiserum. Antibodies against the 0-111 antigen conferred virtually complete resistance to induction of homotypic *E. coli* pyelonephritis though resistance was not conferred against infection with Klebsiella type C and three heterotypic strains of *E. coli*.

In contrast, induction of acute pyelonephritis with a strain of Klebsiella failed to evoke vigorous antibody responses. The pyelonephritic process in these animals was more persistent ($>$ 2000 colonies per g at 10 weeks) than that observed with strains of *E. coli* which evoked vigorous immune responses.

In summary, the importance of immunity as a determinant in experimental pyelonephritis has been shown both by prevention of acute hematogenous pyelonephritis

in immune rats and by persistence of infection in rats not eliciting vigorous immune responses.

The Effect of Changes in Atrial Systole on the Relation Between Mean Atrial Pressure and Stroke Work. STANLEY J. SARNOFF,* JERE H. MITCHELL and JOSEPH P. GILMORE, Bethesda, Md.

Experiments were designed to further clarify the relation between mean atrial pressure and stroke work. To this end, the relation between mean atrial pressure and ventricular end diastolic pressure was studied at constant heart rate. Continuous measurements were made of venous, right and left atrial, pulmonary artery, left ventricular and aortic pressures, cardiac output and heart rate, before and during stimulation of cardiac sympathetic and parasympathetic nerves. It was observed that: *1*) sympathetic stimulation augments and vagal stimulation diminishes the vigor of atrial systole; *2*) at any given stroke volume, the level of mean atrial pressure in relation to ventricular end diastolic pressure and ventricular segment length is lowered by sympathetic stimulation and elevated by vagal stimulation; *3*) the higher the stroke volume, the greater were the changes that could be induced by sympathetic and vagal stimulation. It was also found that high heart rates, per se, elevated mean atrial pressure in relation to ventricular end diastolic pressure as well as to stroke work and stroke volume. These data show that the relation between mean atrial pressure and stroke work is determined by the performance characteristics of the atrium as well as the ventricle, whereas the relation between ventricular end diastolic pressure and stroke work is determined only by the performance characteristics of the ventricle. Simultaneously observed changes in venous pressure will be discussed.

Quantitative Studies of a Prolonged Alteration of Serum Cholesterol Induced by Desoxyribonucleic Acid. J. PHILIP SAVITSKY, New York, N. Y. (introduced by Robert Bloch).

It has been previously reported that a single injection of 1 mg of purified, undenatured desoxyribonucleic acids (DNA) produced a significant decline in the serum lipids lasting at least 6 months. The lowered mean serum cholesterol reflected the decline (50 to 70 per cent) in cholesterol levels of those animals with high normal values. DNA preparations from homologous and heterologous mammalian tissues were equally effective. The present study describes the effect in rabbits over 1.5 years of a single injection of varying amounts of heterologous DNA. Groups of 10 normal rabbits received a single injection intravenously of heterologous DNA in the following amounts: 50 μg, 200 μg, 500 μg, 1 mg and 2.5 mg. Serum cholesterol determinations were made prior to the injections and at monthly intervals thereafter. DNA-injected and saline-injected control groups were indistinguishable in nutrition, weight gain and general health.

At the end of 1.5 years, the mean serum cholesterol of each DNA-injected group was significantly (30 to 40 per cent) below control levels. The variation of the cholesterol levels as measured by the standard deviation and coefficient of variation had decreased to one-third control values. The time required for the changes to appear varied with the amount of DNA injected. The animals receiving 2.5 mg changed inside of 2 months, those receiving 500 μg in 5 months, and those receiving 50 μg required 10 months. There was no return to control levels at any time after the serum cholesterol had declined significantly. The serum phospholipid and neutral fat showed similar changes, paralleling the cholesterol.

A single injection, therefore, of minute quantities of pure, undenatured heterologous DNA decreased the serum cholesterol and maintained a significantly lowered serum cholesterol for at least 1.5 years in the rabbit. Considering the known properties of biologically active DNA, the prolonged alteration may be a permanent effect.

Vitamin D and the Active Transport of Calcium. DAVID SCHACHTER, EUGENE B. DOWDLE, HARRIS SCHENKER and DANIEL V. KIMBERG, New York, N. Y. (introduced by John V. Taggart).

The small intestine of several mammalian species can transport calcium against concentration gradients, from fluid bathing the mucosal surface to fluid bathing the serosa. The mechanism was studied with everted gut-sacs and with slices of intestine incubated *in vitro*. The active transfer is relatively specific for Ca^{++}, is limited in capacity, dependent on oxidative phosphorylation, and in several species it is maximally active in the proximal duodenum.

Adaptive changes in the active transport appear to explain the facultative nature of calcium absorption *in vivo*. The active transfer is maximal in younger, growing rats and decreases in older rats. Intestine from pregnant rats transfers calcium more readily than that from nonpregnant controls. Maintenance on a low calcium diet enhances the active transport.

After 4 to 6 weeks on a vitamin D-free diet the active transport is severely impaired, but can be restored by feeding rats vitamin D or AT-10, or by ultraviolet irradiation of the animal. Restoration of the mechanism occurs in 1 to 4 hours following 50,000 IU of calciferol orally, or in 48 hours following 1 to 20 IU. The effect of vitamin D is maximal in respiring, proximal duodenum. The vitamin increases the maximal transport capacity for calcium. Thus, its principal action appears to be on the active transport mechanism rather than on the permeability to calcium of the mucosa.

Lesions in Rats Caused by Serotonin and Histamine Before and After Amine Oxidase Inactivation and Serotonin Inhibition. ARTHUR L. SCHERBEL, ROY L. MCKITTRICK and WILLIAM A. HAWK, Cleveland, Ohio (introduced by A. Carlton Ernstene).

Serotonin and histamine possess multiple pharmacological actions. They are believed necessary for central nervous system function and may produce profound alterations in vascular tone and permeability. Their effect on connective tissue has not been completely defined.

Three hundred ten rats were studied from 1 to 4 weeks while serotonin or histamine, 1 to 30 mg daily, was administered subcutaneously. Connective tissue was studied by the Ivalon sponge implantation technic. When serotonin was administered, a wasting disease resembling the runting syndrome developed. Necropsies were performed weekly and the findings were compared with those in the controls. Gross lesions included renal ischemia, gastric ulcers, enterocolitis, focal areas of submucosal hemorrhage, cardiac dilatation, and splenic infarcts. Microscopic lesions included renal tubular necrosis, myocarditis, focal areas of hepatic necrosis, and occasionally vasculitis. None of these lesions appeared in histamine-treated or in control animals. Serotonin increased inflammation in sponges but did not alter fibroplasia from that seen in controls; heavily granulated mast cells were present. Histamine significantly increased fibroplasia, but inflammation was only slightly increased; mast cells were degranulated. Monoamine oxidase inhibitors stimulated fibroplasia in the sponges. The effect of serotonin was greatly potentiated when animals were pretreated with an amine oxidase inhibitor and it was completely blocked when animals were pretreated with a serotonin antagonist (1-methylmethergine-tartrate, coded UML 491).

It is concluded that inflammation, alterations in fibroplasia and in mast-cell granulation, localized vasospasm and vasodilatation; and increased vascular permeability resulting in ulceration may occur from the administration of these biogenic amines. The lesions are increased when an amine oxidase inhibitor is administered simultaneously, and conversely, they are prevented if a serotonin antagonist is administered simultaneously.

A Sensitive Test for the Detection of Coagulation Abnormalities in Hyperlipemia. JOHANN SCHMIDT and GEORGE O. CLIFFORD, Detroit, Mich. (introduced by Richard J. Bing).

A study was done to investigate the mechanism of coagulation abnormalities possibly induced by lipemia. The thromboplastin generation test, modified by dilution of absorbed plasma 1 : 10 and serum 1 : 40, exhibits markedly accelerated evolution of thromboplastin when lipemia is present. Dilution weakens the system and increases its sensitivity.

Ten normal subjects were studied fasting and at the peak of lipemia following 70 g of butter. Four hyperlipemic patients (1 diabetic, 1 biliary cirrhotic, 2 essential) were studied fasting. At the height of lipemia in normals, the diluted thromboplastin generation test consistently revealed 50 to 100 per cent increase of thromboplastic activity during the first 5 minutes of incubation, while the standard thromboplastin generation test was only slightly accelerated. In the hyperlipemic patients, activity of the diluted thromboplastin generation test was proportional to elevation of serum lipids. Shortening of the Stypven time was the most constant additional finding, with platelet-poor lipemic plasma as active as fasting platelet-rich plasma. Other coagulation tests were variable and inconsistent. Total lipids, optical density and phospholipid phosphorus rose with lipemia.

The diluted thromboplastin generation test is a sensitive tool for study of the effects of lipemia on early phases of coagulation.

The Induction of Heterologous Immunity to Influenza by Aerosol Administration of Inactivated Virus. JEROME L. SCHULMAN and EDWIN D. KILBOURNE,* New York, N. Y.

Influenza viruses may be so treated by the application of heat or ultraviolet or ionizing radiation that their capacity to multiply is inactivated yet their ability to inhibit the multiplication of infective virus is preserved. The cellular resistance induced by inactivated viruses (viral interference) has been demonstrated in *in vitro* cell cultures and in the allantoic sac of the chick embryo. Such topical immunity is heterologous in that antigenically dissimilar influenza viruses may interfere with the multiplication of one another.

The virtual restriction of influenza virus infection to cells lining the mammalian respiratory tract presents a situation peculiarly amenable to the induction of topical immunity with nonreplicating virus. When CFW mice received by aerosol an estimated 250 H A units of concentrated, partially purified suspensions of influenza B (Lee) or influenza A (CAM) viruses inactivated with ultraviolet light, immunity to heterologous viral challenge 24 hours later (also by aerosol) was demonstrated. This "immunity" results in 1 to 4 log reductions in pulmonary virus (as determined by infectivity titrations of individual mouse lungs) and in the prevention or reduction of pulmonary lesions. The concentrations of inactivated virus employed have induced no manifest harmful effects.

The use of antigenically heterologous viruses in these experiments precludes the participation of specific antibody in the immunity induced. The relative nonspecificity of the immunity of "interference" has special potential significance in influenza because strain-specific immunity may be circumvented by antigenic change in this highly mutagenic virus. Furthermore, interference which modifies but does not completely inhibit infection may result not only in immediate nonspecific resistance, but also in lasting strain-specific immunity following the challenge infection so modified.

Relation of Hormone-Receptor Bonding and Antidiuretic Action of Vasopressin. IRVING L. SCHWARTZ,* HOWARD RASMUSSEN, MARY ANNE SCHOESSLER, CONRAD T. O. FONG and LAWRENCE SILVER, Upton, N. Y.

An *in vitro* system has been used to study some aspects of the interaction of an antidiuretic hormone, arginine vasopressin, and a target organ, the toad bladder.

Bladders from *Bufo marinus* were tied as sacs onto glass tubes and filled from within with Ringer's solution diluted with water or concentrated above isotonicity with mannitol. Then the assembly was placed in a bath of isotonic Ringer's solution and weighed periodically. Net water transfer assumed the direction of the osmotic gradient and

increased 20- to 30-fold above control values when neurohypophyseal hormones were added to the outside bath. No difference was encountered in potency or in other parameters of hormone action when the effect of arginine vasopressin (AVP) was compared with that of tritium-labeled AVP (specific activity, 400 μc per mg).

When the bladders were pretreated with reagents which bind SH groups (N-ethylmaleimide, 10^{-3} M; *p*-chloromercuribenzoate, 10^{-3} M; CH_3HgBr, 10^{-5} M), the action of AVP was completely inhibited, although a variety of nonthiol-binding metabolic inhibitors did not block hormone action. In other experiments in which bladders were exposed to H^3-AVP and freed of electrovalently-bonded radioactivity, 60 to 90 per cent of the hormone appeared to be attached to the bladders through disulphide linkage as determined by the removal of radioactivity under mild conditions specific for the reduction of disulphide bonds (L-cysteine, 10^{-1} M, pH 8). However, pretreatment with SH binding reagents prior to exposure to H^3-AVP greatly decreased the amount of hormone attached to the bladder through -S-S- linkage.

These findings, in conjunction with concordant data reported previously for the rat kidney, support the hypothesis that the mechanism of action of autidiuretic hormone on its receptor involves a thiol-disulphide interchange reaction.

Continuous Hemodialysis as a Method of Preventing Uremia in Acute Renal Failure. BELDING H. SCRIBNER,* RACHIT BURI and JOHN E. Z. CANER, Seattle, Wash.

A number of investigators now believe that critically ill patients who develop acute renal failure should not be allowed to develop the uremic syndrome. Preventing the uremic syndrome often requires frequent, even daily, hemodialyses, and this large task led us to attempt continuous hemodialysis. To date 14 patients have been treated by continuous hemodialysis for a total of 48 patient-days. One patient was treated 14 days. A Skeggs-Leonards dialyzer with 6 layers arranged in parallel was used because of its low resistance and adequate clearance. Its low volume obviated priming with blood. The external circuit was cooled to near 0° C to minimize clotting, platelet consumption, and bacterial growth. Preliminary experience demonstrated that a blood flow of only 30 ml per minute permitted removal of 15 to 30 g of nitrogen per 24 hours with the BUN stabilized at or below 100 mg per 100 ml. All fluid and electrolyte imbalances were readily corrected. At this low blood flow, a constant infusion of heparin into the arterial cannula at about 6 mg per hour usually prevented clotting in the extracorporeal circuit while producing little, if any, prolongation of the patient's clotting time. No blood pump was used. The 300 L dialysate tank was changed no more often than once a day. These features resulted in a simplified technique that was monitored by the special nurse on the case. No serious complications attributable to the procedure have been encountered.

Continuous hemodialysis appears to be a safe and practical alternative to repeated intermittent dialysis and has certain advantages. Since no blood priming is required, blood consumption is greatly reduced. Regional heparinization reduces the danger of hemorrhage. Continuous adjustment of electrolyte balance prevents the rapid and often dangerous shifts that can take place with intermittent dialysis. Continuous nitrogen removal prevents recurrences of uremia even in highly catabolic patients.

The Metabolism of Variously C^{14}-Labeled Glucose and an Estimation of the Extent of Glucose Metabolism by the Hexosemonophosphate Shunt Pathway in Man. STANTON SEGAL, MONES BERMAN and ALBERTA BLAIR, Bethesda, Md. (introduced by Donald S. Fredrickson).

Previous studies of glucose metabolism in man have employed uniformly C^{14}-labeled glucose. Because the individual carbon atoms of glucose have different metabolic fates in the known pathways of glucose catabolism, further information may be obtained by studying the metabolic fate of C^{14} derived from glucose labeled in a single carbon atom.

Glucose-1-C^{14} and glucose-6-C^{14} were administered i.v. to 4 normal subjects. In 2 of these subjects, experiments with glucose-2-C^{14} and glucose-U-C^{14} were also performed. Expired $C^{14}O_2$ was collected for 5 hours. Marked differences have been observed in the $C^{14}O_2$ excretion curves derived from metabolism of each of the sugars. A characteristic time lag was seen for $C^{14}O_2$ excretion from glucose-6-C^{14}, and the C^{14} yield was smaller than from glucose-1-C^{14}. From blood collected at intervals up to 3.5 hours glucose was isolated as gluconate from 2 subjects who received glucose-1 and 6-C^{14} and degraded so that C-1 and C-6 were isolated individually. The turnover rate of these carbon atoms was identical. Moreover, up to 3 hours after injection little randomization of C^{14} in blood glucose occurred. Examination of C^{14} incorporation into plasma lipids revealed 40 per cent greater incorporation from glucose-6 than from glucose-1-C^{14}.

The $C^{14}O_2$ excretion and lipid C^{14} data indicate the operation of the hexosemonophosphate shunt pathway in man. A biological model system has been constructed based on a three-compartment glucose and a two-compartment bicarbonate system as well as on the reactions of the glucose pathways. Kinetic solution of the model has revealed that about 8 per cent of glucose is catabolized via the shunt pathway. Glucose metabolism for various endocrine states is now under study employing the above methods of analysis.

Effects of Glucose, Sulfonylureas and Other Hypoglycemic Agents on Insulin Activity in Pancreatic Venous Blood. HOLBROOKE S. SELTZER and WALTER L. SMITH, Dallas, Tex. (introduced by Ben Friedman).

This study compared respective insulin-secreting potencies of glucose and sulfonylureas, and investigated possible insulinogenesis by other hypoglycemia-inducing agents. Phlorizinized dogs were anesthetized, laparotomized and heparinized. Pancreatico-duodenal vein can-

nulation enabled continuous collection of effluent from the right pancreatic limb after acute injection of test agents by femoral vein. Insulin activity of four successive 10 minute aliquots (baseline, A, B, C) was determined by Vallance-Owen's method (rat diaphragm), and mean flow rates were expressed in microunits effective insulin concentration per minute.

Control substances did not increase pancreatic venous insulin activity. Saline: baseline = 42; postinjection = 0. Sulfadiazine (50 mg per kg): baseline = 27; A = 29; B = 0. Glucose (15 g) elicited immediate, maximal, sustained outpouring of insulin activity: baseline = 59; A = 3,530 ($p < 0.001$); B = > 5,000 ($p < 0.001$); C = > 5,000 ($p < 0.001$).

Sulfonylureas increased secretory rate promptly but only transiently. Tolbutamide (50 mg per kg): baseline = 44; A = 785 ($p < 0.02$); B = 713 ($p < 0.05$); C = 68. Chlorpropamide (50 mg per kg): baseline = 66; A = 988 ($p < 0.02$); B = 1,045 ($p < 0.025$); C = 654 ($p < 0.05$). Metahexamide (10 mg per kg): baseline = 84; A = 937 ($p < 0.01$); B = 1,350 ($p < 0.02$); C = 282 ($p < 0.05$).

Pancreatic venous insulin activity remained unchanged after injection of sodium salicylate (50 mg per kg); indole-3-acetic acid (50 mg per kg); L-leucine (50 mg per kg); and "Tris" buffer or THAM (0.3 M).

The data indicate that *1*) both glucose and sulfonylureas cause prompt insulin release; *2*) the greater potency of hyperglycemia suggests that it may actually stimulate new insulin formation, while sulfonylureas may only release preformed insulin; *3*) salicylates, indole-3-acetic acid, L-leucine and "Tris" buffer produce hypoglycemia via extrapancreatic mechanisms.

Effect of Hypotensive Drugs on the Pressor Response to Noxious Stimuli. ALVIN P. SHAPIRO, Pittsburgh, Pa. (introduced by I. Arthur Mirsky).

In spite of numerous clinical assays of hypotensive drugs in therapy, little attention has been given to the effect of their chronic administration in man on responsivity to pressor stimuli. Therefore, during studies of the variables influencing the magnitude and quality of pressor responses to different psychophysiological stimuli, changes in a group of 30 hypertensive patients receiving rauwolfia, chlorothiazide, and mecamylamine in the usual doses, singly or in combinations, were compared with those in 40 untreated patients.

An automatic indirect recorder which permits unattended determinations of blood pressure at 2-minute intervals was used. Results of three tests, designed to be simple, readily reproducible, and to contain minimal "doctor-patient interaction," were evaluated: *1*) maximum rise in blood pressure after the investigator entered room of the resting patient and injected 10 ml of normal saline intravenously; *2*) rise during a modified cold pressor test; *3*) rise during performance of a difficult "color reading" task. Most subjects received all three tests.

Pretest blood pressures in the treatment group were: 178/103, 183/105, and 187/106 for the three tests, respectively, in contrast to 164/101, 172/105, and 178/108 mm Hg in the untreated group. Responses of 33/15, 40/29, and 26/7, on the three tests, respectively, in the treated patients, contrasted with responses of 27/13, 32/26, and 17/6 mm Hg in the untreated group. Apparent differences were statistically significant only between initial systolic pressures in Test 1 ($p = 0.05$).

Two explanations for failure of drugs to depress responses are suggested: *1*) dose was insufficient and the apparently satisfactory management resulted primarily from placebo effects; *2*) during chronic adminstration, the drugs, by sympatholytic effects, may enhance responsiveness to humoral components of the pressor response, as acute studies in animals and man suggest. To further elucidate these observations, paired studies in the same patient before and during treatment are indicated.

Type 2 Poliovirus Infection and Disease in Panama City. ALEXIS I. SHELOKOV, JOHN CRAIGHEAD and JACOB BRODY, Balboa Heights, Canal Zone (introduced by Joseph E. Smadel).

Outbreaks of paralytic poliomyelitis caused by Type 2 strains are uncommon, but such occurred in Panama City in late 1959. Data from an enterovirus survey on children under 3 years of age in Panama City during the half year preceding the outbreak and following it were of particular interest. Thirty to 35 rectal swabs obtained from pediatric clinic patients every 2 weeks were tested for presence of poliovirus.

Two of the clinic children had intestinal infection with Type 2 poliovirus, one in June and the other in September. Thus, only two polioviruses were found in the 292 children examined during the 4 months. The number of polio isolates, all Type 2, increased progressively, i.e., October, 2; November, 7; December, 8; then dropped to 4 in January. During the peak of the spread of virus in the community, about 11 per cent (8/71) of the children examined were shedding Type 2.

Paralytic polio appeared in Panama City in October with 1 case reported, in November there were 2 cases, in December there were 7, and in January there was a drop to 4. Eleven of the 14 paralytic patients studied yielded Type 2 virus; no poliovirus was isolated from 3 cases.

The rough correlation found between the appearance of Type 2 virus in the community, its rapid spread and decline, and the increase and decrease in paralytic disease in the inhabitants is in accord with other epidemiologic aspects of this disease, particularly the fact that paralysis occurs in only a small proportion of infections.

A New Syndrome of Post-Transfusion Immunologic Purpura. N. RAPHAEL SHULMAN, RICHARD H. ASTER, ALFRED LEITNER and MERILYN C. HILLER, Bethesda, Md. (introduced by James A. Shannon).

Two patients who developed fulminant thrombocytopenic purpura with abundant megakaryocytes 1 week after

blood transfusion had a circulating antibody which reacted with platelets of normal individuals to cause complement fixation, agglutination and inhibition of clot retraction. One patient was treated successfully with exchange transfusion, the other recovered spontaneously. Platelets obtained from both patients after recovery did not react with the antibody of either patient, yet no blocking agent was detectable on their platelets. Rather it was found by using platelets obtained from 40 relatives and 90 other individuals that there are 2 antigenically different platelet types unrelated to erythrocyte groups. The most common phenotype (genotypes $Pl^{A}Pl^{A}$ in approximately 74 per cent of the population and $Pl^{A}Pl^{B}$ in 24 per cent) reacts with the antibody, and the rarer patients' phenotype (genotype $Pl^{B}Pl^{B}$ in 2 per cent) does not. Pl^{B} is an autosomal recessive. Immunization studies in animals suggest that $Pl^{B}Pl^{B}$ platelets simply lack the Pl^{A} antigen.

The described syndrome is unrelated to classical idiopathic thrombocytopenic purpura (ITP), for 5 consecutive cases of ITP tested had Pl^{A} platelets. Although approximately 2 per cent of all transfusions involve a $Pl^{B}Pl^{B}$ individual receiving Pl^{A} platelets, immunization does not occur with the expected frequency. Moreover, one patient received 15 transfusions subsequent to the initial immunization without stimulating further antibody formation, and in both patients recovery took place in the presence of a significant antibody titer. This suggests that the donor's blood which provokes the syndrome must be unique. It may contain an unusual amount or form of Pl^{A} antigen which both immunizes a $Pl^{B}Pl^{B}$ recipient and coats $Pl^{B}Pl^{B}$ platelets to render them susceptible to the antibody.

The Enzymatic Defect of Orotic Aciduria. LLOYD H. SMITH, JR. and CHARLES M. HUGULEY, JR., Boston, Mass. and Atlanta, Ga. (introduced by Anne P. Forbes).

The propositus and thus far the only reported patient with orotic aciduria was a child with refractory megaloblastic anemia associated with large amounts of orotic acid in the urine. A hematologic remission and reduction in orotic acid excretion followed the administration of mixed pyrimidine nucleotides. It was suggested that the patient had an enzymatic block in the further metabolism of orotic acid. The patient died several years ago of varicella.

Assay procedures have been developed for orotidylic pyrophosphorylase (O5P'ase) and orotidylic decarboxylase (O5P-decarboxylase), sequential enzymes which convert orotic acid to uridine-5'-phosphate. These enzymes can be measured in circulating erythrocytes and leukocytes, but are absent from serum. A heterozygous defect in both O5P'ase and O5P-decarboxylase was demonstrated in erythrocytes from both parents and two siblings of the propositus. A third sibling demonstrated no enzymatic abnormality. Levels of erythrocyte aspartate carbamyltransferase and dihydroorotase, enzymes prior to orotic acid formation, were normal in all subjects. An additional asymptomatic carrier of the orotic aciduric trait has been discovered in control studies. Preliminary studies indicate that the enzymatic defects can also be demonstrated in saliva. Orotic aciduria can be produced experimentally in animals with azauracil, which inhibits O5P-decarboxlase. Orotic aciduria represents a genetic block in O5P'ase and O5P-decarboxylase, which seems to be transmitted in the pattern of a Mendelian recessive trait. Which, if either, enzymatic defect has primacy has not been established. The finding of an additional carrier in a small control study suggests that the trait may not be uncommon, but that the homozygous state is generally lethal.

Qualitative Differences in the Immune Response of Infants and Adults Receiving Salmonella Vaccines. RICHARD T. SMITH,* DONALD V. EITZMAN and BILLIEVELYN MILLER, Gainesville, Fla.

Previous observations showed that neonatal infants without passively acquired agglutinins produce "H" agglutinins in high titer after immunization with Salmonella vaccines. This study describes the characterization of this agglutinin and comparison of its properties with agglutinins which appear in the serum of older children and adults after a comparable immunological stimulus.

Salmonella vaccines and diagnostic antigens were prepared from strains having a single monophasic flagellar antigen. The antigens for immunization were selected as those for which no detectable flagellar or somatic agglutinins were present in a preimmunization specimen. Serial bleedings were examined by tube agglutination, and for specificity, heat-stability, electrophoretic mobility, sedimentation characteristics, and the effect of thiol reagents on the agglutinin.

The infants examined were found to produce for the first 2 to 4 months of life only "H" agglutinins, which were in each instance heat-stable, highly specific, γ_1, 19 to 20 S globulins. The agglutinin activity was intact after treatment with 2-mercaptethanol, but in smaller active fragments (approximately 7 S). In older infants this neonatal type of antibody was found during the first days of the response, but soon thereafter a 7 S, γ_2, heat-stable globulin antibody-resisting thiol reagent also appeared.

Young adults were found to produce early in the immune response a γ_1, heat-stable, macroglobulin, which variably lost agglutinin activity when treated with thiol reagent. After 7 days agglutinins of lower sedimentation coefficient, probably 7 S appeared. "Naturally" occurring Salmonella agglutinins in adults were found to be of the thiol labile macroglobulin type, as has been previously reported.

These data suggest that the neonatal infant differs qualitatively in his immune response to this stimulus from the mature individual.

Reticuloendothelial Clearance of Blood Thromboplastin in Rats. THEODORE H. SPAET,* HERBERT I. HOROWITZ and DOROTHEA ZUCKER-FRANKLIN, New York, N. Y.

The hypothesis has been proposed that blood clotting intermediates are cleared during *in vivo* circulation, and that this clearance is a major factor in the preservation of blood fluidity. The present study concerns the removal of blood thromboplastin.

Experimental animals were 250 g Sprague-Dawley rats, and thromboplastic reagents used were of rat origin. Reagents were: rat blood thromboplastin (RBT), a thromboplastin generation mixture prepared in the usual manner; and sedimented rat blood thromboplastin (SRBT), similar to product II of Bergsagel and Hougie except that activity was sedimented on crude "cephalin" instead of platelets. RBT given into the jugular vein or aorta caused a marked increase in the prothrombin time and drop in fibrinogen, but injection into the mesenteric vein had a greatly reduced effect. The protective effect of mesenteric injection was partially reversed by previous reticuloendothelial blockade with Pelikan Ink. Addition of I^{131}-labeled albumin to RBT showed that the injected material was not grossly trapped in the liver following mesenteric injection. Similar results were obtained when the RBT was prepared with crude "cephalin," aqueous platelet extract, or intact platelets. SRBT was labeled with I^{131}, injected into the jugular vein, and radioactivity was determined in various organs. About 75 per cent was found in the liver. With crude "cephalin" lacking thromboplastic activity only about 50 per cent was found in the liver. Injection of SRBT during a carbon clearance produced significant depression of carbon removal; the effect of crude "cephalin" without thromboplastic activity was considerably less.

The data indicate that blood thromboplastin as prepared above is cleared from the circulation as a "foreign" particle, by the reticuloendothelial system. Although these findings are in conformity with the original hypothesis, it remains to be determined what relationship the laboratory thromboplastin has to coagulant material that develops *in vivo* under physiological conditions.

Demonstration of Increased Proactivator in Induced Fibrinolytic States. NORTON SPRITZ, BURTON D. COHEN and ALBERT L. RUBIN, New York, N. Y. (introduced by Robert F. Watson).

The present concept of the fibrinolytic system is that plasminogen is converted to the fibrinolytic agent, plasmin, by a group of proteolytic substances (activators). Both plasmin and plasminogen activators possess lysine ester-splitting activity. Streptokinase lacks this activity, and its capacity to activate plasminogen results from its interaction with an inactive precursor (proactivator) producing an activator with esterase activity. It is controversial whether or not proactivator and plasminogen are separate substances.

Epinephrine and electroconvulsive shock (ECS) have been shown to produce increased euglobulin fibrinolytic activity, resulting from an increase of plasminogen activation. Lysinemethyl esterase activity of the euglobulin fraction was not increased by these procedures, although such activity is characteristic of plasminogen activators. Esterase activity was significantly increased, however, in postepinephrine and post-ECS specimens when streptokinase was added to the reaction mixture. Since streptokinase alone lacks this activity, these results indicate that euglobulin following epinephrine and ECS contains an increase in material activated by streptokinase, either proactivator or plasminogen. Further studies demonstrated that the material increased was not plasminogen. This material was assayed by conversion to plasmin and the activator used for this conversion removed either by acid precipitation or heat. Esterase activity of the activator-free plasmin was unchanged following epinephrine or ECS. It was consistent regardless of the activator used or the method of its removal, and was regularly less than that due to streptokinase when activator was not removed. These findings indicate that streptokinase-induced esterase is due to the conversion of both proactivator and plasminogen and that the former alone is increased by epinephrine and ECS. The findings also indicate the separate identities of these substances. The value of an artificial substrate, without clot lysis, in the measurement of these components of the fibrinolytic system is demonstrated.

Cholesterolemia, Hypertension and Coronary Disease in Negroes Compared with Whites. JEREMIAH STAMLER,* HOWARD A. LINDBERG, DAVID M. BERKSON, YOLANDA HALL and WILDA A. MILLER, Chicago, Ill.

It has been clearly demonstrated that hypertension is associated with a several-fold increase in the risk of coronary disease in middle-aged men. It has also been unequivocally established that prevalence rates of hypertension are grossly higher in Negroes than in whites in the United States. Nevertheless, available data indicate that the incidence of coronary heart disease is no greater, and may even be lower, in middle-aged Negro men than in white men. This apparent paradox stimulated an epidemiologic investigation on patterns of cholesterolemia —another major variable associated with susceptibility to coronary heart disease—in Negroes and whites aged 40 to 59 in Chicago. Population samples for study were obtained from the labor force of a Chicago industrial corporation (1,245 white males, 84 Negro males) and from a community survey in a residence area of lower income Negroes. Data were obtained on such variables as age, sex, race, height, weight, occupation, industry, employment status, place of birth and time of migration North, if born in the South. Mean serum cholesterol level in the white males was 239 mg per 100 ml; in the Negro men employed in the Chicago company, 227 mg per 100 ml; in the Negro men in the community, 207 mg per 100 ml. The white men in different socioeconomic subgroups had generally similar mean serum cholesterol values; for the Negro males, serum cholesterol levels were lower in the unemployed than in the employed, lower in the "blue collar" than in the "white collar" workers. These lower levels of serum cholesterol in middle-aged Negro men compared with those in white men may be significantly related to the comparative pat-

terns of coronary disease incidence rates in the two races.

The Effects of Acetylcholine on Pulmonary Circulation and Alveolar Gas Exchange. C. ALPHEUS STANFIELD, MILTON N. LURIA, JAMES K. FINLAYSON, FRANK W. LOVEJOY, JR. and PAUL N. YU, Rochester, N. Y. (introduced by Nolan L. Kaltreider).

Pulmonary blood flow, pulmonary vascular pressures, arterial blood gases, ventilation and pulmonary diffusing capacity for carbon monoxide were measured in 17 patients with rheumatic or congenital heart disease before and during the continuous infusion of acetylcholine into the pulmonary artery at rates of 2 to 5 mg per minute.

The changes in oxygen consumption, pulmonary blood flow, respiratory frequency and minute ventilation were inconsistent during acetylcholine infusion. Pulmonary artery mean pressure declined significantly in 6 of 9 patients with elevated control values, although pulmonary "capillary" mean pressure decreased in only 2 patients. No change in pulmonary vascular pressures was observed in patients with normal pulmonary hemodynamics. There was no significant change in pulmonary diffusing capacity for carbon monoxide.

A decline in arterial oxygen saturation which occurred in 8 patients was not necessarily accompanied by a significant fall in pulmonary artery pressure, although pulmonary vascular resistance usually decreased slightly. Inhalation of 100 per cent oxygen while acetylcholine infusion was continued resulted in an increment in arterial oxygen content of more than 2 vol per cent in each of the 8 patients.

Our observations confirm the reports by other workers that acetylcholine decreases pulmonary artery pressure without appreciable change in the rate of blood flow in some patients with pulmonary hypertension. The exact mechanism for this reduction is not clear but most likely the drug causes direct vasodilatation of the pulmonary arterioles.

The occurrence of arterial desaturation during acetylcholine infusion in a certain number of patients deserves special comment. Available evidence indicates that there is neither impairment of alveolar ventilation nor increase in direct pulmonary arteriovenous shunts. The most likely explanation for the arterial desaturation is an increased perfusion of blood through pre-existing hypoventilated areas.

Secretion In Vitro of Recently Absorbed Cholesterol and Absorption of Cholesterol In Vivo and In Vitro by the Rat Small Intestine. MALCOLM M. STANLEY,* C. EDWIN BALL and KENNETH R. JAEGERS, Louisville, Ky.

Fasting rats given free C^{14}-cholesterol by stomach tube were killed after 4 hours. Each small intestine was divided into 24 approximately equal segments. After eversion and washing, every third segment was fixed as a control and the others studied by Wilson's method (J. appl. Physiol. 1958, **12**, 145) using medium containing stable cholesterol (Treadwell, et al., Amer. J. Physiol. 1958, **193**, 34). One of each pair was incubated with O_2 (37° C); the other was refrigerated at 2° C with N_2. The medium was changed after 1 hour and incubation or refrigeration continued 3 hours longer in fresh medium. C^{14}, stable cholesterol contents of media and fixed tissue were determined. *In vitro,* while mucosa actively absorbed cholesterol from medium, there was transfer of recently absorbed (*in vivo*) cholesterol to medium from mucosa. Transfer from mucosa to medium averaged 3 per cent per hour for 37° C specimens; refrigerated anoxic specimens had less than half this rate. Transfer rate during the 3 hour second period was nearly as rapid as that during the first hour. In general, our data concerning absorption confirm those of Treadwell et al. Since segments could not transfer cholesterol through serosa, accumulation of sterol might favor secretion through mucosa. As an explanation desorption could not be excluded although it is unlikely in view of simultaneous active absorption. Cholesterol secretion by intestinal mucosa may be an important means by which body cholesterol content is regulated.

Alterations of Cardiopulmonary Physiology Following Acute Experimental Pulmonary Embolism. MYRON STEIN, CLAUDE E. FORKNER, JR., E. MARTIN SPENCER, EUGENE D. ROBIN and GEORGE S. KURLAND, Boston, Mass. and Pittsburgh, Pa. (introduced by A. Stone Freedberg).

Many discrepancies exist between the physiologic and pathologic findings in acute experimental pulmonary embolism. Using the technique of serum-induced thrombosis for the production of emboli, multiple parameters of pulmonary and cardiac function were measured before and after pulmonary embolization in 32 anesthetized dogs. Massive embolization resulted in a prompt decrease in cardiac output and a fall in arterial O_2 saturation; arterial O_2 content did not rise to control levels after ventilation with 100 per cent O_2 for 20 minutes. One to three hours after embolization, arterial O_2 content following 100 per cent O_2 for 20 minutes returned to control values. These physiologic alterations occurred only in animals in which more than 50 per cent of the main lobar arteries were obstructed by emboli as determined at necropsy. In 16 dogs, dissemination of similar total quantities of emboli to a single lobar artery or diffuse seeding to smaller peripheral pulmonary arteries of several lobes was not associated with a fall in arterial oxygen saturation or a decrease in cardiac output. In both groups, acute pulmonary embolization resulted in an increase in rate, depth and minute volume of ventilation, a disproportionate increase in dead space ventilation, and an arterial-alveolar CO_2 tension gradient. These data, obtained with emboli of the animal's own blood suggest that the physiologic abnormalities induced depend not only on the quantity of emboli but also on the specific pattern of the embolic distribution. The decreased arterial O_2 content breathing 100 per cent O_2 is consistent with the development of a right to left shunt. Its rapid reversal reflects the anesthetized animal's capacity to

compensate for an acutely-induced abnormality in pulmonary perfusion.

Hepatic and Systemic Blood Flow in Man during Intravenous Infusion of Ethanol. SAMUEL W. STEIN, WALTER H. ABELMANN,* CHARLES S. LIEBER, CARROLL M. LEEVY and GILBERT R. CHERRICK, Boston, Mass.

There is conflicting evidence concerning the effect of ethanol on the hepatic circulation. In the present study hepatic blood flow and cardiac output were measured simultaneously in patients without liver disease. Cardiac output was measured by indicator dilution, using Evans blue. Hepatic flow was determined by the Fick principle with constant infusion of indocyanine green. After one hour of saline, intravenous infusion was maintained unchanged for a second hour in some patients and was changed to ethanol in saline at the rate of 0.6 to 0.7 g per minute in a second group.

In 6 patients mean hepatic flow during the first hour of saline infusion was 798 ml per minute per m^2, with a range of 450 to 1,120 ml per minute per m^2. Hepatic flow averaged 24.1 per cent of cardiac output, with a range of 21.5 to 26.4 per cent. In 2 of the 3 patients in whom ethanol was not employed, both hepatic and systemic flow in the second hour changed less than 10 per cent from initial values, leaving the ratio of hepatic to systemic flow unaffected. In the third, hepatic flow increased by 47 per cent and total systemic flow by 28 per cent. This was the only patient who demonstrated a change in the hepatic fraction of systemic flow, from 26.4 to 30.2 per cent.

During infusion of ethanol in 3 patients mean hepatic flow increased by 43 per cent, to 1,127 ml per minute per m^2. Simultaneously determined cardiac output, however, increased by 48 per cent. Thus, the fraction of total systemic flow going to the liver remained essentially unchanged at 23.8 per cent.

Under the conditions of study the hepatic fraction of cardiac output remained remarkably constant. Since infusion of ethanol did not affect arterial blood pressure, the data indicate that it decreased both splanchnic and extrasplanchnic vascular resistance proportionately. There was no evidence for a specific effect on the hepatic circulation.

The Mechanism of Action of MER-29. DANIEL STEINBERG, JOEL AVIGAN and EUGENE B. FEIGELSON, Bethesda, Md. (introduced by Jack Orloff).

MER-29 (1-[*p*-β-diethylaminoethoxy)-phenyl]-1-(*p*-tolyl)-2-(*p*-chlorophenyl) ethanol) has been shown by Blohm and MacKenzie to be a potent inhibitor of cholesterol biosynthesis in animals and to lower serum and tissue levels of cholesterol. Studies in this laboratory, both in animals and in man, show that administration of the drug is accompanied by accumulation in the serum and in the red blood cells of large amounts of 24-dehydrocholesterol (desmosterol). In patients on a dosage of 100 mg per day, it was found that 24-dehydrocholesterol accumulated to a fairly constant level, accounting for 20 to 25 per cent of the circulating sterols in the serum and 17 per cent of the red blood cell sterols. Four hours after the injection of 2-C^{14}-mevalonic acid the specific radioactivity of the circulating 24-dehydrocholesterol was greater than the specific radioactivity of the circulating cholesterol. This, and similar data from animal studies, are compatible with a precursor-product relationship. This conclusion is supported by direct demonstration of the rapid conversion of C^{14}-labeled 24-dehydrocholesterol to cholesterol after intravenous injection into rats and after incubation with whole rat liver homogenates. The conversion is blocked by addition of MER-29 to the homogenate. It is concluded that the major site of action of MER-29 is in the final step in cholesterol biosynthesis, namely, in the reduction of 24-dehydrocholesterol to cholesterol.

The color yield of 24-dehydrocholesterol at 635 mμ in the Liebermann-Burchard reaction is approximately 60 per cent of the color yield of cholesterol. Consequently most standard methods give a falsely low value for the total serum sterol level. The importance of this in properly evaluating clinical results with MER-29 will be discussed in relation to a group of treated patients in whom levels of both cholesterol and 24-deyhdrocholesterol have been determined.

Free and Bound Thyroxine of Serum. KENNETH STERLING* and MILTON TABACHNICK, New York, N. Y.

Thyroxine in serum has been found to migrate with albumin, α-globulin and prealbumin on electrophoresis, as reported by others. The existence of a small moiety of "free" or unbound thyroxine has been previously postulated. In the present work, free thyroxine has been demonstrated by dialysis through cellophane. Tracer amounts of I^{131}-labeled thyroxine added to serum yielded radioactivity in the dialysate which was identified as thyroxine chromatographically. Equilibrium dialysis studies suggested an association constant between serum albumin and thyroxine of the order of 10^5. Experiments with serum on both sides of the cellophane membrane also indicated transfer of free thyroxine, as reported by Christensen. The sera of thyrotoxic patients exchanged labeled thyroxine faster, while sera of myxedematous patients exchanged the tracer more slowly than normal sera.

The uptake of added tracer thyroxine from serum by ion exchange resins varied with the state of thyroid function. With triiodothyronine as a tracer, the uptake by IRA-400, formate cycle, was adapted as a diagnostic test which could be used with sera despite iodine contamination.

Polycythemic Response to Erythropoietine Compared with a Short-Term Assay. FREDERICK STOHLMAN, JR.,* GEORGE BRECHER and ARCHIE A. MACKINNEY, Bethesda, Md.

The relationship between the dose of erythropoietine producing a positive response in the fasted rat and that necessary to induce polycythemia in the intact animal was studied. Sources of erythropoietine were rats exposed for 18 hours to a barometric pressure of 310 mm of Hg, rats with phenylhydrazine-induced anemia, and extracts of urine from a child with congenital erythrocytic hypoplasia (J. B. urine). In a short-term assay (starved rat), the iron incorporation in the fasted recipients of normal plasma was 5 to 8 per cent; that for recipients of high altitude plasma, 21 per cent; phenylhydrazine plasma, 26 per cent; J. B. urine, 33 per cent. A one to three dilution of high altitude plasma resulted in an iron incorporation of 10 per cent; a similar dilution of phenylhydrazine plasma, 13 per cent; and of J. B. urine, 24 per cent. When these substances were administered to normal rats over a 3 to 4 week period, the values for red cell mass were: normal plasma, 4.9 cc; altitude plasma, 5.2 cc; phenylhydrazine plasma, 5.7 cc; J. B. urine, 6.9 cc. Injection of a one to three dilution of J. B. urine resulted in average red cell mass of 4.9 cc. Thus an amount of erythropoietine capable of increasing the output of red cells in a starved animal failed to produce a sustained increase in red cell mass in the normal animal. However, if the amount of erythropoietine was substantially increased, polycythemia ensued. The failure of erythropoietine in lower doses to produce polycythemia indicates that some other regulant re-established homeostasis. This in turn supports our concept of a dual regulation of erythropoiesis.

Fluorescence Microscopy in Diseases of the Thyroid Gland. IRWIN L. STOLOFF, Philadelphia, Pa. (introduced by W. Paul Havens, Jr.).

Hemagglutinins, precipitins, and complement-fixing antibodies for antigens prepared from aqueous extractions of normal human thyroid glands have been found in the sera of patients with a variety of conditions due to or associated with injury of the thyroid. The possibility that the demonstration of affinity by fluorescence microscopy between normal human thyroid and substances found in the sera of patients with disease of the thyroid might be a better indication of the role of autoimmunity in diseases of the thyroid prompted the use of this technique in the following studies.

Sections (cryostat) of normal human thyroid were covered with the sera to be tested for 30 minutes; washed for 10 minutes with phosphate buffer (pH 7.3); covered with rabbit antihuman γ-globulin labeled with fluorescein isothiocyanate for 30 minutes; and washed in running tap water overnight. The sera of 5 normal persons and 10 patients with a variety of diseases of the thyroid were tested. Brilliant fluorescence was seen in the acinar cells and interstitial cells of the sections treated with the sera of 3 patients with Hashimoto's disease and 1 patient with Riedel's struma. Strikingly little fluorescence was seen on the colloid. Traces of fluorescence were seen in the sections treated with the sera of 2 patients with idiopathic hypothyroidism without goiter, 2 patients with euthyroid goiters, and 2 patients with adolescent goiter. The sections treated with the sera of the 5 normal persons were negative. Sections treated with the sera of 2 patients with Hashimoto's disease obtained 7 months after thyroidectomy were also negative, although a serum obtained from one of these preoperatively had been positive. Precipitins (agar diffusion) to thyroid extract were found in varying amounts in 9 out of 10 sera of patients with thyroid disease and in none of 5 controls. The significance of these results will be discussed.

The Hyponatremia Associated with Marked Overhydration and Water Intoxication. JAMES M. STORMONT and CHRISTINE WATERHOUSE,* Rochester, N. Y.

Although hyponatremia in patients without salt depletion is nearly always attributable to increased total body water and dilution, alterations of serum sodium concentration unaccounted for by water or cation balance have been postulated in certain diseases. We have obtained balance data on 2 patients (pneumonia and cerebral vascular lesion) during both the development of and recovery from severe hyponatremia (103,108 mEq per L) in the absence of salt depletion. Over 50 per cent of the change in serum sodium concentration below 116 mEq per L could not be accounted for by fluid or electrolyte balance, whereas above 116 mEq per L the rise in serum sodium was almost entirely accounted for by balance.

To evaluate the role of overhydration in this process, fluid retention with hyponatremia and clinical water intoxication was induced in 5 control patients receiving a constant diet (16 to 86 mEq per day sodium) on a metabolic ward. Vasopressin in oil was administered for 6 to 12 days. Severe symptoms of water intoxication were correlated with serum sodium concentrations of 114 to 100 mEq per L, negative sodium balance (11 to 109 mEq per day), and negative potassium balance (20 to 31 mEq per day after correction for nitrogen). In these patients over 50 per cent of the fall in serum sodium concentration below 114 mEq per L could not be accounted for by cation or fluid balance. With serum sodium above 114 mEq per L, potassium balance was less negative and the change in serum sodium concentration was largely accounted for by balance. Reciprocal changes were consistently noted during recovery from severe hyponatremia. Both symptomatology and metabolic abnormalities were prevented by fluid restriction (1,200 to 1,500 ml per day), or reversed by intravenous 10 per cent mannitol or discontinuance of vasopressin. In 5 other patients with an equivalent degree of vasopressin-induced overhydration, urine volume increased and fluid equilibrium was established with serum sodium concentrations above 114 mEq per L. Symptomatology and metabolic abnormalities were less marked or absent.

These data are interpreted to indicate altered cellular metabolism due to overhydration which is etiologically related to the unusually low serum sodium concentration (below 114 mEq per L) and severe symptomatology in certain patients.

Antimetabolite-Metabolite Cancer Chemotherapy: Effects of Intra-Arterial Methotrexate-Intramuscular Citrovorum Factor Therapy. ROBERT D. SULLIVAN, EDWARD MILLER, A. MICHAEL WOOD, P. P. CLIFFORD and JOHN DUFF, Nairobi, Kenya and New York, N. Y. (introduced by Joseph H. Burchenal).

A method of therapy has been developed to enhance the antitumor activity of chemotherapeutic compounds in the common forms of "solid" neoplastic disease. This consists of the *continuous* 24 hour delivery of massive doses of an antimetabolite into the arterial supply of tumors, together with the *intermittent,* intramuscular administration of the appropriate metabolite. Clinical studies of the effects of methotrexate-citrovorum factor (CF) therapy are reported.

Twenty-two patients with incurable epidermoid carcinoma or sarcoma of the head and neck or carcinoma of the bladder received intra-arterial methotrexate in massive doses for as long as 15 days. Sufficient CF was given by the intermittent, intramuscular route to prevent severe systemic toxicity. The usual dosage schedule employed was: methotrexate, 50 mg per 24 hours (if bilateral catheterizations, 25 mg per 24 hours per side); CF, 3 to 9 mg every 4 to 6 hours intramuscularly. The usual total volume of the infusion was 2,000 cc per 24 hours. Antitumor effects were noted as early as the third day of therapy. There was progressive decrease in size of visible tumor masses, and as ulcerated oral growths regressed, their surfaces became cleaner and less necrotic. Therapy was continued until complete tumor regression had occurred or until early signs of systemic toxicity were noted. Partial or complete tumor regression was noted in 11 patients.

The enhancement of the antitumor effect of methotrexate appears to be related to its continuous arterial administration, possibly increasing the concentration of "antimetabolic effect" in the tumor, and the use of CF, permitting the administration of massive doses of the antimetabolite.

Results suggest that antimetabolite-metabolite chemotherapy, using methotrexate and CF, may prove to be of practical value in the management of certain localized incurable cancer.

Pressure-Flow Relations in a Portion of the Pulmonary Vascular Bed, the Wedge Segment. H. J. C. SWAN and ARTHUR H. KITCHIN, Rochester, Minn. (introduced by Ward S. Fowler).

Cardiac catheters (no. 6L to 8L) were wedged in peripheral arteries of the lung. With a constant-rate syringe, autogenous blood or saline was infused at known rates and the resulting pressure gardient across the "wedged segment" measured. Variations in perfusion pressure at constant flow were noted, the gradient being reduced during inspiration. In 27 experiments in 10 dogs the relation between pressure and flow was apparently linear in the transmural pressure range of 5 to 60 mm of mercury. The average of the mean flow rates in dogs perfused with blood was 0.43 ml per minute per mm of mercury but showed considerable variation between different wedged segments. A serotonin-induced increase in pressure gradient measured at constant blood flow demonstrated the vascular components of the segment to be reactive. Provided that the effective perfusion pressure was less than 50 mm of mercury, no evidence of damage in the perfused area was noted.

In several humans with pulmonary hypertension, no reduction in the pressure gradient was associated with addition of acetylcholine to the infusion medium perfused at constant flow rate.

This method offers a simple assessment of pressure-flow relations in the pulmonary vascular bed and permits the study of drug effects and physiological responses.

Excretion of the Radioactive Catabolic End Products of I^{131}-Albumin in Human Sweat. Y. TAKEDA, I. C. PLOUGH and E. B. REEVE, Denver, Colo. (introduced by Gordon Meiklejohn).

When satisfactory I^{131}-albumin is injected intravenously, the rate of catabolism of plasma albumin can be determined either from the change in specific activity of the plasma albumin over several weeks (Method 1) or from this and the concurrent excretion in the urine of the I^{131} split from the labeled albumin during catabolism (Method 2). For accurate results with Method 2 the radioactivity released during catabolism either must be completely excreted, or a known constant fraction must be excreted, in the urine. Ordinarily in subjects leading a sedentary life the two methods give results that show good agreement and Method 2 then has a number of advantages. However, in subjects doing heavy work in hot environments, the second method gave significantly lower values than the first. A possible explanation was the excretion of catabolic radioactivity through other channels than the urine. When I^{131}-iodide or I^{131}-albumin was given by mouth to subjects made to sweat, I^{131} was excreted in the sweat, and when sweating was near maximal the rate of excretion in the sweat approached one-fourth of the rate of urinary excretion. Thus, in conditions when sweating is increased, measurements by the second method may give underestimates of the catabolic rate of albumin.

Arylsulfatase Activity of Human Leukocytes: Distinctive Pattern in Eosinophils. KOUICHI R. TANAKA, WILLIAM N. VALENTINE* and ROBERT E. FREDRICKS, Los Angeles, Calif.

Arylsulfatase activity of human leukocytes as determined by using 2-hydroxy-5-nitrophenyl sulfate as substrate has not previously been reported to our knowledge. Because of a report of markedly increased activity in the urine of chronic granulocytic leukemia (CGL) patients and because sulfates are metabolically important, arylsulfatase activity has been assayed (spectrophotometrically, 0.006 M substrate concentration, pH 5.7) on separated leukocytes from over 400 patients with a variety of diseases. Values in 16 normal subjects ranged from 10 to 39 with a mean of 26 (expressed as milligrams of 4-nitrocatechol liberated per 10^{10} WBC per hour at 37° C).

There was no detectable activity in the erythrocytes by our method.

The mean of 57 assays on 26 patients with CGL was 52.4. Thirteen polycythemia vera (PV) patients had a mean of 36.9, while the mean of 10 myeloid metaplasia (MM) patients was 41.1. In contrast, 27 assays on 18 patients with chronic lymphocytic leukemia revealed a mean of 5.4. Results in acute and subacute leukemia were variable. The highest arylsulfatase values occurred in those patients with eosinophilia of various etiologies. Twenty-one patients with a mean eosinophil percentage of 31.2 had a mean value of 129.5. There was fairly good correlation between the percentage of eosinophils and degree of activity.

The data indicate that the eosinophil is extremely active in arylsulfatase activity, whereas the basophil is much less active, but more so than the neutrophil. Lymphocytes and lymphoblasts show essentially no activity. The increased mean values in CGL, PV, and MM appear to be due mainly to the increased percentage of basophils and/or eosinophils.

The function of the eosinophil is virtually unknown. However, the finding of high arylsulfatase activity in eosinophils suggests that they may play a role in sulfate metabolism and may provide a clue as to their function(s).

On the Nature of the Hypoglycemic Effect of Tris-(hydroxymethyl)aminomethane (Tris Buffer). R. TARAIL and T. E. BENNETT, Buffalo, N. Y. (introduced by David K. Miller).

Explanation of our previous finding that alkaline Tris $(CH_2OH)_3 \cdot C\text{-}NH_2$ ("THAM") produces significant hypoglycemia in man, massive quantities reducing blood glucose in dogs virtually to zero, may help solve theoretical and clinical problems of carbohydrate metabolism. Eight to 12 mmoles per kg of 0.3 M Tris or 0.3 M $NaHCO_3$ was given intravenously to unanesthetized, fasting normal and diabetic dogs in about 17 minutes. Mean maximum fall of peripheral plasma glucose concentration was 39 per cent with *persistent* depression for 1 hour after alkaline Tris ($R\text{-}NH_2$; pH 10) in 4 studies of normals. Arterialized blood pH rose by 0.27 with marked elevation of plasma CO_2 content. Plasma inorganic phosphate declined 66 per cent. In contrast, Tris acidified to pH 6 ($R\text{-}NH_3^+$) produced a lesser, *transient* fall in plasma glucose of 23 per cent, in 4 studies, with slight reduction in pH and CO_2 content and a 43 per cent fall in phosphate. $NaHCO_3$ (4 experiments) caused no change in plasma glucose despite increase in pH and CO_2 similar to that after alkaline Tris; phosphate fell by 14 per cent.

Alkaline Tris transiently reduced plasma glucose by only 17 per cent in 5 studies of 2 diabetic (pancreatectomized) dogs deprived of crystalline insulin for 18 to 24 hours. pH rose by 0.25, CO_2 increased strikingly, and phosphate fell 52 per cent. $NaHCO_3$ given to diabetics (3 studies) lowered plasma glucose 17 per cent, phosphate 18 per cent, and produced greater rise of pH and CO_2.

The foregoing suggests, but does not prove, that Tris-hypoglycemia is mediated by an islet and/or peripheral insulin mechanism. The slight lowering of glucose in diabetics can be ascribed to alkalinity since $NaHCO_3$ causes similar lowering. Furthermore, the hypoglycemia in normals is not pH-dependent since $NaHCO_3$ does not produce it in normals. That the hypoglycemia is partly conditioned by pH is shown by its greater magnitude and duration after alkaline in comparison to acidified Tris.

A Physiological and Biochemical Comparison of Crystalline Dihydrotachysterol (AT-10) with Hytakerol. A RAYMOND TEREPKA and PHILIP S. CHEN, JR., Rochester, N. Y. (introduced by Ralph F. Jacox).

The "calcemic" action of calciferol and that of the structurally closely related dihydrotachysterol (AT-10) have been extensively utilized in the treatment of hypoparathyroidism. Although originally suggested as the ideal substitute for parathyroid hormone, the commercially available preparation of the latter compound (Hytakerol) has fallen into disfavor in recent years due to its greater cost and apparently variable potency.

In a comparative study of the relative actions of vitamin D, crystalline AT-10, and Hytakerol in rats, it was noted that Hytakerol had significantly less calcium and phosphorus mobilizing activity when compared with pure crystalline AT-10. Since the results suggested that Hytakerol did not contain the labeled quantity of AT-10, reverse-phase paper chromatographic analyses of the constituents of 3 different lots of Hytakerol were made. A compound exhibiting the typical AT-10 ultraviolet absorption spectrum could be isolated. Surprisingly, spectrophotometric assay of this compound, based on the molar absorbance of crystalline AT-10, revealed that Hytakerol contained 2 to 3 times *more* "AT-10" than the label indicated. In addition, 2 substances more polar than AT-10 and giving positive antimony trichloride tests were found. One exhibited a strong ultraviolet absorption at 250 mμ with lesser peaks at 283 and 291 mμ. The other showed weak absorption at 236 and 286 mμ.

By running mixed paper chromatograms, the AT-10-like ultraviolet absorbing material in Hytakerol was definitively shown to be chromatographically different from crystalline AT-10. In each of 4 different reverse-phase systems, crystalline AT-10 migrated faster than the Hytakerol sterol.

It is concluded that the commercially available Hytakerol does not contain AT-10 but rather an isomer of this sterol which is of significantly lower potency. Available evidence suggests that the compound is dihydrovitamin D_2 II.

Prevention of Stokes-Adams Attacks with Chlorothiazide and Salt. LOUIS TOBIAN,* Minneapolis, Minn.

It seemed feasible to prevent Stokes-Adams attacks by mildly depleting body potassium with chlorothiazide and

sodium salts. Six patients were all having frequent syncopal episodes which occurred whenever their atrial impulses were temporarily blocked at the auriculo-ventricular nodal area. None had permanent complete heart block. When 5 of these patients began taking 500 to 1,000 mg of chlorothiazide and 4 to 9 g of NaCl daily, the Stokes-Adams attacks ceased to occur in every one of them. When the medication was temporarily discontinued in 2 patients, the attacks began to reappear. Resumption of medication again completely prevented the attacks. In the one other patient, the combination of 750 mg of chlorothiazide and 10 g of $NaHCO_3$ daily completely prevented the syncopal seizures without producing any significant changes in plasma pH, bicarbonate, or potassium. So far the regimen has provided excellent improvement in every patient with Stokes-Adams seizures who does not have complete heart block (6 out of 6). Moreover, in all 6 patients there was electrocardiographic evidence of improved conduction. The regimen has not caused any annoying side effects. The medications produced no significant alterations in pH or potassium or sodium concentration in plasma. Only 2 patients had a slight elevation of plasma bicarbonate, never higher than 33 mEq per L. Balance studies indicated that the regimen caused mild depletion of body potassium. A lowering of potassium in the cardiac conduction tissue may be partly responsible for the improved cardiac conduction. To summarize, patients whose conduction system is not irrevocably damaged can often obtain striking relief from Stokes-Adams syncopal seizures by taking generous amounts of chlorothiazide and NaCl or $NaHCO_3$. The efficacy of the regimen may or may not be related to a mild depletion of potassium in the conduction fibers.

Absorption of I^{131}-Labeled Triolein Following Intraduodenal Administration: The Relative Effect of Intestinal Hormones, Secretagogues and Pancreatic Enzymes. MALCOLM P. TYOR, RICHARD R. HORSWELL and EDWARD E. OWEN, Durham, N. C. (introduced by Julian M. Ruffin).

The intraduodenal administration of I^{131}-labeled triolein (3 ml per minute) to normals results in increased fecal and decreased blood radioactivity. This induced defect, which is primarily digestive, may be corrected by the oral administration of a fat meal 30 minutes before the intraduodenal infusion. The present report deals with the effect of intestinal hormones, carbohydrate, peptone and pancreatic enzymes on intraduodenal administration of labeled triglyceride.

When carbohydrate (12 subjects) and peptone (11 subjects) were given orally 30 minutes before the intraduodenal infusion of I^{131}-triolein, 72 hour fecal radioactivity averaged 12.7 ± 9.5 and 10.8 ± 9.0 per cent, respectively. Both values were significantly reduced ($p = <0.05$) when compared with those obtained from subjects who were not prefed, mean value 22.8 ± 12.5 per cent. However, fecal radioactivity in each group was significantly greater ($p = <0.01$) than the mean value observed when lipid was prefed, mean value 2.8 ± 2.8 per cent. Similar results were obtained when 4.0 g of Viokase (11 subjects) was mixed with the labeled lipid prior to intraduodenal administration, mean value 11.0 ± 7.5 per cent. Of particular interest was the significant reduction in fecal radioactivity which followed the intravenous administration of secretin and pancreozymin-cholecystokinen. When these materials were given over a 20 minute period prior to the I^{131}-triolein infusion (7 subjects), fecal radioactivity averaged 3.41 ± 1.83 per cent. This value bore similar statistical significance to lipid prefeeding when compared with other groups. Blood radioactivity, measured from 1 to 6 hours, was significantly greater when lipid was prefed than with any other maneuver.

These observations provide further evidence in support of the digestive nature of this induced defect and point up the importance of intestinal hormones in the normal digestion of I^{131}-triolein. The data also suggest that the secretagogue effect of carbohydrates and peptone is significantly less than that of fat.

The Hormonal Role of Endogenous Glucagon in Blood: Glucose Homeostasis as Demonstrated by a Specific Immunoassay. R. H. UNGER, A. M. EISENTRAUT, MARY S. MCCALL and L. L. MADISON, Dallas, Tex. (introduced by R. W. Berliner).

Despite extensive knowledge of the actions of exogenous glucagon, the lack of a specific method for measuring circulating endogenous glucagon has left its hormonal status and physiological role in doubt. The recent development in this laboratory of a specific glucagon radiochromatographic immunoassay, sensitive to 50 $\mu\mu$g, made possible the following studies of glucagon secretion during acute and chronic hypoglycemia.

Acute hypoglycemia was induced in 9 dogs by i.v. injection of glucagon-free insulin, and chronic hypoglycemia in 4 dogs by phloridzinization. Glucagon concentration was measured radioimmunologically in pancreatic and femoral venous blood. In normal dogs fasting glucagon levels in pancreatic plasma averaged 653 $\mu\mu$g per ml (50 to 1,300), always exceeding the corresponding femoral levels which averaged 443 (0 to 900). Insulin hypoglycemia below 45 mg per 100 ml was invariably followed by a rise in pancreatic venous glucagon to a peak averaging 160 per cent (36 to 290 per cent) above preinjection values. This hyperglucagonemia coincided with blood glucose rebound; it was abruptly reversed by rapid glucose injection. Chronically hypoglycemic phloridzinized dogs had high fasting glucagon levels averaging 1,993 $\mu\mu$g per ml (680 to 3,100) which rose further after insulin injection. In 8 normal and 11 diabetic humans peripheral venous fasting glucagon averaged 273 $\mu\mu$g per ml (0 to 700). Only a small rise followed insulin hypoglycemia in normals but a tenfold rise occurred in a hyperlabile insulin-sensitive diabetic.

The hormonal status of glucagon and its role in glucose homeostasis have been demonstrated by direct specific measurements which indicate its pancreatic origin, its

secretory responsiveness to hypoglycemia, and its counter-regulatory action thereupon.

The Pathogenesis of Viruria: Animal Kidney Infection by Viruses Isolated from Human Urine. JOHN P. UTZ, Bethesda, Md. (introduced by Vernon Knight).

Using a technique of ultracentrifugation of urine, we have been able to demonstrate viruria in over 30 patients with infection due to 7 viruses: mumps, Coxsackie B2, B3, B4, ECHO 9, ECHO 14 and another untyped enterovirus. Virus has been found as early as the first day of illness and as late as the thirteenth day in patients with mumps. Five urinary isolates representing ECHO 9 (2 isolates), Coxsackie B2, Coxsackie B3, and Coxsackie B4 viruses were inoculated subcutaneously into suckling mice to determine whether animal kidneys could be infected. $Ten^{2.5}$ to $10^{4.0}$ $TCID_{50}$ per mg (infective dose for 50 per cent of tissue cultures per milligram of wet tissue) amounts of virus were found in kidneys of animals killed 6 to 8 days after inoculation. This amount was less than that found in the hind leg, approximately the same concentration as that found in brain and heart, and 10 to 300 times that found in blood of the same animals. A technique of perfusion of the living animal with saline to remove blood from viscera before determining the degree of viral infection provided additional evidence that the presence of virus was not accounted for on the basis of the viral content of blood in the kidney. The finding of viruria in man and the evidence that viral propagation may occur in renal tissue of animals points to the possibility that renal viral infection may be a concomitant of several acute viral illnesses of man.

Restoration by Growth Hormone of Normal Responses to Fasting in a Pituitary Dwarf. W. P. VANDERLAAN* and D. P. SIMPSON, La Jolla, Calif.

The effects of 48 to 60 hours of fasting on free fatty acid (FFA), blood ketone, and sugar levels were measured in 22 volunteers. There were rises in FFA and ketone levels to average maxima of 1,850 μEq and 2,426 μmoles per L. Blood sugars fell as low as 45 mg per 100 ml. The fast was broken with an oral glucose tolerance test, FFA, ketone and glucose levels being observed as long as 7 hours. After glucose administration, FFA fell sharply (2 hours after glucose average = 723 μEq per L). The extent of fall varied directly with dosage. Despite 100 g of glucose after 5 to 7 hours, FFA levels again rose sometimes to exceed the fasting peak. Ketone levels frequently rose one-half hour after glucose, then fell and again rose later. Occasionally glucose intolerance occurred.

A pituitary dwarf, receiving thyroid U.S.P. and 10 mg cortisone, fasted 48 hours and had a maximal rise in FFA levels to 480 μEq per L. Ketonemia was minimal until 50 g of glucose was given; then a fourfold rise occurred over 3 hours. In a subsequent fast he received human growth hormone, 4 mg on alternate days; a rise in FFA levels to 1,620 μEq per L and a ketonemia tenfold greater than before occurred. After 50 g of glucose, FFA levels fell in 3 hours below 600, then rose sharply. Ketonemia increased at one-half hour, then fell toward normal. Glucose intolerance was not observed. Human growth hormone, therefore, appeared to speed mobilization of fatty acids, increase fasting ketosis, and revert to normal the influence of glucose administration on ketonemia.

Physiological and Pharmacological Studies on the Reversibility of Endotoxin Shock in Dogs. JAMES A. VICK, Minneapolis, Minn. (introduced by Wesley W. Spink).

Peripheral vascular collapse (endotoxin shock) in humans is most frequently due to coliform bacteria. Management is difficult to evaluate because combinations of procedures and drugs are employed simultaneously in critically ill patients. More precise information has been obtained in adult mongrel male dogs anesthetized with sodium pentobarbital, given lethal doses of endotoxin (*E. coli*), and then treated with a single agent or combinations *after* the onset of progressive hypotension. Over a period of 12 hours systemic blood pressures were recorded continuously; urinary outputs were evaluated qualitatively and quantitatively; and hematocrit concentrations and femoral arterial pH were determined at intervals.

1) The average survival time for 12 control dogs was 11.5 hours. Irreversible shock usually occurred when the hematocrit exceeded 60 vol per cent, and the blood pH fell below 7.0. Renal failure was a prominent feature. *2*) The survival time of 10 dogs given 250 to 750 cc of canine plasma was 14.7 hours. *3*) When a pressor agent, metaraminol, was administered to 10 dogs so that systolic pressures were maintained around 90 to 100 mm Hg, the survival time was 13.1 hours. *4*) The survival time of 11 dogs given large intravenous doses of cortisol was 15.1 hours. *5*) The most significant results were obtained with a combination of cortisol and metaraminol. When a large infusion of cortisol was administered, only one-tenth the usual amounts of metaraminol were necessary to sustain pressure. Good renal function was also sustained throughout in the surviving dogs. Seven out of 10 animals not only survived, but resumed their normal activities.

The results in dogs with a combination of large doses of hydrocortisone and metaraminol simulate observations made in human patients.

The Role of Nutrition in the Alcoholic Neurological Diseases. MAURICE VICTOR, Boston, Mass. (introduced by Raymond D. Adams).

This communication proposes to define the etiological role of nutritional factors in *1*) delirium tremens and related disorders and *2*) the Wernicke-Korsakoff syndrome. Two types of data were secured: *1*) dietary history and nutritional status on examination; *2*) observations on the mode of recovery under strict dietary control.

Of 213 patients with delirium tremens and related disorders, symptoms developed on a background of inadequate diet in 148, but adequate in 65. Signs of nutritional deficiency were present in 37 of the former, in none of the latter. Thirty patients (3 groups of 10) with de-

lirium tremens recovered completely in 24 to 96 hours, while receiving a diet lacking in all vitamins, protein and fat (water *ad. lib.*, 5 per cent glucose/saline, 45 ml whiskey in water every 4 hours. Ten patients with acute auditory hallucinosis recovered in 1 to 8 days while ingesting only water *ad. lib.* (3 patients), 20 per cent dextrose/water (6 patients), and 45 ml whiskey in water every 3 hours (1 patient). These rates of recovery did not differ significantly from those of 101 patients with delirium tremens and 65 patients with acute auditory hallucinosis who received a full diet and all vitamins.

In 186 patients with Wernicke's disease prolonged dietary inadequacy had been present in all; 171 showed signs of malnutrition on examination. Nine patients were given a diet composed only of glucose, minerals and water, and 27 an unfortified rice diet, for periods up to 14 days. Only when thiamine was added did the apathy, ophthalmoplegia, nystagmus and ataxia improve. With respect to memory defect and confabulation (Korsakoff's psychosis), observations were made on 12 patients given the purified diet for 8 weeks. This defect remained unchanged in 4, improved in 6, and disappeared completely in 2, an outcome similar to that in 29 patients given a full diet and all vitamins.

Reciprocal Relationship Between Magnesium and Calcium Metabolism in Tetany. WARREN E. C. WACKER and DAVID D. ULMER, Boston, Mass. (introduced by Bert L. Vallee).

Magnesium or calcium deficiency tetany can only be distinguished by chemical means. The establishment of simple and direct relationships in the biological response and interactions of these elements has encountered great difficulty due to the multiple and unknown equilibria between their various phases and compartmental allocations. Specific and reciprocal relationships between the metabolism of magnesium and calcium under marginal nutritional conditions have now been identified.

In intestinal malabsorption concurrent magnesium and calcium deficiency are observed as evidenced by the low concentrations of both ions in serum. The administration of calcium will restore the concentration of this element in serum to normal values, but simultaneously the concentration of magnesium is depressed further and tetany eventually ensues. The correlation of these events is reflected in a concomitant and marked loss of magnesium in urine. Reciprocally, parenteral administration of magnesium, while abolishing tetany, similarly results in enhanced urinary losses of calcium.

The therapeutic response to the administration of a metabolite is a potential method to diagnose a deficiency state clinically. In the present instance, however, calcium operates as a conditioning factor, aggravating the preexistent subclinical, magnesium deficiency. The observed potentiation is an example of biological antagonism which presents a therapeutic hazard. Therapy cannot be relied upon to serve a diagnostic function here—diagnosis, therefore, depends on precise, quantitative measurements of both ions.

The Removal of Norepinephrine (NE) from the Blood Stream by the Extremities of Man. JOHN M. WALLACE, Durham, N. C. (introduced by Eugene A. Stead, Jr.).

Plasma levels and identity of small amounts of material measured as NE in normal human blood are uncertain. Infused NE disappears rapidly from blood. Observations on arterio-venous differences of material present in normal plasma and comparison of the behavior of this material with NE have not been available.

An ethylenediamine method sufficiently sensitive to deal quantitatively with known epinephrine and NE in concentrations believed present in normal plasma was developed. For NE determinations, standard solutions contain 0.004 to 0.008 μg NE per 5 cc plasma (0.8 to 1.6 μg per L); 0.004 μg gives 23 galvanometer deflections above plasma. NE infusions of 4 μg per minute were given to 5 subjects. Slight increases in blood pressure and decreases in pulse rate occurred. Two sets of samples were drawn from a brachial artery (BA), antecubital vein (ACV) and femoral vein (FV) before and during infusion.

Resting plasma contents of apparent NE did not differ, mean values in μg per 5 cc plasma being 0.0038 (BA), 0.0033 (ACV) and 0.0040 (FV). During infusion mean levels of added NE (control levels subtracted) in μg per 5 cc plasma were 0.0088 (BA), 0.0063 (ACV) and 0.0015 (FV). In every subject ACV and FV plasmas contained significantly less added NE than BA plasma and in 4 subjects FV plasma contained significantly less than ACV plasma. In 2 subjects FV plasma content during infusion showed no increase over resting levels.

When arterial levels of NE are increased to produce minimal changes in pulse and arterial pressure most of the NE is destroyed in the leg; less is destroyed in the arm. Behavior of infused NE in both arm and leg is different from that of normal plasma material commonly called NE. NE might be both removed and added by tissues. Efficient destruction of NE by the leg makes it questionable whether NE made there escapes destruction and appears in effluent blood.

Changes in Phage Types of Staphylococcus aureus in a General Hospital, 1952 to 1959. GOSTA WALLMARK and MILDRED W. BARNES, Boston, Mass. (introduced by Maxwell Finland).

Four series of coagulase-positive strains of *Staphylococcus aureus* collected from various sources in patients at the Boston City Hospital in the years 1952, 1955, 1958, 1959 and 1960 were subjected to phage-typing. The same set of phages was used and all the tests were done within the last few months. The results indicate that about one-half of the 400 strains isolated in 1952 were lysed by phages of Group III and that a few phage patterns predominated. This is in contrast to the later series in which those of phage Group III accounted for only about one-fifth of the strains. Staphylococci lysed by phages of Group I, on the other hand, increased in frequency from about 15 per cent among the 1952 strains to 50 to 60 per cent in the later series. There were only 2 strains of type 80/81 among those tested in 1952, whereas in 1955

at least one-third of the strains were of this type, and this incidence has since remained about the same. The strains were also tested for susceptibility to the antibiotics that are in common use, and the results correlated with the phage types will be presented. Of special interest was the almost universal resistance of type 80/81 strains to penicillin, streptomycin and tetracycline in contrast to the rarity with which strains of this type were found to be resistant to erythromycin.

Hypoxia and Ammonia Toxicity. KENNETH S. WARREN and STEVEN SCHENKER, Bethesda, Md. (introduced by Robert S. Gordon).

Ammonia toxicity was strikingly potentiated by short periods of hypoxia. Although the toxicity (LD_{50}) of ammonium chloride administered intravenously to mice after 2 minutes in 15 per cent oxygen was the same as that in 21 per cent oxygen, its toxicity began to increase when they were exposed to 13 per cent oxygen. The lethal effect from that point on was markedly enhanced by each 2 per cent decrement in oxygen concentration. In comparison with 21 per cent oxygen, 2 minutes in 7 per cent oxygen increased the toxicity of ammonium chloride 4.4 times.

The lethal effect of a dose causing 100 per cent mortality in 7 per cent oxygen could be completely reversed in a matter of seconds by transferring the mice to 21 per cent oxygen. Oxygen concentrations as high as 99 per cent did not alter the LD_{50}, but significantly prolonged life. The effect of hypoxia on the toxicity of another compound with analeptic properties, Metrazol, was relatively slight. Blood pH was not altered by the short periods of hypoxia utilized in these experiments. In addition, measurements of brain ammonia concentrations following a standard dose of ammonium chloride at 21, 11, and 7 per cent oxygen revealed that hypoxia had no effect on ammonia uptake or detoxication by the brain. It appeared therefore, that hypoxia directly potentiated the toxic effect of ammonia on the brain. The problem of hepatic coma will be discussed in view of these findings.

The presence of elevated blood ammonia concentrations in patients with severe liver disease has often been related to the genesis of hepatic coma. Hypoxemia and reduced cerebral blood flow are also frequent concomitants of marked liver pathology. In addition, there are definite neurological similarities between hepatic coma and chronic pulmonary insufficiency. Therefore, measures to improve cerebral oxygenation might be a useful adjunct to the therapy of hepatic coma.

Binding of Papain Protease by Alpha-globulin of Rabbit and Human Serum. GERALD WEISSMANN, JACOBUS L. POTTER, FRANK MCELLIGOTT, MARTIN MELTZER and ROBERT T. MCCLUSKEY, New York, N. Y. (introduced by Lewis Thomas).

It has been shown previously that crystalline papain protease produces widespread depletion of cartilage matrix when injected intravenously into young rabbits, provided that the enzyme is injected in an inactive, disulfide, form (PSSP). Fully active, reduced papain (PSH) has no effect on cartilage *in vivo*. Studies with isolated rabbit ear cartilage *in vitro* have shown that normal rabbit serum impairs the entry into cartilage of PSH, but not of PSSP. For these reasons, the rate of disappearance from serum of PSH and PSSP *in vivo* has been studied using papain labeled with I^{131}. Approximately 50 per cent of the injected enzyme is lost from serum within 30 minutes after an intravenous injection of 2 to 5 mg of PSH or PSSP in 1 kg rabbits. However, only a minute fraction of the injected material enters cartilage, the concentration being 1 to 2 μg per g of wet cartilage in the case of PSSP, and less than 0.5 μg following PSH. On the basis of immunoelectrophoresis and zone electrophoresis in barbital buffer, 0.1 M, pH 8.0 and 8.6, papain was found to be in the α-globulin region of serum. The localization was identical for PSH and PSSP, both after intravenous administration, and following incubation with serum *in vitro*. Under these conditions, the electrophoretic mobility of papain alone was toward the cathode, and it was not modified by albumin or γ-globulin obtained by Cohn fractionation of serum. However, the mobility of papain was modified by isolated α_2-globulin exactly as in whole serum. *In vitro* studies with a variety of human sera have shown a similar localization of papain in the α-globulin region. Since inhibition of trypsin, hyaluronidase and deoxyribonuclease is associated with such α-globulins, it is suggested that binding of papain by serum proteins may represent a similar mechanism preventing damage to tissue by a potentially harmful enzyme entering the circulation.

Bile Salt Transport in the Dog. HENRY O. WHEELER, PIER L. MANCUSI-UNGARO and ROBERT T. WHITLOCK, New York, N. Y. (introduced by Stanley E. Bradley).

Bile salt transport and its effect on bile flow and electrolyte composition and on transport of anionic dyes was studied in 4 unanesthetized, cholecystectomized dogs (20 to 25 kg) equipped with duodenal Thomas cannulae. Intravenous infusions of 1.5 per cent sodium taurocholate at rates of 170 to 230 μmoles per minute resulted in arterial plasma concentrations of 0.6 to 1.2 mmoles per L at 30 minutes which continued to rise, reaching 1.0 to 2.3 mmoles per L at 90 minutes. A constant and reproducible maximal rate of biliary taurocholate excretion was achieved within 30 minutes (mean 132 μmoles per minute, range 111 to 141 μmoles per minute). The resulting choleresis (0.70 to 1.35 ml per minute) was accompanied by chloride (17.9 to 90.0 μmoles per minute) and bicarbonate (9.3 to 41.5 μmoles per minute), outputs much greater than those reported previously at lower taurocholate excretory rates.

The excretory transport maximum of sulfobromophthalein sodium (BSP)—mean 4.0 μmoles per minute—was not reduced when BSP was infused during periods of maximal taurocholate output. However, the uptake of BSP from plasma (or the hepatic BSP storage) was altered by taurocholate since *1)* taurocholate administration during constant BSP infusion produced abrupt in-

creases in plasma BSP concentration, and *2*) unexpectedly high plasma concentrations and delayed achievement of maximal BSP excretion occurred when BSP was given during taurocholate infusions. The hepatic uptake of indocyanine green was similarly affected. Effects of these dyes on taurocholate uptake and excretion were equivocal.

Thus biliary taurocholate secretion is active, rate-limited and not obviously related to BSP secretion, although taurocholate does interfere with hepatic uptake of BSP and indocyanine green. In response to secretion of osmotically active bile salt, water and diffusible electrolytes probably enter bile passively. Diminished reabsorption of these constituents could also contribute to their enhanced excretion during taurocholate choleresis.

Blood ACTH in Cushing's Disease. W. C. WILLIAMS, JR., R. A. A. OLDFIELD, JR., D. P. ISLAND and G. W. LIDDLE,* Nashville, Tenn.

The present study was undertaken in search of a definitive answer to the long-debated question of whether the pituitary secretes abnormal quantities of ACTH in Cushing's disease (hypercortisolism due to bilateral adrenal hyperfunction). It was reasoned that if the primary disorder in Cushing's disease were a derangement of pituitary function, then it would not be corrected by removal of the target organs, the adrenal glands. It was postulated that the reason the adrenals were overactive in this disease was that ACTH secretion could not be suppressed by *normal* levels of cortisol. Two groups of patients were studied: 6 patients who had been treated for Cushing's disease by bilateral adrenalectomy and 4 patients who had Addison's disease without prior history of Cushing's disease. Both groups were maintained at normal blood levels of cortisol by administration of oral cortisol every 8 hours. None of the patients exhibited evidence of pituitary tumor.

ACTH was extracted from plasma by a method utilizing adsorption onto and elution from an ion exchange resin, Amberlite XE-64. ACTH activity in this extract was determined in hypophysectomized rats by a bioassay technique in which corticosterone was measured in adrenal vein blood following injection of graded doses of standard ACTH or plasma extract into the femoral vein. With this method 0.06 mU of ACTH could regularly be detected and a rectilinear log-dose response relationship was obtained with doses up to 0.25 mU per rat.

Although cortisol treatment and plasma 17-hydroxycorticoid values were similar for both groups, blood ACTH levels were 23 ± 16 (mean and standard error) mU per 100 ml for the patients with Cushing's disease and 0.3 ± 0.2 mU per 100 ml for the patients with simple Addison's disease. Conclusion: in Cushing's disease ACTH secretion is not suppressed by normal levels of cortisol.

Absence of the Normal Peripheral Venoconstrictor Response to Infusions of Synthetic Angiotensin II in Patients with Essential Hypertension. J. EDWIN WOOD, Augusta, Ga. (introduced by Thomas Findley).

Patients with essential hypertension have normal peripheral venous tone despite the increase in arteriolar tone; normal subjects with acute hypertension induced by infusions of angiotensin II have increased venous tone. The effect of infusions of synthetic angiotensin II (Ciba) upon responses of the peripheral veins of patients with essential hypertension were studied.

Eight normotensive subjects received infusions of angiotensin II of 2 to 4 μg per minute for 30 minutes. Arterial blood pressure averaged 123/75, σ 8/6 before infusion and 165/108, σ 20/9 during infusion. Venous volume of the leg measured plethysmographically in milliliters per 100 ml of leg tissue per 30 mm Hg rise in local venous pressure averaged 4.3, σ 1.0 before infusion and 3.4, σ 0.9 during infusion. Twelve patients with essential hypertension received angiotensin II infusions of 2 to 4 μg per minute for 30 minutes. Arterial blood pressure averaged 163/90, σ 30/17 before infusion and 208/122, σ 25/14 during infusion. Venous volume averaged 4.0, σ 1.0 before infusion and 3.7, σ 1.1 during infusion. Venous volume was lowered significantly ($p < 0.01$) in the normal individuals by angiotensin II; this material failed to alter venous volume significantly in the hypertensive patients. Rise of venous pressure with infusion of angiotensin II in the normotensive individuals averaged 9, range 4 to 15 cm of water and averaged 2, range 0 to 3 cm of water in the hypertensive individuals. Angiotensin infusions invariably caused headache, dyspnea, chest discomfort, nausea or abdominal cramps in normotensive subjects. Hypertensive subjects suffered none of these symptoms with infusion. Venoconstrictor responses to norepinephrine infusions in 6 patients with essential hypertension were comparable to those of normotensive individuals.

These experiments indicate that the veins of patients with essential hypertension are specifically insensitive to angiotensin, implying that these responses are so modified by previous experience with a substance at least similar to synthetic angiotensin II.

Normal Serum Hemolysins. STANLEY YACHNIN and FRANK H. GARDNER,* Boston, Mass.

Normal human red cells (RBC) treated with various agents which alter the stroma become susceptible to acid hemolysis in human serum (HS). Such hemolysis is complement- and properdin-dependent. Sera vary widely in their capacity for hemolysis. Eighty HS samples have been studied for hemolysis and agglutination with treated RBC. These altered RBC are to varying degrees panagglutinable. The agglutinin titer of sera has been related to their capacity to hemolyze altered RBC. RBC treated with receptor-destroying enzyme (RDE) and periodate are most susceptible to hemolysis in sera having the highest agglutinin titer. Bromelin and ficin-treated RBC are less consistently correlated, and trypsinized RBC show poor correlation between serum hemolytic and agglutinating capacity. Serum hemolysis is not related to complement titer or lysis of paroxysmal nocturnal hemoglobinuria (PNH) RBC. There is some

relationship between serum hemolytic capacity for RDE and trypsin-treated red cells. Bromelin and ficin-treated RBC are more closely related as regards hemolysis, but this relationship is not absolute.

Serum hemolytic activity can be inhibited by absorbing serum with specific types of treated RBC at 2° C. Eluates of these RBC can be bound to the identical treated RBC in saline media. Suspension of these eluate-coated RBC in absorbed acid serum results in restoration of hemolysis. Marked specificity of serum factors for each type of altered red cell may be demonstrated in HS. These antibody-like substances are distinct from properdin and complement, but dependent on these agents for acid hemolysis. These factors for treated red cells do not participate in PNH hemolysis. Such natural HS hemolysins emphasize the potential opportunity for pathologic RBC destruction in diseased states. In addition the recognition of these HS factors may aid in understanding the use and pitfalls of enzyme-treated RBC in serologic tests.

Immunoassay of Plasma Insulin Concentrations in Normal and Diabetic Man: Insulin Secretory Response to Glucose and Other Agents. ROSALYN S. YALOW and SOLOMON A. BERSON,* Bronx, N. Y.

An immunoassay procedure requiring only 10 to 20 μl plasma and capable of detecting less than 10^{-6} U insulin has been employed to measure plasma insulin concentrations in more than 100 subjects during a standard glucose tolerance test. Human insulin added to plasma *in vitro* is recovered quantitatively and 2- to 100-fold dilution of plasma reveals no evidence for nonspecific plasma substances reacting like insulin or inhibiting insulin in the immunologic reaction. Endogenous plasma insulin and insulin-I^{131} were similarly adsorbed by cellulose columns and destroyed by cysteine.

Fasting insulin concentrations did not differ significantly in untreated maturity-onset diabetic and nondiabetic subjects (range, 0 to 70 μU per ml; means 27, 21 μU per ml); 100 g glucose p.o. elicited peak insulin concentrations in nondiabetics at 0.5 hour (mean, 136 μU per ml) or 1 hour (mean, 128 μU per ml) with decline by 2 hours (mean, 105 μU per ml). Insulin concentrations in diabetics were lower at 0.5 hour (mean, 97 μU per ml) but continued to rise to a high peak at 2 hours (mean, 243 μU per ml) showing a significantly greater than normal integrated insulin output. Diabetics showed still higher insulin concentrations on further glucose loading. In 5 of 7 patients with functioning islet cell tumors (courtesy Drs. H. Epstein, J. Field, E. D. Furth, E. Gordon, A. Renold, J. Steinke) fasting insulin exceeded 118 μU per ml but response to glucose was normal in 1 patient studied.

Plasma insulin increased abruptly following L-leucine p.o. in 6 of 9 studies in 6 leucine-sensitive hypoglycemic patients (courtesy Drs. A. DiGeorge, M. Goldner, M. Grumbach, I. Rosenthal and S. Weisenfeld). Increases in plasma insulin were observed consistently following sodium tolbutamide (i.v. or p.o.) in all subjects but were much less marked than those following glucose in the same patients.

The high endogenous insulin secretion rate observed in maturity-onset diabetics on glucose loading suggests that deficient glucose utilization in this condition is not due to inadequate synthesis or release of insulin but is related to poor tissue responsiveness and/or inhibitors of the hormonal action of endogenous insulin.

The Mechanism of the Steroid Inhibition of Pyruvate Oxidation. K. LEMONE YIELDING, GORDON M. TOMKINS and JANET S. MUNDAY, Bethesda, Md. (introduced by Harry Eagle).

The DPN-dependent oxidation of pyruvic acid to acetyl coenzyme A and CO_2 is the metabolic step which permits the entry of glucose carbon into the Krebs cycle, and its rate might be important in the regulation of carbohydrate metabolism. Steroid hormones have been shown to inhibit this reaction *in vitro,* and the elevated blood levels of pyruvic acid in patients with Cushing's disease and upon steroid administration suggest some such action of these hormones *in vivo.*

We have shown recently [Proc. Nat. Acad. Sci. (Wash.) 1959, **45**, 1730] that the oxidation of DPNH to DPN by the DPNH oxidase reaction is strongly inhibited by various steroid hormones. Since DPN is essential for the oxidative decarboxylation of pyruvate, these findings suggested that the steroid inhibition of pyruvate oxidation might be due to curtailment of DPN arising from DPNH oxidation. Pyruvate oxidation in homogenates of rat tissues was stimulated by the addition of cytochrome C and/or liver microsomes, which specifically stimulate DPNH oxidation. This showed that the rate of pyridine nucleotide oxidation could control that of pyruvate. Alpha tocopherol, which prevented steroid inhibition of the DPNH oxidase, also overcame the steroid suppression of the pyruvate reaction. When DPNH oxidation was impeded by other inhibitors of electron transport, such as amytal or antimycin A, there was a corresponding depression of the oxidation of pyruvate.

These findings illustrate that steroids can regulate the oxidative decarboxylation of pyruvic acid, by virtue of their ability to inhibit oxidation of DPNH.

Glycolytic and Citric Acid Cycle Metabolites Related to Intracellular Ion Composition in the Experimental Nephrotic Syndrome. T. YOSHIDA, F. YAMASHITA, E. KAISER and J. METCOFF,* Chicago, Ill.

The experimental nephrotic syndrome, induced in rats by 6-dimethylamino-9-(3-amino 3-deoxy β-d-ribofuranosyl)-purine (aminonucleoside), causes disturbance of protein, lipid and electrolyte metabolism and of some enzyme activities in kidney tissue. The present study was designed to explore the influence of intracellular electrolyte concentration on intermediary cellular metabolism.

Fourteen nephrotic rats are compared with their pair-fed controls (nephrotic versus control value). In muscle,

the intracellular water content (352 > 260 ml per 100 g DFFS) and composition in the edematous nephrotic rats differed significantly from the controls (mmoles per L ICW: Na 50 > 19, K 121 < 182, Mg 19 < 22, $P_{organic}$ 46 < 76, $P_{inorganic}$ 44 < 54). Suggestive differences in cellular contents of glycolytic and Krebs cycle intermediates also occurred (μmoles per g NCN phosphoenolpyruvate 3 < 7, pyruvate 18 > 12, oxalacetatic 21 > 15). Lactate, citrate and α-keto-glutarate contents were similar. *In vitro* uptakes of pyruvic acid by isolated diaphragm (9.2 > 3.7 μmoles per g NCN per minute), and by slices of kidney (10.9 > 8.0) and liver (6.6 > 5.1) of nephrotic rats were increased.

Significant differences in activities (μmoles per g NCN per minute) of glutamic dehydrogenase (8.4 > 5.6) and glutamic-pyruvic transaminase (8.7 > 6.2) were found. Pyruvic kinase, lactic dehydrogenase, isocitric dehydrogenase and malic dehydrogenase activities were similar. Substrates were constructed containing ion concentrations approximating those found in ICW of nephrotic and control rat muscle and activity of purified pyruvic kinase tested. At appropriate dilutions, activity in the substrate simulating the "nephrotic" muscle was significantly less than that in "control" substrate.

Apparently, distortion of essential intracellular electrolyte concentrations characterizing edema of the experimental nephrotic syndrome is associated with, and may condition, altered pathways of terminal glycolysis and subsequent metabolism of intermediates in the Krebs cycle.

The Effects of Hypercalcemia on the Renal Concentrating Mechanism in Man. J. LESTER ZEFFREN and HENRY O. HEINEMANN, New York, N. Y. (introduced by Alfred Gellhorn).

The effects of hypercalcemia on the renal concentrating mechanism has been studied in 7 patients with neoplastic disease and skeletal metastases, and a patient with milk alkali syndrome. Observations were made during periods of hypercalcemia and normocalcemia in 5 patients at intervals of 1 to 10 weeks. The results indicate that hypercalcemia is associated with a reversible defect in the renal concentrating mechanism which is disproportionate to the simultaneous reduction in glomerular filtration.

The renal concentrating ability was defined by the maximal and minimal osmolal U/P ratio, U/P_{osm}, and by the calculation of $T_{H_2O}{}^c$ during osmotic diuresis with 10 per cent mannitol in a hydropenic state. $T_{H_2O}{}^c$ expressed as a percentage of glomerular filtration rate ($T_{H_2O}{}^c$/GFR) permitted comparison between different observations.

Hypercalcemia resulted in reduced GFR, lowering of maximal U/P_{osm}, preservation of the minimal U/P_{osm}, and formation of urine iso- or hypotonic to plasma during osmotic diuresis, despite adequate vasopressin. With normocalcemia, GFR and U/P_{osm} increased and hypertonic urine was produced during osmotic diuresis. At high flow rates, however, $T_{H_2O}{}^c$/GFR tended to decrease significantly.

One patient with hypercalcemia and normal GFR produced persistently hypotonic urine despite large amounts of vasopressin, resulting in a free water clearance of 5.0 ml per minute. This study suggests that the defect in renal concentrating ability is not dependent upon changes in glomerular filtration and may be related to relative insensitivity of the distal tubule to antidiuretic hormone, as well as to changes in intramedullary osmolality.

The Poorly-Permeable Membrane as an Alternative to the Carrier Hypothesis of Facilitated Diffusion. KENNETH L. ZIERLER,* Baltimore, Md.

Diffusion facilitated by unidentified carrier molecules is a process hypothesized for movement of certain substances across cell membranes in order to account for three phenomena—saturation, competition and specificity—which occur even though net movement is in the direction of the chemical or electrochemical potential gradient. However, this hypothesis may be unnecessary because saturation and competition are inherent properties of poorly-permeable membranes, and biological membranes, in general, are poorly permeable. For example, it has been estimated that for mammalian skeletal muscle only 10^{-8} of the membrane surface is available for diffusion of water-soluble substances. When a relatively large number of molecules, whether of the same or different species, bombard such a membrane there will be competition, not for a limited amount of carrier, but for a limited area of permeable sites. Saturation is exhibited when the molecules are of a single species; competition when there are two species. Specificity occurs when the molecules and the permeable sites are matched conformationally. This hypothesis was tested in a simple mechanical analog in which steel bearing balls were shaken in a chamber separated from a second chamber by a wall in which were drilled one or more holes. With increasing concentration, flux became constant; there was saturation. Addition of a second size of bearing balls reduced the flux of the first species; there was competition. Both saturation and competition increased: *1*) as mean velocity of particles decreased, *2*) as the ratio of area of a single permeable site to particle diameter decreased, and *3*) as total permeable area decreased. It is concluded that the observations for which the facilitated diffusion hypothesis was invoked can be explained more simply by the properties of a system in which the membrane is poorly permeable.