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ABSTRACTS

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Research Article

A Previously Unrecognized Disorder of Metabolism of Sulfur-containing Compounds—Abnormal Urinary Excretion of S-Sulfo-L-Cysteine, Sulfite, and Thiosulfate in a Severely Retarded Child with Ectopia Lentis. LEONARD LASTER, FILADELFO IRREVERRE, S. HARVEY MUDD, AND WILLIAM D. HEIZER, Bethesda, Md. (introduced by Robert S. Gordon, Jr.*).

An infant born with neurological abnormalities deteriorated to an almost decorticate state by 9 months and was noted to have bilateral ectopia lentis at 1 year. We studied him between age 30 months and his death, 1½ months later. His urine contained abnormally increased amounts of an amino acid shown to be S-sulfo-L-cysteine. Inorganic sulfate in his urine was decreased to below 5% of total sulfur. Normal subjects excrete over 60% as inorganic sulfate. Oral administration of L-cysteine failed to increase his urinary sulfate, whereas it does increase it in normal subjects. His urine also contained abnormally elevated amounts of sulfite and thiosulfate, the latter accounting for more than 15% of total sulfur. The concentration of S-sulfo-L-cysteine in plasma was abnormally increased. ¶ These findings suggest that the underlying disorder was metabolic rather than renal. The simplest hypothesis is a deficiency of activity of sulfite oxidase, the enzyme normally catalyzing conversion of sulfite to sulfate. This would lead to sulfite accumulation which in turn could cause formation of S-sulfo-L-cysteine and thiosulfate via known reactions. A dependence on exogenous sulfate would also ensue. The near absence of sulfate from the patient's urine suggests that he was not absorbing enough dietary sulfate to meet his metabolic needs. Accumulation of metabolites proximal to the sulfite oxidase reaction could also contribute to the pathology. That three of seven siblings died in infancy with neurological manifestations resembling the patient's suggests the disorder is hereditary, but as yet we have found no evidence of heterozygosity in the parents or remaining siblings. Determinations of sulfite oxidase in tissue obtained at autopsy from the patient are in progress.

Studies on the Mechanism of ACTH-mediated Insulin Release. HAROLD E. LEBOVITZ AND KAREN POOLER, Durham, N. C. (introduced by Eugene A. Stead, Jr.†).

Previous studies have documented the direct action of ACTH in stimulating insulin secretion in small laboratory animals. Additional investigations have shown that agents which interfere with protein synthesis at the ribosomal level such as puromycin and cycloheximide greatly potentiate ACTH-mediated insulin secretion. This is in marked contrast to the action of these same agents in blocking ACTH-mediated adrenal steroidogenesis. Since puromycin has been shown to inhibit cyclic AMP phosphodiesterase in rat hemidiaphragm *in vitro*, the present study was designed to determine whether the puromycin potentiation of ACTH-mediated insulin secretion is due to an interference of this enzyme activity rather than to the inhibition of protein synthesis. ¶ Mice were pretreated with either saline or theophylline ethylenediamine (Aminophylline), a known inhibitor of cyclic AMP phosphodiesterase, and then treated with either ACTH, 5 μg, or saline. Ten minutes after treatment, the animals were sacrificed and blood glucose and plasma insulins were measured. Plasma insulin levels in a typical experiment were *1*) pretreatment with Aminophylline, treatment with saline 26 ± 3 μU per ml; *2*) pretreatment with saline, treatment with ACTH 96 ± 16 μU per ml; *3*) pretreatment with Aminophylline, treatment with ACTH 426 ± 48 μU per ml. In all experiments, there was a significant ($p < 0.01$) potentiation of ACTH-mediated insulin secretion by Aminophylline. Additional findings were *1*) Aminophylline had no effect on the ACTH-induced hypoglycemia, *2*) the Aminophylline effect occurred in corticosterone-maintained adrenalectomized mice, and *3*) the dose of Aminophylline that potentiated ACTH-mediated insulin secretion had no effect on pancreatic protein synthesis as measured by the incorporation of leucine-^{14}C. ¶ The data clearly show that Aminophylline potentiates ACTH-mediated insulin secretion in a manner quite similar to puromycin and suggest that the puromycin effects may be through an action on the cyclic AMP system rather than through an inhibition of protein synthesis.

Isolation and Identification of Prostaglandin-E_2 and Prostaglandin-$F_{2\alpha}$ from Rabbit Kidney Medulla. JAMES B. LEE, KEITH CROWSHAW, AND BERTIL H. TAKMAN, Worcester, Mass. (introduced by Harold Jeghers†).

The isolation of medullin from rabbit kidney medulla and its subsequent identification as dehydrated prostaglandin E_2 (PGE_2-217) suggested the presence of a potent antihypertensive and vasodepressor lipid in kidney that does not exhibit significant intestinal stimulating activity. Our earlier studies also demonstrated two prostaglandins in rabbit renal medulla that possess marked intestinal stimulating activity: one without vasodepressor properties (compound 1), the second with potent vasodepressor activity (compound 2). ¶ In the present study, compounds 1 and 2 were isolated from rabbit renal medulla by a combination of solvent extraction and column and thin layer chromatography (TLC). Direct comparison of compound 2 and authentic prostaglandin-E_2 (PGE_2) revealed identical TLC mobility on $AgNO_3$-impregnated plates. The mass spectrum of compound 2 revealed a molecular ion at 352 with fragmentation patterns identical with authentic PGE_2. In addition, the nuclear magnetic resonance spectrum of compound 2 was consistent with PGE_2. Compound 1, previously described as a prostaglandin-F (PGF), has now been shown to be indistinguishable from authentic $PGF_{2\alpha}$ by a combination of column and thin layer chromatography. Since we have been unable to detect significant quantities of PGE_1, PGE_3, PGF_1, and PGF_3, it is likely that the prostaglandins of the rabbit renal medulla are derived from arachidonic acid (Van Dorp and associates, Bergström and associates). ¶ In contrast to the potent vasodepressor activity of PGE_2-217 and PGE_2, $PGF_{2\alpha}$ revealed pressor activity when injected into the vagotomized pentolinium-treated rat. Since PGE_2-217 has been shown by Barger and co-workers to

result in a redistribution of renal blood flow in dogs from outer medulla to cortex, and since we have observed it to produce a marked natriuresis in hypertensive man, the present findings suggest that renomedullary prostaglandins may possess complex intrarenal regulatory functions.

Cerebrospinal Fluid Buffering in Respiratory Acidosis. JOHN E. LEE, FRED PLUM,* AND JEROME B. POSNER, New York, N. Y.

This study compares the buffering capacities of blood and cerebrospinal fluid (CSF) during acute respiratory acidosis. Anesthetized dogs were ventilated with various concentrations of CO_2 in oxygen. After each successive level of CO_2 had been maintained for 2 hours, samples of arterial blood and cisternal CSF were drawn and analyzed for pH, P_{CO_2}, and bicarbonate. To determine the *in vitro* buffering capacity of CSF, we equilibrated in a microtonometer samples drawn before CO_2 inhalation with four different concentrations of CO_2 in oxygen. In an additional group of dogs, 10^{-4} M ouabain was infused into the lateral cerebral ventricle while hypercapnia was induced. ¶ The buffering capacities of blood and CSF were demonstrated by plotting H^+ concentration in millimicromoles per liter against P_{CO_2} in millimeters Hg, obtaining a statistically straight line in each group of experiments. The slope of this line *in vivo* was 0.50 for CSF and 0.75 for blood ($p < 0.001$). The increment in bicarbonate ion during CO_2 inhalation was greater in CSF than in blood. *In vitro*, the buffering slope of CSF was 0.85, not significantly different from blood. Intraventricular ouabain reduced the increment of CSF bicarbonate during hypercapnia and consequently reduced CSF buffering capacity (slope 0.67) to a level not significantly different from blood. Of interest is that during respiratory acidosis, CSF bicarbonate bears the same arithmetic relationship to arterial blood pH (−30 mEq per L per pH U) as does the electrical potential difference between CSF and blood (−31 mv per pH U) found by Held and associates.

Presence of B-Protein (Apoprotein of β-Lipoprotein) in Normal Plasma and in Abetalipoproteinemia. ROBERT S. LEES, New York, N. Y. (introduced by Zanvil Cohn*).

Since a lipid-free protein that reacts with antiserum to β-lipoproteins has never been demonstrated in plasma, it has been assumed that lipid-free B-protein does not exist in plasma and is not synthesized at all in abetalipoproteinemia. If B-protein differed antigenically from β-lipoprotein, its presence in plasma might not be demonstrable by the use of anti-β-lipoprotein antisera; immunologic study of structurally modified β-lipoprotein might, however, reveal it. ¶ To test this hypothesis, we altered the conformation of β-lipoprotein by formation of arsanilazo and acetyl derivatives. Both altered proteins were still precipitable by anti-β-lipoprotein antisera. This precipitation, however, was inhibited *1*) by plasmas from five subjects with abetalipoproteinemia, *2*) by $D > 1.21$ proteins from normal plasma, or *3*) by $D > 1.21$ proteins from abetalipoproteinemic subjects (three types of protein mixture hereafter designated "test antigens"), whereas that of native β-lipoprotein was not. Antisera to the test antigens did not precipitate native β-lipoproteins but did precipitate both azo- and acetyl-β-lipoproteins. On immunoelectrophoresis of whole normal plasma or of the test antigens against antisera to the test antigens, a β-globulin precipitin line was produced which was not identical with β-lipoprotein and which did not stain for lipid; it was immunologically identical with azo-β-lipoprotein. This precipitin line was also demonstrable with antisera to normal lymph chylomicrons; its identity with acetyl-β-lipoprotein was shown on Ouchterlony plates. ¶ It is concluded that B-protein in the lipid-free state is antigenically different from native β-lipoprotein and antigenically similar to azo- and acetyl-β-lipoproteins. It is present in lymph chylomicrons, in normal plasma, and in abetalipoproteinemia as well. The biochemical defect in abetalipoproteinemia cannot be failure to synthesize B-protein.

Decreased Renal Tubular Calcium Reabsorption during Chronic Metabolic Acidosis in Man. EDWARD J. LENNON AND JACOB LEMANN, JR., Milwaukee, Wis. (introduced by William W. Engstrom†).

The mechanism that causes hypercalciuria in chronic metabolic acidosis has not been elucidated. We have previously shown that bone mineral participates in the defense against chronic metabolic acidosis. We assumed that hypercalciuria during acidosis resulted from an increase in serum ultrafilterable calcium concentration (UF_{Ca}) and thus increased glomerular filtration of calcium. However, these earlier studies did not exclude the alternative possibility that hypercalciuria was due to decreased renal tubular calcium reabsorption. To determine which of these mechanisms is involved, we performed clearance studies in nine normal adults on constant diets before and 6 to 10 days after inducing stable metabolic acidosis with NH_4Cl (six subjects) or acetazolamide (three subjects). Serum UF_{Ca} was separated at each subject's *in vivo* blood $[H^+]$. During acidosis, as compared to control, blood $[H^+]$ rose ($+12.4 \pm 5.9$ nEq per L; $p < 0.001$) and serum bicarbonate fell (-8.8 ± 2.7 mEq per L; $p < 0.001$). UF_{Ca} rose ($+0.05 \pm 0.05$ mmole per L; $p < 0.025$). However, presumably because of the diuresis occurring during the induction of acidosis, inulin clearance fell (-13 ± 12 ml per minute; $p < 0.025$). Thus the filtered load of calcium actually fell (-13 ± 16 μmoles per minute; $p < 0.05$) despite which urinary calcium excretion rose ($+3.2 \pm 2.0$ μmoles per minute; $p < 0.005$). Clearly, hypercalciuria must have resulted from decreased renal tubular calcium reabsorption. This could not be explained by the known influence of variations in the rate of sodium excretion, since sodium excretion did not change (mean change $= 0 \pm 62$ μEq per minute; NS). Urine flow rates exceeded 8 ml per minute in all studies, and thus any effects of changing concentrations of anions capable of complexing calcium were minimized. Since acidosis caused an increase in UF_{Ca} (and presumably

serum ionized calcium), the secretion of parathyroid hormone or thyrocalcitonin or both may have been altered. Thus, reduced tubular reabsorption of calcium could have been hormonally mediated. Alternatively, metabolic acidosis may directly alter renal tubular cell metabolism.

Effect of an Acute Salt Surfeit in Proximal Tubular (PT) Reabsorption in Normal Man. PAUL R. LENZ, MARVIN H. GOLDSTEIN, AND MARVIN F. LEVITT,† New York, N. Y.

It has been demonstrated by a number of investigators that administration of saline promptly diminishes fractional PT reabsorption in dog and rat. The effect of this stimulus on PT function in man is less clear. Recent studies in this laboratory have suggested that during sustained hydration distal tubular (DT) urea reabsorption virtually ceases, so that in this state changes in C_{Ur}/C_{In} ratio may provide an index of changes in fractional PT reabsorption. This parameter was used to evaluate the effect of a saline load on PT activity in normal, maximally hydrated subjects. Administration of 7 L of 0.45% saline over 4 hours to 11 subjects produced an inconstant and meager effect. In only 4 of 11 subjects did a modest increase in $C_{osm}/C_{In} \times 100$ and in $C_{Ur}/C_{In} \times 100$ occur, averaging 5.2% and 3.0%, respectively. In a second series of 6 studies, the same protocol was employed, except that known DT diuretic agents (mercaptomerin, chlorothiazide) were administered coincident with the saline load. In contrast to the first group of studies, $C_{osm}/C_{In} \times 100$ rose consistently, the increase averaging 12.7%, so that the salt surfeit achieved in each experiment was considerably smaller. Despite the smaller salt surfeit in these combined studies, each of the 6 subjects showed an increase in $C_{Ur}/C_{In} \times 100$, averaging 13.0%. The administration of the same DT diuretic without coincident saline infusion to the same 6 hydrated subjects did not increase $C_{Ur}/C_{In} \times 100$, although $C_{osm}/C_{In} \times 100$ increased 9.0%. ¶ These experiments suggest that, in man, a major change in fractional PT reabsorption does not occur consistently after an increase in extracellular volume, unless some subliminal drug effect is first produced on the PT, or PT function is somehow altered by a major DT diuresis (perhaps by feedback).

Enzymatic Synthesis of Urobilinogen. ROGER LESTER, NANCY H. DAWBER, AND ROBERT F. TROXLER, Boston, Mass. (introduced by Chester S. Keefer†).

It has been demonstrated previously that mixed cultures of living intestinal bacteria can reduce bilirubin to urobilinogen. The present communication describes the *in vitro* reduction of bilirubin to urobilinogen by broken cell preparations of mixed fecal bacteria. ¶ Fecal bacteria grown anaerobically for 48 hours were harvested, washed, and sonicated. The sonicate was incubated with bilirubin in 0.1 M phosphate buffer, pH 8.0, for 5 hours, and urobilinogen formation was determined by the method of Fischer. ¶ Bilirubin was converted to urobilinogen by intact and sonicated preparations of intestinal bacteria. Anaerobic conditions were essential for the reaction when performed *in vitro*. Per cent conversion of bilirubin to urobilinogen depended in part on the relative concentrations of substrate and "enzyme" (sonicate), but as much as 7.9 μmoles of urobilinogen was produced from 8.4 μmoles of bilirubin. Mesobilirubin was converted to urobilinogen as effectively as bilirubin, which is consistent with the accepted concept that mesobilirubin is an intermediate in urobilinogen biosynthesis. ¶ Separate incubations of the supernatant and pellet obtained from centrifuged ($105{,}000 \times g$) sonicated cells formed no urobilinogen, but recombined supernatant and pellet produced urobilinogen as effectively as uncentrifuged controls. When enzyme was dialyzed for 24 hours at 2° C against distilled water, 70% of the activity was lost. Urobilinogen was not produced by intact or sonicated cells incubated without bilirubin, by bilirubin incubated without cells or with boiled cells, or by bilirubin incubated with rat liver homogenates. ¶ These results establish that bilirubin can be reduced to urobilinogen *in vitro*. They suggest that the reaction is enzymatic, that the enzyme is bound to bacterial membranes, and that soluble cofactors are required by the system.

The Induction of Hemoglobin Formation in Erythroid Cell Culture by 5β-H Steroid Metabolites. RICHARD LEVERE, ATTALLAH KAPPAS,* AND S. GRANICK, New York, N. Y.

Porphyrin and heme synthesis is regulated by δ-aminolevulinic acid synthetase (ALAS), the initial and rate-limiting enzyme in this biosynthetic pathway. Certain 5β-H steroid metabolites stimulate porphyrin production in liver cells by inducing this enzyme. The possibility that these steroids could also induce heme and hemoglobin formation in erythroid cells was studied with *in vitro* cultures of de-embryonated chick blastoderms. ¶ Blastoderms obtained before the 6-somite stage permit study of erythrocyte precursors from a time before initiation of hemoglobin synthesis until full maturation, and simulate a phased erythroid culture since growth on glucose-agar inhibits maturation of all cells except the hemopoietic mesoderm. The blastoderms were bisected, with one half serving as control and the other half being treated with steroid. After incubation, the hemoglobin formed in each half (>0.10 μg) was quantitated by difference spectrometry of the reduced versus CO derivative. ¶ The formation of hemoglobin in cultures treated with certain 5β-H steroid metabolites was significantly increased over that in controls. Hemoglobin-inducing steroids include pregnanolone, pregnandiol, 11-ketopregnanolone, etiocholanolone, and 17α-hydroxypregnanolone; all strongly stimulate porphyrinogenesis in liver as well. Precursors of these metabolites, and 5α-H steroids, have weak or no activity. C-21 hydroxylation and glucuronidation abolish inducing action. Steroid induction of hemoglobin formation is not due to erythroid cell replication and is blocked by inhibitors of nucleic acid and protein synthesis. ¶ These experiments define a new biological property of 5β-H steroid metabolites and demonstrate that steroids

can stimulate hemoglobin production directly, by a mechanism independent of renal erythropoietin. Since hemoglobin formation in proerythroblasts is limited by ALAS, steroids probably induce *de novo* synthesis of this enzyme. This steroid action may be important in the physiological control of hemoglobin production.

Virus Complementary Ribonucleic Acid (RNA) in Hamster Kidney Cells Transformed by Adenovirus-Simian Virus 40 (SV40) Hybrid Viruses. MYRON J. LEVIN, PATRICK H. HENRY, PAUL H. BLACK,* AND SHERMAN M. WEISSMAN, Bethesda, Md.

Nucleic acid homology techniques have been used to demonstrate the presence of virus specific nucleic acids in cells transformed *in vitro* by the oncogenic DNA viruses —polyoma, SV40, and adenovirus (adeno) 12. Recently several hybrid viruses containing adenovirus and SV40 genetic materials were discovered. Several hamster cell lines derived from *in vitro* transformation studies with these viruses were examined for RNA complementary to virus DNA. Uridine-^{3}H-labeled RNA extracted from normal and transformed cells was hybridized with virus or hamster DNA by a modification of the Nygaard and Hall method. We demonstrated adenovirus complementary RNA in eleven of twelve transformed cell lines, and SV40 complementary RNA was also detected in several lines. Cells transformed by the adeno 2-SV40 "passage hybrid" virus contained adeno 2 complementary RNA, and in adeno 3-SV40 transformed cells both adeno 3 and SV40 complementary RNA were detected. In cells transformed by a "transfer hybrid," adeno 12-SV40, which acquired its SV40 DNA from the adeno 7-SV40 hybrid virus, RNA complementary to both adeno 12 and SV40 were present. Some of the lines transformed by this hybrid also contained adeno 7 complementary RNA. This finding confirms previous antigenic studies which indicated that adeno 7 DNA accompanied the SV40 genome during transfer from one adenovirus to another. Cell lines transformed by adeno 12 alone contained only adeno 12 complementary RNA. No homology was detected between radioactive normal hamster RNA and virus DNA. ¶ These findings indicate that genetic material from more than one virus may be permanently associated with cells transformed by these hybrid viruses. Although adenovirus 2 alone does not transform cells, the presence of adenovirus 2 complementary RNA in adeno 2-SV40 transformed cells indicates that the genome of this non-oncogenic adenovirus is permanently associated with the host cell.

Immune Mechanisms of Penicillin-induced Coombs Positivity in Man. BERNARD B. LEVINE* AND ANTHONY P. REDMOND, New York, N. Y.

Various groups of patients treated with benzylpenicillin were studied. Red blood cells (RBC) were assayed for surface benzylpenicilloyl (BPO) groups and immunoglobulins by Coombs techniques with monospecific antisera. In one case antibody eluates were prepared. Patients' sera were assayed for penicillin-specific antibodies by hemagglutination (HA) of penicillin-reacted O$^+$ RBC. Haptenic specificity was studied by univalent hapten inhibition. ¶ The findings were as follows: *1*) All patients treated with large amounts of penicillin had benzylpenicilloyl (BPO) haptenic groups covalently coupled to their RBC. Similarly, washed RBC reacted with penicillin in low concentration at pH 7.4 *in vitro* to form BPO-coupled RBC. *2*) Only those patients who also had high titered IgG antibody responses to penicillin had Coombs-positive RBC; patients with only IgM antibodies or very low titered IgG responses did not have Coombs-positive RBC. *3*) Coombs positivity was demonstrated with anti-IgG, IgM, and IgA sera. *4*) Antipenicillin antibodies were in part BPO specific (by specific inhibition with BPO-propylamine), and in part apparently specific for the benzylpenaldic acid hapten group. The latter HA antibodies were not inhibitable by BPO-haptens, but were specifically inhibitable by a crude preparation of benzylpenaldoyl-propylamine (a degradation product of BPO-propylamine). ¶ The results indicate that penicillin-induced Coombs positivity is mediated by antibodies specific for penicillin haptens (BPO and apparently benzylpenaldic acid), and not for RBC structures alone. The development of BPO-specific Coombs positivity appears to involve the covalent coupling of RBC with BPO groups by chemical reaction with penicillin *in vivo,* followed by specific binding of BPO-specific antibodies from plasma. Both relatively intense BPO coupling of RBC and a relatively high-titered (or high avidity or both) BPO-specific IgG antibody response to penicillin appear necessary for the development of BPO-specific Coombs positivity.

Force-Velocity Relations in Failing and Nonfailing Hearts of Subjects with Aortic Stenosis. HERBERT J. LEVINE, KEVIN M. MCINTYRE, JOSE G. LIPANA, AND OSCAR H. L. BING, Boston, Mass. (introduced by Samuel Proger†).

In nine subjects with pure aortic valvular stenosis, left ventricular (LV) force-velocity relations were determined at 20-msec intervals throughout systole. Three subjects had overt clinical and hemodynamic evidence of LV failure; six did not. The stenotic valve was used as an orifice flowmeter and fiber shortening calculated from the flow rate and ventricular volume (thermodilution). Series elastic velocity was derived from the time derivative of force and the series elasticity (extrapolated from studies of the intact canine LV). Inverse force-velocity curves were observed from the time of peak velocity to the time of peak force. Time to peak contractile element (CE) velocity (mean 45 msec), time to peak force (mean 190 msec), and CE work (mean 34×10^6 dyne-cm per beat) did not differ significantly in the nonfailing and failing groups. The same was true for the ratio of internal/total CE work (mean 41%) and the fraction of fiber shortening work performed by the recoiling series elastic (mean 18.5%). Velocities were normalized for unit length, and V_{max} was estimated by linear extension of the inverse force-velocity curve to zero force. In the non-

failing group, V_{max} averaged 1.69 (1.4 to 1.96) and peak velocity 1.18 (0.84 to 1.40) muscle lengths per sec. In the failing group V_{max} averaged 0.84 (0.70 to 1.07) and peak velocity 0.61 (0.51 to 0.69) muscle lengths per sec. Thus, in these subjects with afterloaded ventricles, the mechanism for force generation is not impaired by the presence of heart failure. CE velocity, on the other hand, is depressed in chronic failure, indicating a negative inotropic state.

The Clinical and Biochemical Definition of Two Forms of Familial Hyperbetalipoproteinemia. ROBERT I. LEVY, RORERT S. LEES, AND DONALD S. FREDRICKSON,* Bethesda, Md.

In study of 121 kindreds with familial lipoprotein abnormalities, a genetically determined hyperbetalipoproteinemia (type III) has been sharply delineated from the type II syndrome, the more common form of "familial hypercholesterolemia." ¶ Both types II and III are associated with xanthomatosis, premature atherosclerosis, and hypercholesterolemia manifested by increased concentrations of lipoproteins with beta mobility and immunochemical identity to normal beta lipoproteins. In contrast to type II the bulk of beta lipoproteins in type III have abnormally low density, appearing in the supernatant after centrifugation at density 1.006, where normally only prebeta lipoproteins and chylomicrons are found. ¶ Study of 120 affected members (57 kindreds) indicates that, whereas triglyceride concentrations in type II are often slightly elevated (to 500 mg per 100 ml), the serum lipids are stable and respond sluggishly to diet or drugs. The heterozygote has elevated beta lipoproteins in childhood and often develops tuberous and tendon xanthomas and coronary vessel disease between ages 20 and 60. ¶ By contrast, in type III the concentrations of triglycerides, which average 500 mg per 100 ml (range 80 to 2,000), and cholesterol are extremely labile. As observed in 34 affected individuals (25 kindreds) type III is probably inherited as an autosomal recessive, with delayed expression, particularly in females. Tendon xanthomas (11/34) and "tubero-eruptive" lesions (25/34) occur, but palmar xanthomas (27/34) are a peculiar feature. Nine of 11 males over age 45 had coronary and peripheral vascular disease. ¶ The beta lipoproteins in type III have normal proportions of free/esterified cholesterol, lecithin/sphingomyelin, and cholesterol/phospholipid/protein, but appear bound to excessive amounts of glyceride. This defect persists even when plasma concentrations of glyceride and beta lipoproteins have been lowered to normal. The removal mechanisms for glyceride, both endogenous and exogenous, seem defective in type III, but the basic abnormality remains to be determined.

The Effect of Erythropoietin on Erythroid Committed Cells. JERRY P. LEWIS, LOIS F. O'GRADY, ROBERT D. LANGE, AND FRANK E. TROBAUGH, JR., Chicago, Ill. (introduced by Mark H. Lepper†).

While studying the early stages of erythropoiesis, we have gathered evidence suggesting that erythropoietin is required for maturation of erythroid tissue, but not for differentiation of hematopoietic stem cells. When hematopoietic tissue is injected into nonplethoric irradiated mice, stem cells seed in the spleen and produce colonies of erythroid, granulocytic, or megakaryocytic cells. During the first 10 days the number of cells in erythroid colonies doubles every 12 to 14 hours, but it is only after the fifth day post-transplantation that stem cell renewal occurs. However, when hematopoietic tissue is injected into plethoric irradiated mice, no erythroid colonies develop and there is no increase in the number of nonerythroid colonies. ¶ In the present studies, plethoric CAF_1 mice were irradiated, transfused with isogenic marrow cells, and given 0.2 U of erythropoietin daily either for 8 days or for 4 days at varying time intervals after transplantation. Animals were sacrificed on the fifth or eighth day, and their spleens were prepared for analysis of hematopoietic colonies. ¶ These studies demonstrate that *1*) erythropoietin is required to form erythroid colonies; *2*) erythropoietin given for 4 days early after transplantation effects small colonies that disappear by day 8; *3*) erythropoietin given for 4 days immediately preceding sacrifice produces colonies similar in size to those produced by 8 days of stimulation. ¶ If the colonies that were stimulated for only 4 days had started from a single cell, their cells would have had to double in number every 6 hours, a figure inconsistent with the measured growth rate of erythroid colonies. These colonies must have originated from a cluster of cells, not functioning stem cells, which differentiated into erythroid committed cells at the time of seeding and doubled several times in the absence of erythropoietin, but required erythropoietin to complete their development into recognizable erythroid elements.

Difference in Hepatic Metabolism of Long and Medium Chain Fatty Acids; the Role of Fatty Acid Chain Length in the Production of the Alcoholic Fatty Liver. CHARLES S. LIEBER,* ANDRE LEFEVRE, AND LAWRENCE FEINMAN, New York, N. Y.

We found previously, both in men and rats, that ethanol ingestion results in hepatic deposition of dietary long chain fatty acids (LCFA). To assess the role of fatty acid chain length in the production of fatty liver, we fed rats, over 24 days, ethanol (or isocaloric carbohydrate in the controls) in liquid diets, with 41% of total calories as either an olive-corn oil mixture (comprising LCFA) or medium chain triglycerides (composed of medium chain fatty acids: MCFA). Compared to controls, in the rats given ethanol, hepatic triglycerides increased eight times with LCFA but only three times with MCFA. ¶ The mechanism of this significant reduction in steatosis was studied in isolated perfused rat livers by comparing oxidation to CO_2 and incorporation into esterified lipids of chylomicron-^{14}C and palmitate-^{14}C (representing LCFA) or octanoate-^{14}C (as MCFA). Ethanol depressed $^{14}CO_2$ production from all substrates, but MCFA were oxidized much more and esterified much less than LCFA: the ratio of $^{14}CO_2$ to esterified lipids was one hundred times

greater with MCFA than with LCFA. Similar results were obtained in liver slices incubated with palmitate-^{14}C or octanoate-^{14}C. Furthermore, in hepatic microsomal preparations incubated with α-glycerophosphate, octanoate was esterified at a significantly lesser rate than palmitate. The propensity of MCFA to oxidation rather than to esterification provides a likely explanation for the reduction in alcoholic steatosis through replacement of LCFA by MCFA. This is further supported by the observation that in rats fed MCFA, hepatic triglycerides comprised endogenously synthesized fatty acids, with only traces of MCFA, both in ethanol and control groups. ¶ In conclusion, the striking reduction of alcoholic fatty liver upon replacement of dietary LCFA by MCFA can be explained by the observation that in isolated perfused livers, liver slices, and microsomal preparations, MCFA are much less esterified than LCFA, whereas conversely, oxidation of MCFA to CO_2 is much greater.

Specific Binding and Sphering of γG-Globulin-coated Red Cells by Human Monocytes and Splenic Macrophages. ALBERT F. LOBUGLIO AND JAMES H. JANDL,* Boston, Mass.

It is unknown how antibodies that do not cause spherocytosis, agglutination, or hemolysis *in vitro* readily induce spherocytosis and sequestration of red cells *in vivo*. In exploring this, we have evaluated interactions between antibody-coated red cells and leukocytes using surface monolayer preparations of human leukocytes. Whereas C′-fixing γM antibodies caused mixed agglutination and erythrophagocytosis, involving granulocytes and monocytes, red cells coated with non-C′-dependent, γG isoantibodies and "autoantibodies" were bound selectively and very firmly by monocytes. After monocytes had been detached from the monolayer surface with EDTA, sensitized red cells remained bound to monocytes in rosette patterns. Monocytes clutched γG-coated red cells by multiple foot processes, but phagocytosis seldom followed despite optimal conditions. Human spleen macrophages were identical to blood monocytes in their interactions with sensitized red cells. Bound red cells rapidly became spheroidal, and their osmotic fragility increased greatly, whereas unbound red cells nearby were unaffected. ¶ Specific binding to monocytes was peculiar to red cells coated with γG antibodies and did not require C′ or Ca^{++}. Binding was inhibited completely by free γG-globulin or sulfhydryl blocking agents, but only partially by γM-globulin or heparin. Red cells coated by nonimmunological means with γG-globulin adhered similarly to monocytes, whereas cells rendered strongly Coombs positive by coating with C′ or with other plasma proteins did not adhere or form rosettes. ¶ It is concluded that monocytes and splenic macrophages possess unique surface receptors for γG-globulins and thereby for apprehending red cells coated with incomplete antibodies. The tight binding of antibody-coated cells by monocytes is not usually a prelude to phagocytosis, but it is rapidly injurious as indicated by spherocytosis. This specific interaction provides a mechanism for the sphering and destruction *in vivo* of red cells coated with γG-antibodies or "autoantibodies" and may be instrumental in the catabolism of γG-globulin itself.

Distribution of Maternal and Fetal Placental Blood Flow during Hypoxia. LAWRENCE D. LONGO, GORDON G. POWER, AND ROBERT E. FORSTER,† Philadelphia, Pa.

Previous studies of placental circulation in sheep showed uneven distribution of maternal placental blood flow ($\dot{Q}_M$), fetal flow ($\dot{Q}_F$), and maternal flow/fetal flow ($\dot{Q}_M/\dot{Q}_F$). About 55% of the placenta had a $\dot{Q}_M$ and $\dot{Q}_F$ of between ±50% of the mean flow per unit weight. Fifty per cent of the placenta had a $\dot{Q}_M/\dot{Q}_F$ within ±50% of its mean value. The coefficient of correlation of maternal to fetal flow was low ($r = 0.066$). (These findings were based on the assumption that the distribution of radioactive labeled macroaggregates of albumin, injected into the ewes' left ventricle and the umbilical vein of the fetus, reflects the distribution of blood flow.) ¶ Our present studies were performed to test the hypothesis that the distribution of placental flow is influenced by the maternal or fetal P_{O_2} or by both. Five ewes were studied after they had breathed 10 to 12% O_2 for 10 minutes. The maternal arterial P_{O_2} was 40 to 47 mm Hg (normal 88 to 95 mm Hg), and P_{CO_2} was 25 to 27 mm Hg (normal 28 mm Hg). The P_{O_2} of the umbilical vein was 16 to 20 mm Hg (normal 25 to 30 mm Hg) and of the umbilical artery was 9 to 15 mm Hg (normal 10 to 15 mm Hg). During maternal hypoxia the distribution of placental blood flow became more uniform. The percentages of placental weight with between ±50% of the mean flow per unit weight were, for the maternal placental flow, 70%, and for the fetal placental flow, 85%. Seventy-seven per cent of the placenta by weight had a $\dot{Q}_M/\dot{Q}_F$ within ±50% of the mean value. The coefficient of correlation of maternal to fetal flow was high ($r = 0.53$, $p < 0.01$). In two experiments while the ewe breathed room air, when a major branch of the umbilical artery was ligated, the distribution of $\dot{Q}_M$, $\dot{Q}_F$, and $\dot{Q}_M/\dot{Q}_F$ became more uniform in the remaining $\frac{2}{3}$ to $\frac{3}{4}$ of the placenta. We conclude that in response to maternal hypoxia or compromise of the placental circulation the distribution of $\dot{Q}_M$, $\dot{Q}_F$, and the $\dot{Q}_M/\dot{Q}_F$ becomes more uniform. This redistribution of flows increases the efficiency of placental O_2 exchange.

The Effects of Aflatoxins on CFW Mice. DONALD B. LOURIA* AND J. KELLY SMITH, New York, N. Y.

Aspergillus flavus elaborates several fluorescent toxins (aflatoxins) that produce profound hepatic damage in experimental animals. Studies were performed to analyze the tumorigenic effect of aflatoxins in CFW mice. A combination of aflatoxins B-1, B-2, and G-1 was given subcutaneously or by force feeding; 100 μg daily 5 days a week for 1 year was administered. Other mice were given aflatoxin, 100 μg per ml, by aerosol for 5 minutes, twice daily. Of 7 mice treated subcutaneously, all developed sarcomas between the fifth and tenth months. Electron microscopic examination of 2 of the tumors

showed no virus particles attached. The sarcomas could be readily transferred to weanling CFW mice by injection of 10% tumor cell suspensions. When tumor extracts were studied by thin and thick layer chromatography and ultraviolet absorption, aflatoxin-like substances were readily identified. Three of the 7 also showed leukemia or lymphoma or both invading liver, lung, spleen, and kidneys. All 12 controls remained well; 6 were sacrificed between the eighth and eleventh months, and none showed evidence of leukemia, lymphoma, or sarcoma (other studies show that white Swiss mice have a spontaneous leukemia-lymphoma incidence of up to 12%). Of 7 mice force-fed aflatoxin, 1 became ill and showed lymphoma-leukemia at autopsy; the other 6 remained well. Three of these were sacrificed after 11 months and showed no cirrhosis or hepatomata. Preliminary data from 20 mice given aflatoxin aerosol suggest a substantial increase in the incidence of leukemia-lymphoma in mice dying or sacrificed 4 to 12 months after initiation of the experiment (28% thus far). No lung carcinomas have been found. These studies suggest that aflatoxins more readily cause local sarcomas than liver damage in certain strains of mice and that they also produce systematic neoplasms either due to a direct effect on host cells or by enhancing latent tumor viruses.

An Electrical Method for Assessing the Degree of Digitalization. BERNARD LOWN* AND ROBERT CANNON, Boston, Mass.

Previous studies have indicated that digitalization to near toxic levels lowers the electrical energy necessary to provoke ventricular tachycardia (VT) by a factor of 2,000. In these earlier investigations electrical shock was external and triggered to fall within the QRS complex. ¶ In ten dogs recovering from ouabain-induced VT, the entire cardiac cycle was explored by electrical stimuli through either a unipolar right intraventricular or a left intramyocardial electrode. A zone of marked sensitivity was found, characterized by repetitive ventricular response (RVR) to a single stimulus. This zone was unrelated to the ventricular vulnerable period and extended from the downslope of the T-wave far into diastole. Whereas before digitalization it required 5.9 joules to evoke RVR, after ouabain the threshold for RVR was reduced by 6 orders of magnitude to a mean energy of 4.2 μjoules. Results were similar whether stimulation was through a cavitary or intramyocardial electrode. With recovery from digitalization the zone of RVR receded by a moving inward of the outer boundary toward the T-wave. Sensitivity persisted for a mean duration of 270 minutes after the animals recovered from overt ouabain intoxication. ¶ In ten other animals a similar study was carried out during digitalization with acetylstrophanthidin (AS). RVR appeared after 54% $\pm$ 13% of the toxic dose. Again the reduction in threshold was 6 orders of magnitude and extended far through diastole. As digitalis toxicity was approached, a single stimulus resulted in paroxysms of VT identical in morphology to those evoked by AS alone. ¶ To date there has been no method for determining the degree of digitalization; administering small electrical pulses during diastole provides information not otherwise obtainable.

An Unusual X Chromosome. HERBERT A. LUBS, New Haven, Conn. (introduced by F. D. Gray†).

A group C chromosome with an unusual secondary constriction near the end of the long arm has been observed in a normal mother and two retarded sons. It was present in 13 to 33% of cells and most characteristically gave the appearance of very large satellites. In other cells it appeared as a single terminal chromatid break or satellite. The chromosome was most frequently between no. 7 and no. 8 in size, and the short arms were relatively long. These findings suggested that it might be the X chromosome. Thymidine-^{3}H labeling studies in the mother showed that of 20 cells in which both the unusual secondary constriction and a single late replicating C chromosome were present, 6 had a late replicating chromosome with the secondary constriction. These results constitute strong evidence that the secondary constriction is located on the X chromosome and permitted a number of additional observations to be made concerning the behavior of the human X chromosome. In both maternal cells where this chromosome was not the differentially late replicating chromosome and in her sons' cells, the long arm was relatively late replicating, usually being as heavily labeled as the most heavily labeled B chromosome. The short arm was early replicating. In maternal cells where both X chromosomes could be identified, one by the presence of the secondary constriction and the other as the latest replicating C chromosome, no consistent difference in size or arm ratio between the homologues could be detected. ¶ The presence of mental retardation and other anomalies in the two males with this chromosome but not in their mother suggests that this chromosome lesion, like an X-linked recessive gene, may be expressed in the hemizygous male and therefore may have clinical as well as biologic significance. In addition, by demonstrating that either of the two X chromosomes could be late replicating, these studies provide additional cytological confirmation of the Lyon hypothesis.

Regulatory Control of Pyrimidine Synthesis during Tranformation of Lymphocytes *In Vitro*. ZOLTAN J. LUCAS, Durham, N. C. (introduced by Ivan Brown, Jr.†).

Lymphocytes cultured from human blood can be stimulated to undergo transformation into blast cells by a number of agents. The morphologic changes induced by one such mitogen, phytohemagglutinin, are preceded by a period during which the rate of incorporation of uridine-^{3}H into RNA increases exponentially. The kinetics of incorporation of uridine-^{3}H into RNA parallel the rate of labeling of the intracellular pool of acid-soluble nucleotides and coincide with a tenfold increase in the specific activity of uridine kinase in extracts prepared by sonication. The specific activities of cytidine and adenosine

kinase also increase in stimulated cells, whereas the uridylate, cytidylate, and adenylate kinases remain constant. Puromycin and actinomycin inhibit morphologic transformation, uridine incorporation, and the increase in uridine kinase activity. ¶ The incorporation of uridine by lymphocytes and the induction of uridine kinase are subject to regulatory control by cytidine ribonucleotides. Cultures grown in the presence of 10^{-4} M cytidine do not incorporate uridine-^{3}H into RNA and do not show the increase in uridine kinase activity; morphologic transformation and RNA synthesis as measured by adenosine-^{3}H incorporation are not inhibited. The activity of uridine kinase is completely inhibited by 10^{-4} M CTP. These observations suggest that an early consequence of stimulation by phytohemagglutinin is the depletion of the intracellular levels of CTP, which normally represses uridine kinase synthesis. The increase in uridine kinase synthesis then allows for a re-expansion of the nucleotide pool via the "salvage" pathway of pyrimidine nucleotide synthesis. This hypothesis would predict that in lymphocytes undergoing transformation *in vitro* there is no fluctuation in the activities of the enzymes of the *de novo* pyrimidine biosynthetic pathway.

Reduced Cardiac Myosin ATPase Activity in Dogs with Spontaneously Occurring Congestive Heart Failure. ROBERT J. LUCHI AND EVE M. KRITCHER, Philadelphia, Pa. (introduced by Hadley L. Conn, Jr.*).

Several biochemical abnormalities have been described in human and animal congestive heart failure. Reduced myofibrillar ATPase activity has been demonstrated in human heart failure, and di- or trimerization of myosin molecules has been suggested as a cause of the decreased force of myocardial contraction in dog congestive heart failure. A detailed study of cardiac myosin was undertaken with the LiCl-$(NH_4)_2SO_4$ technique for purifying myosin. Cardiac myosin was obtained from five dogs with congestive heart failure (F) secondary to spontaneously occurring mitral insufficiency. Fifteen dogs without heart failure served as controls (C). The following results were obtained: molecular weight: C = 500,000 ± 20,000, F = 528,000 ± 36,000 ($p > 0.1$); % helix: C = 58.2, F = 57.7 ($p > 0.1$); intrinsic viscosity: C = 2.08 dl per g, F = 2.00 dl per g; $S_{20,w}$: C = 6.0, F = 6.0; ATPase activity: C = 4.44 μmoles P per mg per 5 minutes, F = 3.66 μmoles P per mg per 5 minutes ($p < 0.01$); SH groups: C = 41.5 per mole, F = 41.0 per mole; and tyrosine-tryptophan content: C = 1.78×10^{-4} mole tyrosine per g and 0.35×10^{-4} mole tryptophan per g, F = 1.94×10^{-4} mole tyrosine per g and 0.39×10^{-4} mole tryptophan per g ($p > .01$). Thus, the myosin molecule in congestive heart failure is different from the normal only in its enzyme activity. This difference cannot be the result of a gross configurational change in F myosin in view of the similarity of the hydrodynamic data and helical content to those of the control. The significantly lower ATPase activity in cardiac myosin of dogs with established heart failure undoubtedly plays a role in the impaired cardiac muscle performance observed in that state.

Effect of 2,4-Dinitrophenol on Thyroid Response to Thyrotropin. M. L. MAAYAN AND I. N. ROSENBERG,† Boston, Mass.

Previous studies have shown that administration of 2,4-dinitrophenol (DNP) depresses thyroid function; among the proposed mechanisms have been inhibition of pituitary TSH secretion and decreased thyroid responsiveness to circulating TSH. Recent findings of Bakke and Lawrence suggest that TSH secretion is unimpaired in DNP-treated rats. The present study was undertaken to learn whether DNP affects thyroid responses to TSH. ¶ Rats were fed Purina chow containing 0.2% DNP for 7 to 14 days; this resulted in substantial but proportional decreases in body and thyroid weight; serum PBI and thyroidal ^{131}I uptake were significantly lowered. Thyroid content of pyridine nucleotides and their oxidoreduction state were unaffected. ¶ The DNP regimen prevented *1)* goitrogenesis from 0.03% PTU added to the diet; *2)* increase in thyroid weight after 7 daily injections of TSH (2 U); and *3)* a significant rise in thyroid total NADP and in NADPH/NADP ratio 3 hours after a single iv injection of TSH (4 U), all of which occurred in control rats not given DNP. By contrast, TSH (0.1 to 3 U) stimulated glandular ^{131}I release in prelabeled DNP-treated rats as promptly and effectively as in controls. Thyroidal 1-hour ^{131}I uptake and total NADP content rose significantly in both DNP-treated and control rats after 7 daily injections of 2 U TSH. Since the TSH-induced thyroidal release of ^{131}I was unimpaired by DNP, the absence of a significant rise in serum PBI 3 hours after TSH in the DNP group as compared with the controls reflects a shortened plasma half-time of hormone secreted from the thyroid. ¶ The results suggest that the thyroidal growth response to TSH is blocked in DNP-treated animals, but other glandular responses to TSH, especially the enhancement of glandular uptake and release of iodine and rise in NADP content, are little affected.

Iodination of Human Intrinsic Factor. IAIN L. MACKENZIE, ROBERT M. DONALDSON, JR.,* AND ROBERT F. SCHILLING,† Madison, Wis.

A radioactively labeled intrinsic factor (IF) preparation would be of value in studying IF function. Human IF saturated with ^{60}Co-labeled cyanocobalamin (B_{12}-^{60}Co) was purified by the method of Chosy and Schilling to yield IF preparations that bound 1.0 μg B_{12}-^{60}Co per 10 to 16 μg N. The method of Hunter and Greenwood was modified to label these with iodine-^{125}I. Preparations studied had a specific activity of 0.4 to 3.0 μc ^{125}I per μg N. Column chromatography of the iodinated IF-B_{12}-^{60}Co complex on Sephadex G200 and DEAE-cellulose showed coincidence of the ^{60}Co peak and the major ^{125}I peak. Starch gel electrophoresis of the iodinated complex demonstrated coincidence of the ^{125}I and ^{60}Co peaks at the same anodal distance as the ^{60}Co peak of the noniodinated complex. Preincubation of both iodinated and noniodinated complexes with intrinsic factor antibody II

(AB2) abolished electrophoretic migration of both ^{125}I and ^{60}Co. Coombs serum precipitated equal fractions of ^{60}Co from the iodinated complex incubated with AB2 compared with the noniodinated complex similarly incubated. The fractions of ^{125}I and ^{60}Co precipitated from the iodinated complex were also similar. After exchange of B_{12}-^{60}Co from the iodinated complex with intrinsic factor antibody I by incubation at 30° C for 24 hours, Coombs serum precipitated ^{125}I. Urinary ^{60}Co excretion in pernicious anemia patients after oral administration of 0.5 μg B_{12}-^{60}Co bound to freshly prepared iodinated IF was similar to that obtained with noniodinated IF. Urinary ^{60}Co excretion after oral administration of the iodinated complex stored at −20° C for 1 to 2 months, although lessened, was still greater than after free B_{12}-^{60}Co. Starch gel electrophoresis of these stored preparations demonstrated no change in antigenicity. These results show that iodination of partially purified human IF does not markedly alter its chromatographic, electrophoretic, antigenic, or biologic properties. This tracer may be of value in studying IF metabolism.

Studies of the Relationship of Metabolism to Active Transport. Roy H. Maffly* and Wallace W. Jones, Palo Alto, Calif.

Hormones, drugs, and metabolites that affect active membrane transport may act on *1*) the transport mechanism itself, *2*) the link between the transport mechanism and energy, or *3*) the metabolic pathways that supply energy. If the action of the agent is on either *1* or *2*, a change in rate of transport should precede a change in metabolism; whereas if the action is at *3*, changes in transport should follow changes in metabolism. ¶ Such an analysis has been applied to Na transport by the toad bladder, mounted *in vitro*. Rate of Na transport was measured as the short-circuit current (scc) while CO_2 production was simultaneously monitored with a continuous flow, conductometric method. After the action of a variable, the increment in scc was related to the increment in CO_2 production, and was expressed as equivalents Na per mole CO_2. To produce an action of presumed type *1*, we stimulated Na transport by changing the mucosal bath from choline to Na Ringer. The result ($N = 5$) was an initial high ratio [60 ± 12 (SE) Eq per mole] that fell curvilinearly and leveled off at 15 minutes (20 ± 3 Eq per mole). To produce an action of presumed type *3*, we stimulated Na transport by addition of glucose or pyruvate. With glucose ($N = 5$), the initial ratio was low (3 ± 2 Eq per mole) and rose to a plateau at 30 minutes (15 ± 4 Eq per mole). Results with pyruvate ($N = 3$) were qualitatively similar. ¶ When Na transport was stimulated by vasopressin ($N = 4$), the pattern of the ratio conformed to type *1* rather than type *3* (60 ± 10 Eq per mole dropping to 12 ± 3 Eq per mole in 20 minutes). Since it has previously been concluded from permeability studies that vasopressin increases mucosal permeability to Na and thereby provides a higher Na concentration at the transport site (= type *1*), our results, reached by an entirely different approach, offer strong support of those conclusions. Further studies utilizing this approach are in progress.

The Plasma Disappearance and Erythrocyte Uptake of ^{54}Mn. John P. Mahoney and Kathleen Sargent, Boston, Mass. (introduced by John W. Athens*).

To further elucidate the hematopoietic function of manganese, we have examined the plasma disappearance and erythrocyte uptake of ^{54}Mn. ¶ Carrier-free ^{54}Mn (half-life 310 days) preincubated with autologous plasma was injected intravenously into nine subjects who had elevated reticulocyte counts in response to therapy for treatable anemias. Blood was withdrawn from the opposite arm at 20-second intervals. Disappearance of radioactivity from the plasma was determined. Thereafter, blood was withdrawn at intervals, and erythrocytes and isolated hemin were analyzed for radioactivity. ¶ Plasma disappearance curves plotted on semilogarithmic paper revealed that 80% or more of the radioactivity disappeared from the plasma as a simple exponential with a $t_{\frac{1}{2}}$ of between $1\frac{1}{2}$ and 5 minutes, and that the remainder of the radioactivity declined at a much slower rate. ¶ Radioactivity in erythrocytes increased with maximal uptake between 9 and 35 days. Between 0.5 and 9.0% of the total administered radioactivity was incorporated irreversibly into erythrocytes, and 60 to 70% of the erythrocyte radioactivity was recovered in a crystalline hemin preparation. ¶ The erythrocyte uptake was of the same order of magnitude as that reported by Borg (1958), and the radioactivity remained throughout the life-span of the cell. ¶ Radiomanganese was incubated for 4 hours *in vitro* with a hemolysate of chicken erythrocytes (Goldberg, 1958). Heme crystallized from the incubation mixture contained between 0.01 and 1.7% of the added radioactivity. Cyanide totally inhibited this process. ¶ These data suggest that manganese is rapidly cleared from the plasma and then exchanges slowly with at least one extra plasma pool. The metal is irreversibly incorporated into erythrocytes, and available evidence indicates that it is present in erythrocytes as a manganese porphyrin.

Metaphase Arresters: Reversible Effects on the Living Mitotic Spindle, and a Possible Mechanism for Side Effects on Nondividing Cells. Stephen E. Malawista, Hidemi Sato, and Klaus G. Bensch, New Haven, Conn., and Philadelphia, Pa. (introduced by J. W. Hollingsworth*).

Fresh unfertilized oocytes of the marine annelid, *Pectinaria gouldi*, persist for several hours at the first meiotic metaphase. The metaphase spindle is a highly organized protoplasmic gel, and the content of oriented spindle material in these oocytes can be measured in polarized light as retardation induced by spindle birefringence. We have studied spindle effects of some metaphase-arresting agents both in live cells and in cells fixed for electron microscopic study. ¶ Perfusion with the *Vinca* alkaloid, vinblastine (1×10^{-5} M), resulted in a decrease in birefringence and size of the spindle and, within 6 to 12

minutes at 24° to 25° C, complete dissolution. On subsequent perfusion with artificial sea water, recovery of spindles began in about 20 minutes and was complete by 50 minutes. This reversible effect was repeatable in the same preparation and was at least as efficient as that of *N*-desacetyl-*N*-methylcolchicine (Colcemid, 1×10^{-5} M). Neither dissolution nor recovery was affected by glutamate, 1×10^{-3} M. In contrast, vincristine and colchicine each required much higher concentrations for similar efficiency of dissolution, and recovery was often incomplete, leaving small tri- and tetrapolar spindles. ¶ In response to griseofulvin (1×10^{-5} M), the spindle became less birefringent and smaller, disappeared in 3½ to 6½ minutes, and recovered completely in 5½ to 11 minutes. This rapid, reversible dissolution could be carried out repeatedly in a single oocyte; recovery was complete even with a tenfold increase in concentration of griseofulvin. ¶ Electron micrographs showed a loss of microtubular elements in spindles treated with vinblastine, Colcemid, and griseofulvin. ¶ Examining effects on spindle architecture may provide insight into the mechanism of action of these drugs in other gelated cellular systems. We have recently shown that colchicine affects microtubular elements in mature polymorphonuclear leukocytes. Thus, "metaphase arresters" may possibly interfere with the protoplasmic gels necessary for stability of form, motion, and perhaps other functions in nondividing cells.

Hexokinase Isoenzymes in Human Erythrocytes. JOHN I. MALONE, EDWARD W. HOLMES, JR., ALBERT I. WINEGRAD,* AND F. A. OSKI, Philadelphia, Pa.

Multiple molecular forms of hexokinase with differing physical and kinetic properties are known to exist in mammalian tissues. ¶ On starch gel electrophoresis hexokinase activity in normal adult erythrocytes was found to be associated with two electrophoretically distinct proteins designated types I and III. Type II hexokinase was not observed in normal adult erythrocytes. No difference in hexokinase isoenzyme pattern was observed in "young" and "old" cells prepared by ultracentrifugation of normal adult erythrocytes. Red cells from 17 of 17 newborn infants studied within the first 5 days of life contained type II hexokinase; in five instances it was the only isoenzyme demonstrated. Thus, hexokinase, a probable rate-limiting step in glycolysis, may be mediated by distinct proteins in fetal and adult erythrocytes. Types I and II hexokinase have been separated from hemolysates by DEAE-cellulose chromatography and have Km for glucose of 5.3×10^{-5} and 1.4×10^{-4} M, respectively. ¶ An association of type II hexokinase and fetal hemoglobin was suggested by the observation that electrophoresis of hemolysates from a newborn "dysmature" infant demonstrated only hemoglobin A and types I and III hexokinase. Samples were obtained from a well-studied family at Johns Hopkins that includes a homozygote (age 7) for the persistent fetal hemoglobin trait; type II hexokinase was present in the homozygote's erythrocytes and in those of the adult heterozygotes. The presence of type II hexokinase is the first evidence that the erythrocytes in hereditary persistence of fetal hemoglobin differ from normal adult cells in any aspect other than hemoglobin type. The data suggest a relationship between the regulation of type II hexokinase and γ-chain synthesis. ¶ Adults homozygous for hemoglobin S have been found to have a distinctive erythrocyte hexokinase isoenzyme pattern that includes an intensely staining band in the region of type II.

Beta-Adrenergic Blockade and Left Ventricular Performance in Patients with Heart Disease. HAROLD W. MARCH AND HENRY T. LEW, San Francisco, Calif. (introduced by Elliot Rapaport*).

Propranolol, a beta-adrenergic blocking agent, was administered intravenously to 29 patients with aortic and mitral valve disease during right and left heart catheterization at rest and exercise. The dose was 0.13 mg per kg. There were significant differences between mean heart rates at rest before (76 per minute, SE ± 3) and after (67 per minute, ± 3) propranolol ($p < 0.05$) and at exercise before (124 per minute, SE ± 6) and after (95 per minute, ± 6) the drug ($p < 0.01$). There were no statistically significant differences after propranolol in the group as a whole for the following parameters of ventricular performance at rest or exercise: oxygen consumption, arteriovenous difference, cardiac index, left ventricular stroke or minute work, LV diastolic or mean left atrial pressures or both, LV end-diastolic volume, pulmonary and systematic vascular resistances, and LV dp/dt. Since patients with heart failure may be catecholamine depleted, the patients with normal cardiac indexes and normal LV diastolic or mean LA pressures or both were compared with those who had low cardiac indexes and elevated pressures. There was no significant difference between these subgroups. It is concluded that except for heart rate, intravenous propranolol does not have a significant effect on left ventricular performance in patients with valvular heart disease whose circulatory status is stable. This is probably due to catecholamine depletion. Moreover, their exercise performance is not highly dependent on adrenergic reserves.

Evidence for a Hormonal Inhibitor of Proximal Tubular Reabsorption. M. MARTINEZ-MALDONADO, N. A. KURTZMAN, F. C. RECTOR, JR.,* AND D. W. SELDIN,† Dallas, Texas.

The principal mechanism mediating saline diuresis is inhibition of proximal tubular reabsorption. It has been suggested that a humoral factor ("third factor") is responsible for the inhibition, and some evidence for this has been obtained from cross circulation experiments. To determine the presence of such a hormone, we examined the effect of infusing plasma from antidiuretic animals and from animals undergoing saline diuresis on the intrinsic reabsorptive capacity of the proximal tubule of rats. Intrinsic reabsorptive capacity was measured by the stop-flow microperfusion technique of Gertz. Antidiuretic plasma had no effect on intrinsic reabsorptive

capacity. By contrast, both rat and dog plasma after saline produced a 30% inhibition of proximal tubular reabsorption. Evisceration, and nephrectomy before saline infusion, did not abolish the inhibitory effect of the plasma obtained from such animals. To characterize the nature of the substance, we carried out the following: *1*) Freezing-thawing of the plasma abolished its inhibitory effect; however, storage at 4° C for 5 days did not. *2*) Activity was lost by dialysis and could be demonstrated in the dialysate. *3*) The substance had an effect when infused intravenously or when placed in the tubular lumen. These studies indicate that saline infusion releases a humoral substance that inhibits proximal tubular reabsorption.

Demonstration That Renal Hemodynamics and Plasma Oncotic Pressure Are Mediators of the Natriuretic Response to Volume Expansion. JOSEPH A. MARTINO AND LAURENCE E. EARLEY,* Boston, Mass.

Studies from this laboratory have demonstrated that tubular sodium absorption can relate directly to plasma oncotic pressure and renal vascular resistance, and inversely to perfusion pressure. We suggested that these effects are mediated via changes in the renal interstitial volume. However, causal roles for these factors in the diuretic response to volume loading have not been demonstrated. The present studies were undertaken for this purpose by systematically controlling each of these physical variables. Dogs were infused with either isotonic saline, "plasma" (5% albumin in saline), or donor blood from a reservoir in equilibrium with the experimental animal. When 30% albumin solution was infused to restore plasma oncotic pressure in saline-loaded animals, sodium excretion decreased strikingly despite increases in GFR, RBF, and arterial pressure. Reduced vascular resistance and ipsilateral natriuresis were produced by renal arterial infusions of acetylcholine before infusing plasma or blood, and perfusion pressure was controlled by an aortic clamp. Iso-oncotic plasma produced natriuresis and profound decreases in renal vascular resistance in nonvasodilated control kidneys. In already vasodilated kidneys plasma produced little or no additional natriuretic effect when perfusion pressure was constant, despite increases in GFR and RBF and continued natriuresis in control kidneys exposed to the same pressure. Infusing blood did not reduce renal vascular resistance, and at controlled arterial pressure there was virtually no natriuretic effect in either the vasodilated or control kidneys despite unchanged or increased GFR. ¶ These results indicate that saline infusion depresses tubular sodium absorption in part by decreasing plasma oncotic pressure, and that the entire natriuretic response to plasma or blood loading may be mediated through reduced renal vascular resistance or increased perfusion pressure or both. Therefore, either alone, or in combination, these physical changes appear to be the major physiologic pathways through which volume expansion increases the excretion of sodium.

Stimulation of Aldosterone Biosynthesis in Adrenal Mitochondria by Sodium Depletion. ELISA T. MARUSIC AND PATRICK J. MULROW,* New Haven, Conn.

A unique step in the biosynthesis of aldosterone is the conversion of corticosterone (B) to aldosterone. The present experiments demonstrate that this step occurs in the adrenal mitochondrial fractions of the rat, cow, human, and also of an aldosteronoma. Further studies with rat adrenal mitochondria have shown that Krebs-cycle intermediates, NADPH, calcium, and increasing the B concentration in the incubation media can stimulate the conversion of B to aldosterone, whereas changes in sodium and potassium concentration have no effect. ¶ *In vivo,* sodium depletion stimulates aldosterone biosynthesis. Three possible mechanisms are *1*) stimulation of early steps in the biosynthetic pathway, thus delivering more B to the mitochondria for conversion to aldosterone; *2*) hyperplasia or hypertrophy of the cells or mitochondria of the zona glomerulosa; *3*) stimulation of a specific function of glomerulosa mitochondria, conversion of B to aldosterone. ¶ To study this, we depleted male rats of sodium by dietary sodium restriction. Cell homogenates and mitochondria of adrenal capsules, which contain chiefly glomerulosa cells, were incubated. The mitochondria, from 1-, 2-, and 4-day sodium-depleted rats, converted 60, 231, and 235%, respectively, more B to aldosterone than the control rats. Endogenous B production by homogenates of 2-day sodium-depleted rats was the same as the control rats (5.1 μg per 100 mg tissue), as well as succinic dehydrogenase and 11β hydroxylase activity (DOC to B) of the mitochondria from 4-day depleted rats. Therefore, it is unlikely that early steps in biosynthesis are stimulated by short term sodium depletion. Although a small increase in zona glomerulosa cells or mitochondrial mass is possible, it cannot account for the striking increase in the conversion of B to aldosterone. It is concluded that sodium depletion stimulates the last step in aldosterone biosynthesis by causing a specific enzymatic change in adrenal mitochondria.

The Interrelationship between Serum Calcium, Sodium Excretion, and the Parathyroids on Renal Handling of Calcium. SHAUL G. MASSRY, JACK W. COBURN, LLOYD W. CHAPMAN, AND CHARLES R. KLEEMAN,* Los Angeles, Calif.

Calcium clearance is closely related to that of sodium when excretion of sodium ($U_{Na}V$) is altered by various procedures. This correlation remains unchanged during saluresis even when GFR and filtered calcium are acutely reduced. ¶ In this investigation the relationship between renal handling of calcium and sodium was studied during hypo-, normo-, and hypercalcemia, in the absence and presence of parathyroid hormone (PTE), and with and without saline infusion. Clearance studies were performed in anesthetized dogs [10 normal and 17 thyroparathyroidectomized (T-PTX)] receiving NaCl or $CaCl_2$ or both in different sequences and rates, and, in some, PTE. This relationship is expressed in terms of the clearance

ratios of diffusible calcium/sodium (C_{Ca}/C_{Na}). ¶ In normals this ratio (0.97 ± 0.12, mean $\pm$ SEM) was unchanged during saluresis (0.85 ± 0.06). After T-PTX, C_{Ca}/C_{Na} was significantly increased during hypocalcemia (5.10 ± 0.94) and during normocalcemia (5.15 ± 1.60) and hypercalcemia (3.43 ± 0.69) induced by calcium infusion. With PTE administration to T-PTX dogs, C_{Ca}/C_{Na} became normal during normocalcemia but remained high during hypercalcemia. Natriuresis invariably followed calcium infusion, and $U_{Na}V$ exceeded control rates during hypercalciuria despite acute reduction of GFR and F_{Na} by inflation of an intra-aortic balloon in 7 out of 7 T-PTX dogs. Saluresis produced by saline infusion resulted in lowering of C_{Ca}/C_{Na} regardless of serum calcium, although C_{Ca}/C_{Na} remained above normal during hypercalcemia. ¶ These data indicate that *1*) the normal relation between calcium and sodium reabsorption is altered in the absence of PTE; *2*) proximal sodium rejection is enhanced by calcium infusion; and *3*) during hypercalcemia, either the increased filtered calcium or the high tubular fluid calcium concentration may primarily alter C_{Ca}/C_{Na}. The results are consistent with PTE influencing a small fraction of calcium transport in the distal nephron, but this effect is obscured during the augmented distal delivery of calcium and sodium that follows saline infusion or hypercalcemia.

Bacterial Interference: Its Effect on Reticuloendothelial Function in Embryonated Eggs. WILLIAM R. MCCABE, Boston, Mass. (introduced by Franklin G. Ebaugh, Jr.*).

Prior allantoic infection of embryonated eggs with avirulent staphylococci protects against subsequent challenge with egg-virulent strains of *Staphylococcus aureus, Diplococcus pneumoniae,* various gram-negative bacilli, and influenza virus (A_2J 305). Earlier studies have indicated that host defense mechanisms contribute to the protection induced by an interfering infection. A slight, but statistically significant ($t_{38} = 3.1$, $p < 0.01$), increase in bactericidal activity of whole blood is produced by interference, but this does not appear to be sufficient to explain the marked protection observed. Further attempts to elucidate the mechanism of protection have demonstrated that bacterial interference induces a substantial increase in reticuloendothelial clearance of intravenously injected *S. aureus* by embryonated eggs. The reticuloendothelial system of 12- to 14-day-old normal control eggs clears 95% or more of 10^8 to 10^9 intravenously injected staphylococci within 15 minutes. The initial clearance of intravenously injected staphylococci is exponential, but the rate of clearance decreases after 15 to 30 minutes. Recrudescence of bacteremia occurs, however, and significantly greater numbers ($p < 0.001$) of bacteria are present in the blood of controls 24 hours after intravenous challenge than are present 1 or 3 hours after challenge. Significantly greater clearance ($t_{\infty} = 4.0$, $p < 0.001$) was observed in eggs, previously infected intra-allantoically with interfering strains, at 15 minutes, 1 hour, 3 hours, and 24 hours after intravenous challenge. In addition, no bacterial regrowth occurred in embryos previously infected with an interfering strain, and the number of staphylococci present in the blood progressively decreased throughout the 24- to 48-hour study period. The enhanced reticuloendothelial activity induced by interference was not associated with the appearance or increase in serum opsonins but appeared to reflect increased reticuloendothelial cellular function.

Erythrocyte G-6-PD in Primates. PAUL R. MCCURDY, JOHN SWITZER, AND HANS J. HUSER, Washington, D. C., Kensington, Md., and Atlanta, Ga. (introduced by Laurence H. Kyle†).

In a search for a genetic polymorphism, red cell glucose 6-phosphate dehydrogenase (G-6-PD) was examined from males of 8 species of primates. From 22 *Macaca mulatta* (rhesus), 41 *M. irus* (cynomolgus macaque), 20 *Cercopithecus aethiops* (African green), 4 *Cynopithecus nigra* (black ape), 2 *Zati radiata* (bonnet monkey), and 1 *M. speciosa* (stump-tailed macaque) the G-6-PD had fast electrophoretic mobility on starch gel comparable to that of human G-6-PD A. On the other hand, samples from 2 chimpanzees had G-6-PD with mobility similar to human G-6-PD B. Total G-6-PD activity (single tube technic) was similar to that found in human red cells, e.g., *M. mulatta,* 167 ± 47 IU per 100 ml red cells (μmoles NADPH per minute at 25° C); *M. irus,* 220 ± 59; *Cercopithecus aethiops,* 166 ± 48; human, 174 ± 45 ($n = 160$). No animal had deficiency comparable to primaquine-sensitive Negro males. Kinetic characteristics of G-6-PD, which was partially purified from hemolysates of 4 *M. mulatta* and 4 *Cynopithecus nigra,* were studied. For the *M. mulatta,* the Km G-6-P was 37.8 ± 6.8 μmoles; 2 deoxy G-6-P was used at 5.9% the rate of G-6-P; and the single slightly truncated pH optimum peaked at pH 9.0 to 9.5. For the *Cynopithecus nigra* the Km G-6-P was 53.2 ± 7.8 μmoles; 2 deoxy G-6-P utilization was 4.3%; and the single slightly truncated pH optimum peaked at pH 9.0. For human erythrocyte G-6-PD, comparable figures are Km G-6-P, 52.3 ± 3.3 μmoles ($n = 7$); deoxy G-6-P utilization, less than 4%; the single truncated pH optimum curve peaked at pH 8.5 to 9.0. These kinetic constants are quite similar suggesting that no mutations have seriously affected the active site of this enzyme during the course of evolution. No genetic polymorphism was found in this small series.

A Factor V Anticoagulant: Clinical, Physiological, and Biochemical Observations. WILLIAM G. MCGEHEE, DONALD I. FEINSTEIN, SAMUEL I. RAPAPORT,† AND MARY J. PATCH, Los Angeles, Calif.

A Factor V anticoagulant is exceedingly rare; only one prior patient is described in the English language literature. We have studied an anti-Factor V found transiently in a patient with pancreatitis. Bleeding and laboratory evidence of the anticoagulant persisted for 10 days; studies 7 days thereafter and subsequently were normal. Screening studies at the height of anticoagulant activity showed Quick test 110 seconds (control 17 sec-

onds), PTT 270 seconds (control 48 seconds), thrombin time 40 seconds (control 32 seconds). Specific Factor V level was < 2.5%. Other specific Factor assays were normal except for a Factor XI of 40 and a Factor XII of 28%. Incubating undiluted patient's plasma with normal plasma reduced its Factor V level to 3%; incubating dilutions of patient's plasma revealed that the anticoagulant reacted stoichiometrically with Factor V. Besides native Factor V, the anticoagulant inhibited Factor V that had been exposed to thrombin and to Russell's viper venom. The anticoagulant interfered with the prothrombin-converting activity generated in the TGT test, confirming that Factor V participates in prothrombin conversion. The anticoagulant inhibited platelet-bound Factor V and was effective against rabbit, beef, and dog Factor V. It migrated between gamma and beta on starch block electrophoresis and was found in the second peak on column chromatography with Sephadex G-200. Incubation of patient's plasma with aged serum and with aged plasma (both deficient in Factor V activity) markedly reduced the anticoagulant activity on subsequent incubation with normal plasma. Incubation of patient's plasma with hereditary Factor V deficiency plasma from two patients failed to "consume" anticoagulant activity. Thus, hereditary Factor V deficiency probably represents a failure to synthesize Factor V rather than the presence of a defective Factor V molecule.

***In Vitro* Behavior of Lymphocytes from Patients with Pernicious Anemia.** JAMES E. MCGUIGAN, St. Louis, Mo. (introduced by Sol Sherry†).

Circulating autoantibodies to intrinsic factor and gastric mucosal cells, association with autoimmune thyroid disease, and partial remission after administration of glucocorticoids have suggested an immune basis for the pathogenesis of pernicious anemia (PA). In this study the immunological behavior of lymphocytes from patients with pernicious anemia was examined. Immunologically sensitized lymphocytes undergo morphologic transformation when cultured *in vitro* in the presence of specific antigen. Lymphocyte transformation appears to be correlated with cell-mediated hypersensitivity and is often impaired in diseases associated with relative anergy. Peripheral blood lymphocytes from 13 patients with pernicious anemia and controls were cultured *in vitro* with phytohemagglutinin, extracts of normal gastric mucosal cells, normal human gastric juice, or highly, though not completely purified human gastric intrinsic factor. After culture for 96 hours the lymphocytes were harvested and stained with Wright's stain. The per cent undergoing morphologic transformation was recorded. Cultured lymphocytes from PA patients did not differ from controls in rates of spontaneous transformation and transformation in the presence of phytohemagglutinin. Lymphocytes from each PA patient exhibited increased transformation rates when cultured with each of the three gastric antigen preparations ($p < 0.005$). None of the cultures of lymphocytes from the controls underwent transformation at a greater rate than that of cultures without added antigen. The average per cent transformation of lymphocytes from PA patients when cultured with gastric mucosal cell extracts was 38.2, with normal human gastric juice 43.2, and with intrinsic factor 47.5. Antibodies to gastric parietal cell cytoplasmic constituents detected by immunofluorescent techniques were found in 11 of 13 PA patients. Antibodies inhibiting binding of vitamin B_{12} by intrinsic factor were present in the serum of 3 PA patients. These data indicate the presence of an immune mechanism in patients with pernicious anemia and suggest that cell-mediated hypersensitivity may play an important role in this disease.

Thyroid Function after LATS Production in Rabbits. J. M. MCKENZIE,* Montreal, Quebec.

As recently reported, the long-acting thyroid stimulator (LATS) was produced in rabbits, as shown by assay of the γ-globulin fraction of rabbit antiserum to human thyroid homogenate. In several rabbits protein-bound iodine (PBI) and thyroxine in serum were increased although thyroidal ^{131}I uptake was not. Further studies with those sera disclosed multiple antithyroid antibodies, including antithyroglobulin determined by tanned-red cell agglutination. Paper electrophoresis and radioautography with thyroxine-^{131}I showed binding of thyroxine to γ-globulin as well as to the normal postalbumen protein; agar-gel immunoelectrophoresis and radioautography established that binding was to antithyroglobulin antibody. Free thyroxin was normal in these sera; e.g., in eight control sera (antiliver microsomes), PBI (μg per 100 ml) = 6.0 ± 1.5 SD; free thyroxine (mμg per 100 ml) = 8.1 ± 2.6 SD; in six antithyroid antisera, PBI = 24.5 ± 8.4; free thyroxine = 6.8 ± 3. Therefore hyperthyroidism was not initially present. ¶ Four rabbits have maintained LATS production (< 420% on bioassay of 0.5 ml serum per mouse) for 6 months; repeat radioiodine studies showed increased 24-hour thyroid uptake of ^{131}I in comparison with uptake in eight control rabbits, viz., 50,639 ± 8,107 (cpm, mean ± SD) vs. 17,273 ± 6,835, $p < 0.001$; thyroid ^{131}I release in two was significantly faster than in all controls. Whether enhanced radioiodine uptake and turnover reflect thyroiditis (confirmed at necropsy in one other rabbit) or stimulation by LATS is being tested by attempted thyroid suppression. ¶ Conclusions: *1*) A long-acting thyroid stimulator may be produced by immunizing rabbits against a human thyroid preparation; this supports the antibody nature of LATS. *2*) Antithyroglobulin coincidentally formed can bind thyroxine and may increase serum thyroxine without hyperthyroidism. *3*) Prolonged maintenance of antibody production by "booster" immunization can lead to increased thyroid function; whether "clinical" hyperthyroidism—experimental Graves' disease—will result is being studied.

Kinins—Possible Mediators of Circulatory Changes. K. L. MELMON, A. M. RUDOLPH,* T. HUGHES, A. S. NIES, AND M. J. CLINE, San Francisco, Calif.

At birth immediate circulatory adaptations are necessary for extrauterine life. Among the most important are

constriction of the ductus arteriosus and umbilical vessels, and dilatation of the pulmonary arteries. The mechanism by which these rapid adjustments occur has been obscure. ¶ The kinins are potent vasoactive peptides capable of mediating changes in blood flow. Bradykinin potently constricts the human umbilical artery and vein and the ductus arteriosus of the lamb *in vitro* at oxygen tensions above 40 mm Hg (comparable to those in the neonate) and is theoretically capable of effecting some of the circulatory changes required of the newborn. We undertook the characterization of the kinin system in the human newborn and the lamb fetus. We found that minute amounts of bradykinin (1 to 2 ng) injected into the pulmonary artery of the fetal lamb markedly increased pulmonary blood flow. ¶ In human infants bradykinin concentrations in cord blood at the time of birth were significantly higher (12.8 ± 4.3 ng per ml) than levels in adult subjects (< 2.0 ng per ml). Cord arterial blood contained inactive kinin precursor (kininogen) and inactive kinin-releasing enzyme (kallikrein). Plasma kallikrein was activated with subsequent kinin formation and kininogen depletion by exposure to fetal granulocytes or by a fall in temperature from 37 to 25° C. A comparable decrease in the temperature of umbilical arterial blood was observed at the time of birth. The systems for activation of the kallikrein system are different in adult and neonatal plasma; only the former is activated by contact with glass. Kallikrein activation by fetal granulocytes was dependent on cell concentration and required O_2 tension above the range observed in the fetus, but seen in the neonate. Granulocytes of the neonate, unlike those of the adult, lacked kininase activity. ¶ In summary, bradykinin can constrict and dilate the vessels as required for the transition of the fetal to neonatal circulation. Bradykinin can be produced in the fetal plasma by temperature drops equal to those measured in the umbilical blood at birth and by the increased numbers of circulating granulocytes found shortly after birth. Thus, bradykinin may be produced at birth and may be a mediator of neonatal circulatory changes.

Insulinopenia: An Important Accompaniment of Isolated Growth Hormone Deficiency. THOMAS J. MERIMEE, DAVID RABINOWITZ, DAVID L. RIMOIN, J. KENNETH NELSON, AND VICTOR A. MCKUSICK,* Baltimore, Md.

Sexual ateliotic dwarfs have a selective deficit of human growth hormone (HGH) without other detectable disturbances of the pituitary gland. Despite HGH deficiency, they do not exhibit spontaneous hypoglycemia. We have studied 20 sexual ateliotics, and this report deals with an apparently consistent accompaniment of this type of growth hormone deficiency: insulinopenia. Insulin output was studied by measuring the plasma immunoreactive insulin response to the following stimuli: *1)* Oral glucose load: Maximal plasma insulin levels after glucose were approximately half as great in ateliotics as in normal controls (39 μU per ml vs. 77 μU per ml). The poor insulin output was accompanied by a frankly diabetic tolerance curve in about half the ateliotics we studied. *2)* Intravenous arginine infusion: Maximal insulin levels in the ateliotics were about 33% of the normal response (30 μU per ml vs. 91 μU per ml). *3)* Oral protein meal: Whereas ingestion of beefsteak is followed by a definite rise in plasma insulin in normal subjects, it produced a significantly smaller increment in the ateliotics. *4)* Mixed glucose-protein meal: Whereas simultaneous ingestion of the two substrates normally exaggerates the insulin response to each given singly, it failed to do so in the ateliotic group. *5)* Ketogenic diet: Pretreatment of normal subjects with a high fat diet induces a heightened insulin response to a glucose challenge, accompanied by worsening of glucose tolerance. A high fat, low carbohydrate, low protein diet induced ketosis and glucose intolerance in ateliotics, but the insulin response was not exaggerated. It is concluded that an impoverished insulin output is a consistent and prominent feature of isolated HGH deficiency. This probably accounts for their failure to exhibit hypoglycemia and may well contribute to suboptimal protein synthesis and reduced growth potential.

Phagocytosis in Subacute Bacterial Endocarditis: Interaction of Antibacterial Antibody Opsonins and Various Rheumatoid Factors. RONALD P. MESSNER, THROSTUR LAXDAL, PAUL G. QUIE, AND RALPH C. WILLIAMS, JR.,* Minneapolis, Minn.

Thirty-two patients with subacute bacterial endocarditis (SBE) were studied. Nineteen S and 7 S distribution of antibodies was determined by sucrose gradient ultracentrifugation or gel filtration. Agglutinating and complement fixing (CF) antibodies against the patient's own organism were demonstrable in both 19 S and 7 S fractions in the majority of sera studied. Highest agglutinating titers were found most often in 7 S fractions, whereas peak CF titers were evenly distributed between 7 S and 19 S fractions. Bacterial agglutination titers among SBE patients often did not differ from normal serum controls; however, CF titers against infecting organisms were generally higher in SBE than in control sera. ¶ Anti-γ-globulin factors were found in 17 patients. Quantitative estimation of γG, γA, and γM showed striking correlation between marked elevations of γG and the presence of rheumatoid factors (RF). The presence of RF appeared to be related to duration of active SBE rather than type of infecting organism. Phagocytosis was measured by both quantitative bacteriocidal measurements and direct observation. Normal human leukocytes were used with the organism and whole or fractionated serum from the same patient. Opsonic activity of the patient's whole serum against his own organism was generally superior to controls. Opsonic activity was greatest in the 7 S fraction in 80% of the patients studied. This was confirmed with serial dilutions of isolated 19 S and 7 S serum fractions. Autologous and purified heterologous rheumatoid factors were added to isolated 7 S antibody and the corresponding organism to test their effect on phagocytosis. No impediment or acceleration of phagocytosis was noted in two patients in repeated deter-

mination. In one instance heterologous rheumatoid factor selected for reactivity with two of the patient's Gm factors showed potentiation of phagocytosis. Seven S antibody appears to play a major opsonic role among patients with subacute bacterial endocarditis.

Folate Binders in Milk and Human Serum; Their Use in Coated Charcoal Assay of Folic Acid; Their Possible Physiologic Role. JACK METZ AND VICTOR HERBERT,* New York, N. Y.

A coated charcoal radioisotope dilution assay can theoretically be developed for any agent for which there exist a binder and a labeled form. Ghitis reported that cow's milk bound approximately 50 ng of pterylglutamic acid (PGA) per ml. Using a 2.5 g per 100 ml suspension of charcoal coated with dextran 10, we confirmed this. With milk as binder, and H_3-PGA as labeled form, such an assay for PGA was developed, with dextran 10-coated charcoal. As in prior coated charcoal assays, success depended on the fact that the amount of H_3-PGA bound to binder (milk) was reproducibly decreased in direct proportion to the quantity of unlabeled PGA added. A given quantity of milk had a fixed maximal binding capacity for PGA, when the quantity of PGA exceeded the quantity of binder. The milk binder did not appear to bind methotrexate, aminopterin, leucovorin, or N^5-methyl THFA. Because of this failure of milk to bind other types of folate, we turned to serum as a hopeful source of binder. ¶ To date, attempts to demonstrate binders for folic acid in normal human serum, analogous to those present for vitamin B_{12}, have been unsuccessful. Using the same coated charcoal assay system as for milk, but with 50-fold reduction in quantity of charcoal, we were unable to demonstrate unsaturated folate binding capacity of normal sera in the range of 10 to 20 ng per ml. A given quantity of serum had a fixed maximal binding capacity for PGA when the quantity of PGA exceeded the quantity of binder. Past failures to find this binder may relate to its seeming weakness, manifested by the fact that larger quantities of charcoal in relation to the quantity of serum appear to "tear away" bound H_3-PGA from the binder. The serum binder may be involved in transport and delivery of folate to tissue. It is currently being evaluated for possible use in a coated charcoal assay for serum folate. The greater affinity of the milk than of the serum binder for folate may help explain why folate concentrates in mother's milk.

A New Inborn Error of Leukocyte Function. MICHAEL E. MILLER, JOHN M. BUCKLER, AND JOHN R. SEALS, Philadelphia, Pa. (introduced by Alfred M. Bongiovanni*).

An entirely new spectrum of immune disorders has been suggested by the recent demonstration of an inability of leukocytes to kill ingested bacteria in patients with granulomatous disease of childhood. We have now shown a similar defect in a child with repeated infections who ultimately died at the age of 2½ years of a fatal nocardia asteroides pulmonary infection. No apparent defects in immunoglobulin synthesis were found. This condition can be differentiated from granulomatous disease of childhood by genetic, morphologic, clinical, and laboratory criteria. By means of an *in vitro* assay system, it was possible to show a marked impairment in ability of his leukocytes to kill *Staphylococcus aureus,* which had been phagocytosed normally. A distinct difference was noted in the changes in cytoplasmic structure of the patient's cells as compared with controls in the process of bacterial killing. The cytoplasmic vacuoles in the patient's cells were diminished in number, and, when present, were more sharply defined and arranged in a lattice-like configuration. ¶ The importance of these observations is in *1*) the identification of a distinct disease whose cause results from a basic defect in bactericidal activities of the leukocytes, and *2*) the indication that the functional error described in granulomatous disease (Holmes, Quie, Windhorst, and Good, 1966) is a prototype for a group of inborn disorders of leukocyte function. ¶ This enlarges greatly our concept of immunologic disorders in man.

Effect of Vasoactive Substances on Immunoreactive Insulin. DANIEL H. MINTZ, JOSEPH L. FINSTER, AND MICHAEL STEPT, Pittsburgh, Pa. (introduced by Alvin P. Shapiro†).

Epinephrine and norepinephrine infusions in man inhibit immunoreactive insulin responses to intravenous glucose, tolbutamide, and glucagon. To determine the specificity of the reaction to catecholamine infusion, we studied the effect of a nonadrenergic vasoconstrictor, angiotensin II, on the immunoreactive insulin response to glucose and tolbutamide. Normal volunteers served as their own controls, and either oral or intravenous glucose or tolbutamide was administered with and without a continuous infusion of angiotensin II. A marked inhibition of immunoreactive insulin response to oral and intravenous glucose and to tolbutamide occurred during the infusion of angiotensin II. After the infusion, a significant rise in immunoreactive insulin ensued. The immunoreactive insulin response to oral glucose during angiotensin II was significantly greater than to intravenous glucose. The concomitant administration of phentolamine and angiotensin II failed to modify the inhibition of the immunoreactive insulin response. Angiotensin II infusion did not accelerate the plasma disappearance rate of injected insulin. ¶ The effects of vasodilator agents on the immunoreactive insulin responses to intravenous glucose were then examined. The administration of sodium dehydrocholate, aminophylline, and hydralazine hydrochloride augmented the immunoreactive insulin responses to intravenous glucose. ¶ The similar inhibitory effect of catecholamines and angiotensin II and the stimulatory effect of vasodilator agents on immunoreactive insulin responses suggest that changes in pancreatic perfusion, *in vivo,* may influence insulin secretion. These observations may explain, in part, the increased incidence of abnormal glucose tolerance associated with certain hypertensive diseases.

Effects of Physical Training in Young Male Subjects. J. H. MITCHELL,* B. SALTIN, C. G. BLOMQVIST, K. WILDENTHAL, R. L. JOHNSON, JR.,* E. P. FRENKEL, AND C. B. CHAPMAN,† Dallas, Texas.

The effects of 50 days' training were studied in five subjects, age 19 to 21. Three were previously sedentary and two active. All had been exposed to 20 days' bedrest before the training, but the effects of training were compared to control studies before bedrest. Maximal oxygen uptake increased from 2.52 to 3.41 L per minute in the sedentary (+33%) and from 4.48 to 4.65 L per minute in the active subjects (+4%). ¶ Lung volumes, timed vitalometry, and membrane diffusing capacity were not changed by training. Pulmonary capillary blood volume was increased proportional to the increase in blood flow. Red cell mass increased in the previously sedentary subjects from 1.93 to 2.05 L, and the two active showed no change. In all subjects during submaximal work the cardiac output and oxygen uptake did not change. In the sedentary group the heart rate was lower and the stroke volume higher for any given oxygen uptake after training. ¶ During maximal work in the sedentary subjects cardiac output increased from 17.2 L per minute before, to 20.0 L per minute after training (+16.5%). Arteriovenous oxygen difference increased from 14.6 to 17.0 ml per 100 ml (+16.5%). Maximal heart rate remained constant, and stroke volume increased from 90 to 105 ml (+17%). Resting heart volumes were 740 ml before and 812 ml after training. All changes were less marked in the active subjects. ¶ Previous studies have shown increases of only 10 to 15% in the maximal oxygen uptake of young sedentary male subjects after training, with the rise being due primarily to an increase in arteriovenous oxygen difference. The greater increase of 33% in maximal oxygen uptake in the present study was due equally to an increase in stroke volume and arteriovenous oxygen difference. These more marked changes may be due to the extremely strenuous and closely supervised training program.

Ionized Calcium Directly Determined by Ion-Exchange Electrodes: The State of Serum Calcium in Patients with Cirrhosis. EDWARD W. MOORE, Boston, Mass. (introduced by Thomas C. Chalmers†).

Ionized calcium concentration [Ca^{++}] was determined with recently developed ion-exchange electrodes in 31 hospitalized cirrhotic patients and 14 healthy subjects. [Ca^{++}] was measured in a Plexiglas P_{CO_2} chamber at 37° C. Both [Ca^{++}] and pH were continuously monitored at varying P_{CO_2}, and [Ca^{++}] was expressed as that value at original blood pH. Total calcium was determined by EDTA titration and atomic absorption spectroscopy; serum proteins were fractionated by electrophoresis. ¶ Total serum calcium was significantly ($p < 0.001$) reduced in cirrhotics [mean 2.15 ± 0.04 (SE) mmoles per L] when compared with normals (mean 2.66 ± 0.07 mmoles per L), whereas total ultrafiltrable calcium was slightly increased (means 1.65 ± 0.04 and 1.52 ± 0.06 mmoles per L). Ultrafiltrable calcium in the cirrhotic group thus represented a significantly ($p < 0.001$) higher fraction of total serum calcium (means $75.0 \pm 2.25\%$ and $57.45 \pm 2.85\%$, respectively). This was reflected in a significant ($p < 0.001$) reduction in protein-bound calcium [CaProt], with respective means of 0.50 ± 0.06 and 1.15 ± 0.10 mmoles per L, and significant ($p < 0.02$) increase in ultrafiltrable calcium complexes [CaR], with means of 0.54 ± 0.04 and 0.38 ± 0.05 mmole per L, respectively. ¶ Despite these changes in total calcium, [CaProt], and [CaR], mean serum [Ca^{++}] in the cirrhotic group was identical to that in normals (1.12 ± 0.02 mmoles per L in both groups) and was similar in ultrafiltrates (1.12 ± 0.02 and 1.14 ± 0.02 mmoles per L, respectively). ¶ Mean serum albumin concentration in cirrhotics (2.74 ± 0.14 g per 100 ml) was significantly ($p < 0.001$) less than in normals (4.96 ± 0.15 g per 100 ml), whereas serum globulin concentration was significantly ($p < 0.001$) increased, with means of 3.78 ± 0.15 and 2.63 ± 0.15 g per 100 ml, respectively. In both groups, calculated [CaProt] was linearly related to serum albumin concentration ($r = 0.93$), whereas [CaR] was inversely related ($r = 0.79$) to [CaProt]. ¶ These studies demonstrate that serum protein abnormalities in cirrhotic patients are associated with significant reduction in total serum calcium and striking redistribution in protein-bound and soluble-complex pools. In spite of this, ionized calcium is within normal limits, indicating the inadequacy of measuring only total serum calcium and emphasizing the physicochemical complexities in maintenance of calcium homeostasis.

The Syndromes of Diminished Renal Secretion of H^+, The Effect of Na_2HPO_4/NaH_2PO_4. R. CURTIS MORRIS, JR., IRIS UEKI, ANTHONY SEBASTIAN, AND ELISABETH MORRIS, San Francisco, Calif. (introduced by Malcolm McIlroy*).

In children with hyperchloremic acidosis associated with the Fanconi-cystinosis syndrome, we reported previously that Tm HCO_3^- is distinctly subnormal, the capacity to lower urine pH, intact. The excretion of urinary acid at a rate insufficient to prevent acidosis reflects an abnormal restriction on the attainable rate of renal H^+ secretion, not on the attainable lumen-paratubular H^+ gradient as in classic renal tubular acidosis. To investigate further the physiologic character and therapeutic implications of the reduced rate of H^+ secretion, we measured Tm HCO_3^- under different physiologic conditions in eight studies of three patients with hyperchloremic acidosis and the Fanconi-cystinosis syndrome. During intravenous infusion of 0.15 Na_2HPO_4/NaH_2PO_4 (pH 7.4), Tm HCO_3^- increased from 1.4 to 2.0, 2.0 to 2.5, 2.2 to 2.6 mEq per 100 ml glomerular filtrate (N 2.6 to 2.8) in these patients. A phosphate-induced increase in Tm HCO_3^- was measurable over comparable ranges of plasma HCO_3^- and P_{CO_2}. Tm HCO_3^- varied directly with P_{CO_2}: 2.4 at P_{CO_2} of 42 to 45 mm of Hg, 1.7 at P_{CO_2} of 31 to 35. To investigate the possible occurrence of an isolated reduction of renal H^+ secretion less than that resulting in frank acidosis, we measured Tm HCO_3^- and response to NH_4Cl in a nonacidotic, 19-year-old girl with recurrent nephrolithiasis,

idiopathic hypercalciuria, and plasma HCO_3^- of 23 and Cl of 105. Tm HCO_3^- was 2.3. After NH_4Cl, plasma HCO_3^- decreased to 16 mEq per L, and urine pH decreased to less than 5. The administration of $NaHCO_3$ or phosphate chronically and phosphate acutely during HCO_3^- loading resulted in reduced urinary excretion of calcium and regulation of plasma HCO_3^- at significantly higher levels. Abnormalities of renal H^+ secretion were also demonstrated in two other patients with idiopathic hypercalciuria. We suggest that the hypocalciuric effect of phosphate, $NaHCO_3$, and thiazides in patients with recurrent nephrolithiasis relates to regulation of plasma HCO_3^- at higher levels.

Exchange Transfusion Treatment of Fulminating Viral Hepatitis in the Dog. THOMAS Q. MORRIS, DAVID J. GOCKE, AND GIAN F. SARDI, New York, N. Y. (introduced by Stanley E. Bradley†).

The therapeutic efficacy of exchange transfusion in fulminating hepatitis was evaluated during the course of acute viral hepatitis in the dog. Nine purebred beagle puppies, all lacking detectable antibody to the infectious canine hepatitis (ICH) virus, were each infected with 300 $TCID_{50}$ of ICH virus in the anterior chamber of the eye. After 3 to 4 days all displayed clinical and laboratory evidences of severe hepatitis. At that time exchange transfusions of at least 50% of their blood volume were performed under light Nembutal anesthesia with fresh heparinized blood. Five of the nine survived, whereas all of thirty control animals died within 5 to 9 days after infection. Because all donor blood contained a high titer of antibody to ICH virus, a second group of six puppies was infected in order to ascertain the role of immunity. Exchange transfusions were performed with blood from nonimmune donors. In this group there was only a single survivor, which subsequently was found to have been immune at the time of infection. All the others were nonimmune. To verify that antibodies to the ICH virus were the essential elements in this therapy, we infected three more susceptible animals. Treatment consisted only of a single 5-ml injection of serum hyperimmune to the ICH virus. All dogs in this group recovered. These studies have demonstrated that the therapeutic effect of exchange transfusion during fulminating viral hepatitis in the dog is dependent upon the presence of specific neutralizing antibody in the donor rather than the removal of toxins from the blood of the recipient.

Hypokalemia in Cases of Monocytic Leukemia with Lysozymuria. FRANCO MUGGIA, HENRY O. HEINEMANN,† AND ELLIOTT F. OSSERMAN,† New York, N. Y.

Persistent hypokalemia (serum $K^+ = 2.0$ to 3.5 mEq per L) has been observed in seven of twelve patients with monocytic and monomyelocytic leukemia with hyperlysozymuria [urinary lysozyme (LZM) = 0.5 to 2.5 g per day]. LZM is an exceptionally basic (isoelectric pH = 10.5) low molecular weight (mol wt $\cong$ 14,000) protein. ¶ Balance studies in two patients demonstrated inappropriate urinary K^+ loss in the presence of hypokalemia. Partial correction of the hypokalemia was accomplished only if oral K^+ supplements exceeded 120 mEq per day. ¶ Additional studies to elucidate the mechanism of the low serum K^+ and inappropriate renal K^+ excretion revealed normal serum Na^+, Cl^-, CO_2, NPN, and creatinine levels; no acidosis; no impairment of ability to excrete an acid load; normal urinary aldosterone excretion; normal plasma cortisol levels and ACTH response; no aminoaciduria or glycosuria. All patients had hyperuricemia (6 to 10 mg per 100 ml) and hyperuricosuria. Serum Mg levels were low (0.9 to 1.5 mEq per L) in two of four patients. All patients had 0.5 to 2.0 g per day of nonspecific proteinuria (predominantly albumin) in addition to the lysozymuria. ¶ The present data suggest a relationship between the lysozymuria, low serum K^+, and inappropriate renal K^+ loss, but the precise mechanisms remain to be elucidated.

Dissociation of Bradycardia from the Arterial Constrictor Response to Diving. H. V. MURDAUGH,* CARROLL E. CROSS, EUGENE D. ROBIN,† J. EUGENE MILLEN, AND J. BERNARD L. GEE, Pittsburgh, Pa.

The occurrence of bradycardia and arterial constriction during diving has been reported in a number of mammals. In man, it has been suggested that this sequence represents an evolutionary heritage subserving O_2 conservation. ¶ Because of the difficulty in measuring regional blood flow during diving in man, bradycardia has been equated with the dive response. Aquatic mammals serve as classic models for this physiologic adaptation to O_2 lack. Accordingly, the harbor seal, *Phoca vitulina,* was studied to evaluate the relative roles of bradycardia and arterial constriction. Bradycardia was dissociated from arterial constriction during diving with an intracardiac pacing catheter. Aortic pressures and electrocardiograms were continuously monitored. ¶ By controlling heart rate with the pacer, bradycardia could be prevented or stopped during diving. The seal could remain submerged for prolonged periods without bradycardia under these circumstances. The occurrence of arterial constriction during diving without bradycardia was demonstrated by survival of the animal without untoward effects and by aortography. During diving the aortic pressure and pulse pressure progressively decreased when the heart rate was experimentally increased, and increased when the heart rate was slowed. These findings are consistent with the detrimental effect that tachycardia induced during diving would have on the cardiac output in the face of the markedly decreased venous return from the central venous reservoir due to the persistence of the arterial constrictor response. ¶ It is clear that the arterial constrictor response is not dependent upon bradycardia. The bradycardia serves to regulate perfusion pressure in the face of markedly reduced rate of diastolic filling, whereas the arterial constrictor response conserves O_2 stores for O_2 dependent metabolism in critical areas.

Stochastic Models of Blood Platelet Survival. EDMOND A. MURPHY,* MILDRED E. FRANCIS, AND J. F. MUSTARD,* Denver, Colo., and Ottawa and Hamilton, Ontario.

Platelets in clumping sustain damage, not necessarily fatal to them. On this basis we have developed a multiple-hit model of platelet survival outlined elsewhere. The model has been extended to two further cases, where *1*) release of nascent platelets labeled in the marrow had itself a gamma distribution, and where *2*) labeling of both nascent and circulating platelet occurs. The latter may explain very long tails sometimes encountered with DFP labeling (Barkhan). ¶ Iterative nonlinear least squares estimation algorithms for fitting these curves to data from pigs, dogs, and man have been extensively explored with approximate methods for obtaining initial estimates. Estimates of the number of "hits" (i.e., episodes of platelet injury) required for destruction vary in different animals from 1 upwards. The fit of the model is satisfactory though there may be some superimposed time trends as yet unexplored. ¶ The model predicts that drugs such as sulfinpyrazone that depress platelet-surface interaction should increase the number of hits required for destruction, and hence the curve of platelet labeling with ^{35}S against time should have a flatter top among treated than control pigs. This has been verified experimentally. ¶ The model is of value in the light it throws on the economy of the platelet and in providing a more or less rigorous method of comparing groups of observations when treatment modifies the form of the curve. The principles are applicable to survival of any kind of migratory cell.

Hb CC Disease: An Abnormality in Cell Water. JOHN R. MURPHY,* Cleveland, Ohio.

Previous studies indicated that blood from patients with hemoglobin (Hb) CC disease has a greater viscosity than normal blood (Hb AA), and that erythrocytes with Hb CC are more rigid or less deformable than cells with Hb AA when filtered through microfilters of uniform pore size. It was proposed that splenic sequestration and the shortened survival of erythrocytes in Hb CC disease are due to the cell being less deformable or more rigid than normal cells. ¶ The present studies show that in Hb CC disease both the abnormal viscosity of blood and the decreased deformability of erythrocytes are related to an increase in viscosity of the hemoglobin. In addition, the cation content in Hb CC cells is less than in normal erythrocytes. The increased viscosity of Hb C and the low cation content in Hb CC erythrocytes suggested an abnormality in cell water or the relationship of cell water to Hb C or both. ¶ The total water content of erythrocytes with Hb CC is consistently less than in normal erythrocytes, 67.4% as compared to 70.6%. A more significant observation concerned the fraction of cell water available for osmotic equilibrium, which has been termed solvent water. The amount of solvent water in erythrocytes is determined by measuring the change in volume and water content when cells are equilibrated with hypotonic media. In Hb CC cells only 54% of the cell water participates in osmotic equilibrium as compared to 80% in normal cells. This smaller fraction of solvent water in Hb CC cells indicates that a greater amount of cell water is bound to Hb C, 46% as compared to Hb A, 20%. Thus the abnormal rheological property of Hb C appears to be due to the increase in the amount of water bound to the hemoglobin and a proportional decrease in the amount of solvent water within the cell available for dispersion of hemoglobin and dilution of cations.

Growth Hormone Activity in Man with Components of Tryptic Digests of Bovine Growth Hormone. ALLEN C. NADLER, MARTIN SONENBERG,† MARIA I. NEW, AND CHARLES A. FREE, New York, N. Y.

Tryptic digests of bovine growth hormone (TBGH) administered to hypopituitary humans have produced metabolic effects associated with human growth hormone (HGH) administration as well as antibodies reactive with HGH. TBGH digested for 10 minutes (10'TBGH) corresponding to 2.3 to 2.5 peptide bonds hydrolyzed per mole protein (mol wt 22,000) reveals at least six distinct major components pertaining to BGH when characterized by starch gel electrophoresis. These digests were fractionated by gel filtration on Sephadex G-100 with 0.1 M Tris buffer, pH 8.8, or water, and stepwise elution from DEAE-Sephadex with 0.03 N, 0.1 N, and 1.0 N NaCl in 0.1 M Tris, pH 8.8. Using parameters of nitrogen retention as the primary criteria of growth hormone effect, we conducted eleven studies on six hypopituitary humans under conditions of complete metabolic balance. The subjects received fractions of 10'TBGH rich in discrete electrophoretic components present in the digest, and effects on nitrogen metabolism were correlated with the components. Nitrogen retention occurred with five different preparations, all of which contained one, and four of which contained two components of intermediate mobility in common in enriched amounts. No nitrogen retention occurred with preparations in which these components were present in trace amounts or absent. Nitrogen retention in the five positive studies was manifested by decreased urinary nitrogen (five) and creatine (three) excretion and decreased BUN (four). There were associated changes in phosphorus metabolism as reflected by a decreased urinary phosphorus (four studies) or changes in serum inorganic phosphorus (two). Decreased urinary excretion of potassium (three studies) and sodium (two) was observed. Urinary calcium excretion increased in two of the five studies.

Possible Implication of Complement in Acute Gout. GEORGE B. NAFF AND PETER H. BYERS, Cleveland, Ohio (introduced by Oscar D. Ratnoff†).

Sodium urate crystals have been implicated in the pathogenesis of acute gouty arthritis. Two suggested mechanisms by which they might induce an acute inflammatory response are direct cellular injury and the activation of Hageman factor followed by generation of kinin activity. A third possibility might be related to the com-

plement system. Recent investigations have shown that complement is involved in certain aspects of the acute inflammatory response: Anaphylatoxin, a C′ 3 fragment, increases vascular permeability, and complement has been implicated in phagocytosis and in the generation of chemotactic factors. ¶ Participation of the complement system in acute gout is suggested by the following experimental results: *1*) Sodium urate crystals were incubated with normal human serum, and complement activity was measured by immune hemolysis and immune adherence. Complement decreased as a function of time, temperature, and crystal concentration. The activity was decreased by 70% or more after incubation at 37° C for 90 minutes with a 3.5 mg per ml of crystals. Assays for individual components revealed a marked decrease in C′2, C′3, C′4, and C′5 activities, whereas C′1 was only moderately depressed. Soybean trypsin inhibitor did not block this effect of urate crystals; increasing the ionic strength to 0.2 did inhibit. Crystals depleted complement from serum lacking Hageman factor, suggesting that its activation was not important in complement depletion. ¶ *2*) Polymorphonuclear leukocytes, urate crystals, and either normal human serum, heated serum, or R4 serum were incubated together at 37° C, and microscopic counts of the numbers of crystals per field bearing PMN were compared as an index of phagocytosis. The mixture containing active complement had an average count of more than 15 times those of the two controls after 5 minutes' incubation. ¶ *3*) In preliminary experiments, incubation of urate crystals with rat plasma was associated with an anaphylatoxin-like activity as measured with the guinea pig ileum.

Effects of Corticotropin in Patients with Generalized Myasthenia Gravis. TATSUJI NAMBA, MENACHEM S. SHAPIRO, SHIGERU ARIMORI, AND DAVID GROB,† Brooklyn, N. Y.

Corticotropin has a unique effect in patients with generalized myasthenia gravis, producing exacerbation of the disease followed by improvement. Intravenous administration of 100 U daily for 10 days produced a decrease in strength and response to anticholinesterase medication in 23 of 24 courses in 9 of 10 patients. After termination of corticotropin, moderate to marked improvement lasting an average of 71 days occurred after 15 of 24 courses in 6 of 9 patients. Increasing the daily dose to 200 U, or the duration to 15 days, produced little additional effect. Decreasing the dose or duration produced less effect. The serum level of striation-binding globulin decreased during corticotropin administration. ¶ The effects of corticotropin could not be reproduced by cortisone, methylprednisolone, or metyrapone, but were simulated by some respiratory infections or administration of typhoid-paratyphoid vaccine, despite less marked increase in plasma and urinary 17-hydroxycorticosteroids and urinary 17-ketogenic steroids. During exacerbation produced by corticotropin, infection, or vaccine, there were comparable decreases in evoked muscle potentials and tension and responses to intra-arterial acetylcholine and neostigmine. During subsequent improvement, there were comparable increases in these parameters. ¶ Intra-arterial administration to myasthenic patients or to rats of corticotropin (100 to 19 U) or hydrocortisone (200 and 19 mg) produced no change in evoked potentials or tension, or response to acetylcholine or neostigmine. Intramuscular administration to rats of 30 U per kg corticotropin, 75 mg per kg hydrocortisone, or 15 mg per kg deoxycorticosterone daily for 14 days likewise produced no change. Daily intramuscular administration to rats of 60 U per kg corticotropin, or to rabbits of 15 U per kg, produced after 23 days an increase in diameter of motor end plates from 27.9 ± 0.41 μ to 36.7 ± 0.47 μ ($p < 0.001$), and an increase in branching of terminal axions from 20% to 59%. These morphologic changes suggest a mechanism for the effect of corticotropin on neuromuscular transmission.

The Effect of Hydrogen Ion on Intermediary Metabolism in Muscle. ROBERT G. NARINS AND ARNOLD S. RELMAN,* Boston, Mass.

Many of the reactions of intermediary metabolism consume or generate hydrogen ions (H^+). To determine whether such reactions play any role in the regulation of intracellular acidity, we studied the effects of changing P_{CO_2} or HCO_3^- concentration on the levels of key intermediates in isolated intact rat diaphragms incubated in a Krebs-Ringer bicarbonate medium. ¶ Production of lactate from glucose was consistently reduced by high P_{CO_2} and increased by low P_{CO_2}. That this was due to inhibition of the phosphofructokinase (PKF) reaction by H^+ was shown by the fact that intermediates preceding PFK in the glycolytic pathway rose in acid media whereas those following PFK fell. Thus endogenous acid production was increased in alkalosis and decreased in acidosis. Another important step in muscle metabolism, one that consumes H^+, is the breakdown of creatine phosphate (CrP). We found that acidosis induced by a rise in P_{CO_2} resulted in a 30% fall in CrP; comparable acidosis produced by low HCO_3^- caused a slightly smaller change in CrP. Here again variations in pH stimulated a metabolic reaction that would minimize the acid-base perturbation in the cell. Preliminary experiments in intact rats gave similar results. ¶ It can be calculated that the combined changes in CrP and lactate would by themselves "buffer" at least 10 to 12 mEq of H^+ per L of cell water. We suggest that these and other such metabolic mechanisms may play an important role in the stabilization of intracellular acidity.

The Role of Dehydration in Experimental Acute Renal Failure in the Rat. DONALD E. OKEN,* DOUGLAS R. WILSON, GILBERT THIEL, AND MANUEL ARCE, Boston, Mass.

Dehydration predisposes to severe acute renal failure, an effect attributed to high urine concentration and slowed flow in the distal nephron predisposing to cast formation. To test this thesis, we made rats with complete hypothalamic diabetes insipidus hemoglobinuric with 10 ml

per kg 50% glycerol given intramuscularly. Mean BUN 48 hours after glycerol was 226 ± 11 (SE) mg per 100 ml in 35 dehydrated rats without diabetes insipidus and 78 ± 9 mg per 100 ml in 65 comparable nondehydrated rats. Ten DI rats given free access to water had a BUN of 152 ± 28 mg per 100 ml 48 hours after glycerol, a value intermediate between that of dehydrated and nondehydrated animals ($p < 0.01$). These rats had a control urine volume of 163 ± 42 (SD) ml per day. Despite comparably large water intake, they apparently were dehydrated; their control plasma volume was 10% lower and their hematocrit 10% greater than that of nondehydrated rats without DI ($p < 0.01$). Kidneys of hemoglobinuric DI rats were comparable in appearance to those of non-DI hemoglobinuric rats. Micropuncture studies revealed a spectrum of functional abnormality in individual nephron GFR, proximal tubular fluid flow rate, and intratubular pressure in single kidneys of all groups of animals. Eighteen to 26 hours after glycerol, approximately one-half of nephrons of DI rats had minimal proximal tubular flow compared with 75% in dehydrated rats and 25% in nondehydrated rats. DI rats did not become oliguric after glycerol injection, excreting 25 ± 7 (SE) ml per 24 hours and 97 ± 20 ml per 24 hours on the first and second days after glycerol. ¶ It is concluded that the deleterious effect of dehydration on glomerular filtration in acute renal failure is independent of concentrating mechanisms and slow flow of urine in distal segments of the nephron. The great difference in urine volumes of comparably azotemic DI and non-DI rats, however, suggests that the distal tubular absorption of residual filtrate plays a significant role in the development of oliguria in acute renal failure.

Detection of Digitalis in Human Sera. G. Charles Oliver, Jr., Brent M. Parker, Daniel L. Brasfield, and Charles W. Parker,* St. Louis, Mo.

A sensitive, specific, and relatively simple radioimmunoassay permitting measurement of pharmacological levels of digitoxin in human serum has been developed. The assay involves binding of ^{125}I-labeled tyrosine-digitoxigenin (SA > 400 mc per mg) by rabbit antibody to digitoxin. Antibody-bound radioactivity is precipitated by addition of a second antibody (goat antirabbit γ-globulin), and radioactivity of the precipitate is measured. Unlabeled digitoxin is determined by the extent to which it competes with ^{125}I-labeled digitoxigenin and thus reduces precipitation of radioactivity. Before assay, unlabeled digitoxin is extracted from serum by chloroform, and the chloroform solution is evaporated to dryness. ¶ Under current assay conditions approximately 50% of the radioactivity is precipitated with extracts of human serum containing no digitalis. A standard curve is obtained by adding known amounts of digitoxin to normal serum before extraction. As little as 1 mμg of unlabeled digitoxin in 1 ml of serum significantly reduces precipitation of radioactivity. The sera of 5 patients were analyzed before and after oral administration of digitoxin. Average precipitation with extracts of predigitalization sera was 51%, whereas that of postdigitalization sera was 24% ($p < 0.001$), indicating blood levels of digitoxin of approximately 20 mμg per ml. In 17 patients taking digitoxin or digitalis leaf, marked reduction in precipitation of labeled digitoxin was demonstrated uniformly (an average of 25% of counts precipitated). Digoxin did not react equally well; sera of patients receiving digoxin reduced precipitation of radioactivity from 50% in control sera to only 45%. Physiological concentrations of cholesterol, testosterone, and hydrocortisone did not alter precipitation of radioactivity. ¶ Other potential uses of the rabbit antidigitoxin antibody include localization of digitalis in heart muscle by ferritin labeling and its use in treatment of acute digitalis toxicity.

Mechanism of Action of Progesterone in Induction of Synthesis of the Specific Protein Avidin *In Vitro*. Bert W. O'Malley and William L. McGuire, Bethesda, Md. (introduced by Mortimer B. Lipsett*).

To study the process of hormone action, we have developed an *in vitro* system using minced oviduct from estrogen-treated chicks incubated in tissue culture medium. Progesterone added to the medium induced synthesis of a specific protein, avidin, that continued for up to 96 hours. During this period there was no increase in total oviduct protein, ovalbumin, or lysozyme, suggesting the specificity of the progesterone effect. The induction process was dependent on new protein synthesis since cycloheximide (10 μg per ml) inhibited the induction completely. Actinomycin D (1 to 10 μg) in doses that prevented nuclear RNA synthesis but not protein synthesis inhibited avidin production 90%. Avidin synthesis was not affected by 5-fluorouracil. The rate of DNA synthesis examined by thymidine-^{3}H pulse labeling was unaltered during induction. Hydroxyurea (an inhibitor of DNA synthesis) and colchicine (a mitotic inhibitor) did not prevent induction. Studies utilizing uridine-^{3}H pulses showed that rapidly labeled RNA increased coincident with induction in both nuclear tissue fractions and pure isolated nuclei preparations. Nuclear RNA polymerase activity also increased during the avidin induction. In conclusion: *1*) This system represents the first example of *in vitro* steroid-induced synthesis of a specific protein by tissue minces. *2*) The induction is independent of DNA synthesis. *3*) No supportive evidence for a primary cytoplasmic effect (translational depression) of the steroid hormone has been noted in this or previous studies. *4*) The early stimulation of nuclear RNA synthesis and RNA polymerase activity would suggest a mechanism of action for progesterone at the transcription level of protein synthesis.

Introduction of Nucleic Acids into Mammalian Cells. Joseph S. Pagano and James H. McCutchan, Chapel Hill, N. C. (introduced by W. Hollander*).

Isolated nucleic acids are difficult or impossible to introduce into living cells in culture without loss of function or killing the cells. After finding that the polycation, diethylaminoethyl-dextran (DEAE-D), has a strik-

ing capacity to facilitate the entry of intact viral nucleic acids into cells without damage to them, we selected for study a single-stranded viral RNA and a double-stranded viral DNA. These viral systems are seen as potential models for the controlled introduction of nonviral nucleic acids into cells. ¶ Poliovirus RNA and the DNA of simian virus$_{40}$ were extracted from the viruses with phenol. Sensitive plaque assays of the infectivity of both nucleic acids in monkey kidney cells with isotonic solutions of DEAE-D were developed. With DEAE-D the uptake of viral RNA was increased up to 100,000 times in isotonic medium; the functional nature of the nucleic acid was proved by its capacity to replicate. This powerful enhancing effect, which was dependent on the molecular weight of DEAE-D, could be attributed to stabilization of RNA against nucleases, but chiefly to an alteration of the cell surface. Features of the dose-response relations, DNA-induced tumor-antigen formation, and the sensitizing effect of DEAE-D on cells with respect to susceptibility to nucleic acid infections were defined. A means of controlling cell desensitization with heparin was also found. These data strengthen the prospect that functional nonviral nucleic acids can be introduced into viable cells in experimental systems.

Immunological and Chemical Characteristics of Lipoproteins in Human Atherosclerotic Arteries. JOACHIM PAPENBERG AND WILLIAM HOLLANDER,* Boston, Mass.

Previous studies have indicated that lipids accumulate mainly as low density lipoproteins ($D < 1.063$) in human atherosclerotic placques. In the present study the chemical and immunological characteristics of arterial lipoproteins were compared with those of plasma lipoproteins. Lipoproteins were extracted from the diseased arterial intimal layer into saline and then separated and purified by differential density ultracentrifugation. ¶ The arterial low density lipoprotein fraction ($D = 1.006$ to 1.063) comprised about 85% of the extractable lipoproteins and showed the characteristics of a beta-lipoprotein by paper electrophoresis, immunodiffusion, and immunoelectrophoresis. The fraction had a flotation constant of S_f 3 to 9 and an amino acid composition that was comparable to the beta-lipoprotein isolated from the plasma. ^{131}I-labeled beta-lipoprotein, intravenously administered, was isolated from the the arteries. Arterial synthesis of beta-lipoprotein also was indicated by the incorporation of leucine-^{14}C into beta-lipoprotein in incubated arterial intima. ¶ The arterial very low density lipoprotein fraction ($D < 1.006$) comprised less than 10% of the extractable lipoproteins. It also behaved immunologically like a beta-lipoprotein. No α_1-lipoprotein was detectable in the fraction. The arterial beta-lipoproteins contained more cholesterol and less triglyceride per milligram of lipoprotein than the plasma beta-lipoproteins. ¶ The arterial high density lipoprotein fraction ($D = 1.063$ to 1.210) accounted for about 9% of the extractable lipoproteins. It contained an α_1-lipoprotein and trace amounts of albumin and globulin by immunodiffusion and immunoelectrophoresis. Phospholipids and cholesterol but no triglycerides were present in the fraction. ¶ The fraction D > 1.210 contained albumin and globulins by immunoelectrophoresis. This fraction, like the plasma fraction, contained phospholipids but no cholesterol or triglycerides. It is concluded that the lipoproteins found in atherosclerotic arteries are predominantly beta-lipoproteins. They appear to be derived from the plasma as well as to be synthesized in the arteries.

A Thyroid-stimulating Protein from a Bacterium. IRA PASTAN, VINCENZO MACCHIA, AND ROBERT W. BATES, Bethesda, Md. (introduced by Jacob Robbins*).

Thyroid-stimulating hormone (TSH) promptly stimulates many metabolic and morphologic responses in the thyroid. We have purified a bacterial protein that mimics the actions of TSH. From the growth medium of *Clostridium perfringens* a protein fraction was precipitated with ammonium sulfate, dissolved in water, dialyzed, and lyophilized. The bacterial thyroid stimulator (BTS) present in this dry powder was then purified 310-fold by further fractionation with ammonium sulfate, filtration on Sephadex G-100, and chromatography on DEAE-cellulose. The activity of BTS was measured by its ability to stimulate glucose-1-^{14}C oxidation to $^{14}CO_2$ when incubated with dog or beef thyroid slices for 1 hour at 37° C in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 0.1% glucose and 0.1% albumin, or to increase $^{32}P_i$ incorporation into phospholipid of slices incubated for 3 hours. BTS at 1 μg per ml, like TSH, increased glucose-1-^{14}C oxidation and phospholipid synthesis. It also stimulated the formation of pseudopods and intracellular colloid droplets in dog thyroid slices. When injected subcutaneously into chicks, 5 μg of BTS, like TSH, caused a 30% depletion of ^{131}I from the thyroid. BTS appears to be a protein since its activity is destroyed by treatment with pronase but not with RNAase or DNAase. Its behavior on Sephadex G-100 indicates a mol wt of about 30,000. BTS is 75% inactivated by heating at 100° C for 7 minutes and also inactivated by exposure to 10^{-2} M β-mercaptoethanol at pH 7.4. BTS has been separated from neuraminidase, lecithinase C, collagenase, and theta toxin. It has no proteolytic activity on casein, gelatin, denatured hemoglobin, or albumin and does not appear to be a glycosidase capable of releasing mannose, fucose, galactose, *N*-acetylgalactosamine, or *N*-acetylglucosamine from the thyroid slices. Thus a protein with activities like TSH is produced by *Clostridium perfringens.*

"Termination" of Tolerance to Bovine Serum Albumin (BSA) with Dinitrophenyl (DNP)-BSA. WILLIAM E. PAUL, GREGORY W. SISKIND, AND BARUJ BENACERRAF, New York, N. Y. (introduced by Jonathan W. Uhr*).

Rabbits immunologically tolerant to BSA are capable of producing antibodies that bind BSA after immunization with hapten-BSA conjugates. This phenomenon may be interpreted as either *1*) a true termination of the tolerant

state or *2*) an immunization with antigenic determinants to which the animals were not formerly exposed but which cross-react to some extent with determinants on the tolerated BSA. The present study was undertaken to choose between these two possibilities. Adult rabbits were rendered specifically tolerant to BSA. Tolerant and normal rabbits were immunized with DNP-BSA emulsified in incomplete Freund's adjuvant; normal rabbits were similarly immunized with BSA. Antibody capable of precipitating BSA was formed by each rabbit and was specifically purified by the thiolated antigen technique of Singer. The relative binding affinities of such "anti-BSA" antibodies for BSA-^{125}I were determined by a Farr type assay. "Anti-BSA" antibodies produced by tolerant rabbits bound little or no BSA in the presence of an equal amount of DNP-BSA. On the other hand, anti-BSA antibodies produced by normal rabbits immunized with DNP-BSA bound BSA only slightly less well than DNP-BSA in competitive situations. Thus, the capacity to produce antibodies with a high degree of BSA specificity remains inhibited in the tolerant rabbits after immunization with DNP-BSA. These experiments provide a model for the specificity of the immune response in certain autoimmune conditions. Thus autoantibodies in these conditions may represent antibodies produced against substances cross-reactive with native autologous components. Such autoimmune diseases may occur without a true termination of tolerance to autologous components.

Inhibition of Collagen and RNA Synthesis in Isolated Bone Cells by Hydrocortisone *In Vitro*. WILLIAM A. PECK, Rochester, N. Y. (introduced by Seymour Reichlin*).

The mechanism of glucocorticoid-induced osteoporosis and inhibition of bone growth has been studied with bone cells isolated enzymatically from rat calvaria and maintained in primary culture, where they elaborate a collagen-rich organic matrix. Addition of 10^{-6} M hydrocortisone or hydrocortisone hemisuccinate to the incubation medium markedly inhibited protein synthesis in 5 hours, as indicated by a 50 to 60% decrease in the incorporation of proline-^{14}C into both collagen and noncollagen protein and a 40 to 50% increase in the radioactivity of intracellular free proline. Significant effects appeared with concentrations as low as 10^{-7} mole per L. The accumulation of free proline radioactivity and the consistency of hydrocortisone effects when the starting proline concentration was varied from 0.0022 to 0.22 mmole per L indicate that decreased protein labeling was not caused by expansion of intracellular free proline pool. In parallel studies, incorporation of uridine-2-^{14}C into RNA and into the intracellular nucleotide pool decreased 50 to 60% with 10^{-5} M hydrocortisone and 20% with 10^{-8} M. The degree of inhibition was unaffected by the uridine concentration over a wide range (0.0017 to 0.17 mM), suggesting that isotope dilution was not an important factor. That these changes were related to a rapid decrease in RNA synthesis was supported by the presence of a small (10%) but significant decrease in total RNA content per cell after 5 hours of hydrocortisone treatment. RNA breakdown was not accelerated since hydrocortisone failed to decrease the radioactivity of pulse labeled RNA when reutilization of radioactive uridine was blocked by actinomycin D. Effects on RNA and protein metabolism were not associated with significant alterations in DNA metabolism. The DNA content of cell cultures was unchanged, and incorporation of thymidine decreased only slightly with 10^{-5} M hydrocortisone. These results demonstrate at the cellular level that glucocorticoids rapidly and selectively interfere with key metabolic processes required for the formation of organic bone matrix.

Metabolism of Polysomes and Ribosomal Subunits in Growth Hormone Deficient Rats. C. V. PEERY AND K. S. MCCARTY, Durham, N. C. (introduced by J. M. Ruffin†).

Alterations in DNA, RNA, and protein synthesis in rat liver after partial hepatectomy are well known. Growth hormone has been implicated as an important factor in the control of such processes. Since hypophysectomized rats have no source of growth hormone, we studied the incorporation of orotate-^{3}H into liver RNA in these animals 12 hours after two-thirds hepatectomy and at least 14 days after hypophysectomy. Cytoplasmic polysomes, 40 S and 60 S subunits, and polysomal RNA were extracted and characterized by sedimentation and radioactivity on sucrose density gradients. Considering specific activities, as well as optical density differences, we observed that *1*) hypophysectomy did not decrease the rate of transcription of rRNA; *2*) hepatectomy caused an increased transcription of rRNA, the increase being qualitatively similar in both normal and hypophysectomized rats; *3*) the ratio of the specific activities of the 40 S and 60 S cytoplasmic subunits was not altered by hepatectomy; *4*) the specific activity ratios of 40 S/60 S subunits were increased both by hypophysectomy and by hepatectomy plus hypophysectomy; and *5*) a decrease in heavy polysomes resulted from hepatectomy, hypophysectomy, and the two conditions combined. We conclude that hypophysectomy does not decrease the response of rat liver RNA metabolism to partial hepatectomy, and therefore that growth hormone may not be necessary for this response to take place.

Continued Experience with the Oximeter-controlled Induced Anoxemia Test for Coronary Disease. RAYMOND PENNEYS, Philadelphia, Pa. (introduced by F. Curtis Dohan†).

Results of this test on approximately 400 coronary suspects show that *1*) it is a safe, practicable, and quantitative test for the detection of coronary insufficiency. There was no difficulty in producing, and maintaining, the desired levels of blood arterial oxygen saturation. No adverse reactions of any type were encountered. *2*) It can demonstrate the presence of coronary disease even though a selective coronary arteriogram, which does not

necessarily demonstrate disease of very small vessels, is normal; *3*) abnormalities in the ballistocardiogram (Starr, high frequency bed) occurring on low oxygen may constitute the only evidence of coronary disease, thus adding another objective physiological parameter to the test; and *4*) it may provide the only proof that certain atypical symptoms are due to coronary disease.

Anthropological and Genetic Implications of Sticky and Dry Cerumen in American Indians. NICHOLAS L. PETRAKIS* AND KATHERINE T. MOLOHON, San Francisco, Calif.

Matsunaga has demonstrated that human cerumen occurs in two phenotypic forms, dry and sticky, which are controlled by a single pair of genes, in which the allele for the sticky condition is dominant over the dry. Striking racial and ethnic variation was reported in the frequency of the sticky and dry alleles. Mongolians, Japanese, and northern Chinese characteristically had high frequencies of dry cerumen ($q = 0.978$ to 0.915), whereas Caucasians and Negroes had very low frequencies of the dry allele ($q = 0.176$ to 0.069). No data were reported for American Indians. ¶ Since it is presumed that the American Indians migrated from Asia approximately 30,000 years ago, it would be expected that Indian populations would possess a high frequency of the dry allele. In the present study, investigations were made of the type of cerumen present in this racial group and the potential value of this trait as a marker for genetic and anthropologic studies. ¶ Examinations were made of 273 American Indians living in the San Francisco Bay area. The character of the cerumen was determined by simple inspection of both external ear canals. The tribal origins and the presence or absence of non-Indian admixture were recorded. Calculations were made of the frequencies for sticky and dry alleles. ¶ It was found that racially pure Indians from southwestern tribes had high frequencies of the dry allele ($q = 0.901$). Indians with known Caucasian admixture had lower frequencies of the dry allele ($q = 0.326$). Certain Midwestern tribes were found to have intermediate frequencies of the gene, and in these it was possible to estimate the degree of non-Indian admixture and the rate of gene flow. The high value for the dry allele found in unmixed groups is in agreement with theories of the Mongolian origin of the American Indian. The lack of association of either allele with any presently known disease of the ear and the presence of this dimorphism in primates suggest that the quality of cerumen may be employed as a genetic marker in anthropological and genetical studies.

Coordinated Target Organ Responses to Parathyroid Hormone. JAMES M. PHANG, GERALD A. M. FINERMAN, MONES BERMAN, AND LEON E. ROSENBERG, Bethesda, Md. (introduced by Nathaniel I. Berlin†).

Parathyroid hormone (PTH) alters calcium metabolism in bone, kidney, and gut. The contribution of each of these end organ responses to the regulation of calcium homeostasis in man, however, has not been defined. In the present study, such definition has been sought by computer multicompartmental analysis of stable and isotopic calcium dynamics in 1 hyperparathyroid and 10 hypoparathyroid patients. Results were compared with data obtained from 14 control subjects. Deficiency of PTH decreased fractional GI absorption of calcium and rates of skeletal resorption and accretion by about 50%. Renal calcium clearance normalized for filtered load was doubled in the PTH deficient group. These changes in end organ function resulted in a decrease in plasma calcium pool as well as in total exchangeable calcium. Purified bovine PTH given for 1 month to a thyroparathyroidectomized patient reversed these end organ abnormalities. Excessive endogenous PTH in a patient with hyperparathyroidism produced changes that paralleled those seen with exogenous PTH but were of greater magnitude. Thus, PTH appears to control calcium homeostasis by coordinated stimulatory effects on gut, bone, and kidney of approximately equal magnitude. Derangements of this coordinated pattern due to single end organ hypo- or hyperresponsiveness to PTH may be responsible for many metabolic diseases of bone in which pathogenesis remains obscure.

Evidence for Essential Fatty Acid Deficiency in Patients with Abetalipoproteinemia. GERALD B. PHILLIPS* AND JAMES T. DODGE, New York, N. Y.

The phospholipid fatty acid distributions of red cells from three patients with abetalipoproteinemia were determined by gas-liquid chromatography (GLC). The phospholipid fatty acids of red cells from two of the patients were analyzed in more detail by separating the fatty acid methyl esters into groups according to degree of unsaturation by thin layer chromatography of the mercuric acetate adducts before GLC. Changes were observed in the levels of many of the fatty acids in addition to the decrease in linoleic acid reported by others. These changes, however, fell into a pattern consistent with and thus in support of current theory of fatty acid biosynthesis. Members of the linolenate series ($20:5\omega3$, $22:5\omega3$, $22:6\omega3$) of fatty acids were decreased and of the oleate series ($20:1\omega9$, $22:1\omega9$, $24:1\omega9$, $26:1\omega9$, $20:3\omega9$, $22:3\omega9$, and probably $20:2\omega9$, $22:2\omega9$, $24:2\omega9$) increased. Whereas linoleate was strikingly decreased, members of the $\omega6$ series ($22:4\omega6$, $22:5\omega6$, $24:4\omega6$, and possibly $20:2\omega6$, $22:2\omega6$), which are apparently derived from it, were increased. Comparison of these results with data from animal studies indicates that these fatty acid changes were largely if not entirely a consequence of essential fatty acid (EFA) deficiency. This deficiency is probably secondary to the malabsorption and perhaps to the decreased fat intake in these patients. We believe the results support the concept of a requirement for exogenous fatty acid in man and that this study represents the first comprehensive analysis of fatty acids in human EFA deficiency. Several of the fatty acid changes noted, moreover, have not been described previously in any species with EFA deficiency.

A New, Nonisotopic Method for the Measurement of Triglyceride Turnover Rate in Man. D. PORTE, JR., AND E. L. BIERMAN,* Seattle, Wash.

A simple method for quantitating endogenous triglyceride (TG) turnover would facilitate the evaluation of lipemic states in man. Present techniques rely on injection of radioactive TG precursors or labeled lipoproteins. The former method involves unproved assumptions regarding specific activity and turnover rates in precursor pools; the latter method requires injection of foreign or altered native material. In the nonisotopic method for measuring TG turnover, a heparin infusion was utilized to perturb the steady state of TG turnover. As a result TG removal from plasma is accelerated via activation of circulating lipase. Repeated measurements of TG, and of the plasma lipolytic activity induced, were made during a constant infusion of heparin for 165 minutes in subjects who had previously been maintained on fat-free diets to clear plasma of exogenous (dietary) TG. In all ten subjects a new steady (lower) level of TG concentration allowed calculation of TG fractional turnover (k) from the formula, k = lipolytic rate/change in TG concentration from time 0 (modified from Dole and Rizack, 1961). *In vitro* lipolytic rate was measured at pH 7.4 in a 5% CO_2, 95% O_2 mixture by incubation within 10 minutes after blood sampling. The heparin turnover method has been previously validated for measurement of free fatty acid (FFA) turnover rate by comparison with FFA-^{14}C behavior. Simultaneous estimation of FFA turnover rate in the present study gave values between 16 and 27% per minute, in agreement with previous estimates. TG turnover (milligrams per kilogram per hour) was linearly related to TG concentration ($r = 0.84$, $p < 0.01$) in ten subjects with mild diabetes whose plasma TG concentration varied from 67 to 1,000 mg per ml. Half-time for plasma TG ranged from 15 to 43 minutes and was unrelated to plasma TG level. The method is simple, safe, and reproducible. Although isotopic precursor methods had suggested man to be unique among mammals with a low TG turnover rate, the heparin method indicates rapid TG turnover consistent with reported animal studies.

Influence of Acid pH on Lipolysis Activated by ACTH, Glucagon, and Cyclic 3′,5′-AMP. C. POYART, Y. VULLIEMOZ, AND G. G. NAHAS, New York, N. Y. (introduced by E. M. Papper†).

Acidosis inhibits norepinephrine (NE)-induced lipolysis *in vivo* and *in vitro*. This inhibition, which is reversed by theophylline, was attributed to a blocking effect of acid pH on the formation of cyclic 3′,5′-AMP. In the following experiments the interactions of glucagon and ACTH with increased [H^+] were studied *in vitro*. Rat epididymal adipose tissue was incubated in Krebs-Ringer phosphate medium with 5% albumin without glucose, and with glucagon or ACTH added. The rate of glycerol production was linear and was used as the index of lipolytic activity (in micromoles per gram wet tissue ± standard deviation per minute). With ACTH (10^{-1} U per ml) this index was 0.062 ± 0.012 at pH 7.40 and 0.037 ± 0.018 at pH 6.60 (a 40% inhibition). With glucagon (1 μg per ml) the lipolytic index was 0.080 ± 0.015 at pH 7.40 and 0.029 ± 0.012 at pH 6.60 (a 60% inhibition). Addition of 10^{-3} M theophylline produced a marked potentiation of the lipolytic index of ACTH and glucagon at pH 7.40. In contrast, there was not even an additive effect with NE, confirming that NE, ACTH, and glucagon act on lipase activation in a similar way. Addition of 10^{-2} M theophylline with optimal concentration of ACTH or glucagon resulted in similar lipolytic rates at pH 7.40 as well as at pH 6.60 showing that the inhibitory effect of acidosis was reversed by theophylline. These results indicate that H^+ might exert its inhibition on the formation of cyclic 3′,5′-AMP as previously postulated. To test this hypothesis, we added dibutyryl cyclic 3′,5′-AMP (10^{-3} M) to the medium; similar glycerol productions were found at pH 7.40 (3.03 μmoles per g per hour) and at pH 6.60 (2.65 μmoles per g per hour) ($p > 0.1$). When combined with lipolytic hormones at this concentration, dibutyryl cyclic 3′,5′-AMP also reversed the inhibitory effect of acidosis as did theophylline. Conclusions: *1*) Lipolytic activity of ACTH and glucagon (as well as NE) is inhibited by increased [H^+]. This inhibition is reversed by theophylline or cyclic 3′,5′-AMP. *2*) Increased [H^+] does not interact with cyclic 3′,5′-AMP but inhibits its formation, presumably by a direct action on specific sites of the cell membrane.

Zinc and Growth Hormone Interrelationship in Normal and Hypophysectomized Rats. ANANDA S. PRASAD, DONALD OBERLEAS, PAUL WOLF, AND J. HORWITZ, Detroit, Mich. (introduced by Richard J. Bing†).

Decreased growth rate is the most marked manifestation of zinc deficiency in experimental animals. In this study a possible interrelationship between zinc (Zn) and growth hormone (GH) was investigated. Fifty-five normal male rats (60 to 80 g) on a Zn-deficient diet for 3 weeks were divided into 4 groups and treated for 2 weeks as follows: group I received neither Zn nor GH (−Zn−GH); group II received bovine GH 40 U per day subcutaneously (−Zn+GH); group III received supplemental Zn (as carbonate) 55 mg per kg added to basal diet (+Zn−GH); and group IV received both supplemental Zn and GH (+Zn+GH). Two weeks mean weight gain (g) was as follows: group I, +8.6; group II, +7.7; group III, +65.9; and group IV, +68.4. Analysis of liver, bone, testes, muscle, esophagus, and heart for zinc, copper, iron, magnesium, calcium, and manganese revealed a significant decrease in zinc content of bones and testes in the first two groups as compared to the others. Histochemical analysis of various zinc-dependent enzymes revealed reduced activities of lactic, malic, and alcohol dehydrogenases and akaline phosphatase in bones and testes of the first two groups, whereas activity of succinic dehydrogenase (iron dependent) was not significantly different. In another experiment 42 hypophysectomized rats were divided into 4 groups and treated for 2 weeks as follows: group I (−Zn−GH); group II

(−Zn+GH); group III (+Zn−GH), and group IV (+Zn+GH). Two weeks mean gain in weight (g) was as follows: group I, −3.5; group II, +1.5; group III, +8.7; and group IV, +15.8. Except for zinc content of bones and testes, which was reduced in groups I and II, other investigations were unremarkable. In summary, growth depression associated with zinc deficiency does not appear to be mediated through GH activity or production. Most probably Zn and GH regulate protein synthesis and control growth by independent mechanisms.

Renal Concentrating Ability in Cirrhosis of the Liver. IV. Failure of Hypertonic Saline to Improve the $T^c_{H_2O}$ Defect. JORGE PRESSER, LILIANA VAAMONDE, CARLOS VAAMONDE, AND SOLOMON PAPPER,* Albuquerque, N. M.

The mechanism of the concentrating defect exhibited by some patients with cirrhosis of the liver is not clear. A decreased delivery of sodium to the distal concentrating site might be expected in diseases with marked sodium retention. Tubular sodium concentration may also be a critical determinant of the rate of sodium transport at the distal site. ¶ Since hypertonic saline increases the load and concentration of sodium entering the loop of Henle, $T^c_{H_2O}$ was measured separately in the hydropenic state during 10% mannitol and 3% saline diuresis. Three patients with cirrhosis and low $T^c_{H_2O}$ during mannitol diuresis (2.7± 0.7 ml per minute) (mean ± SD) and three chronically ill patients without liver disease ("controls") and with normal $T^c_{H_2O}$ (4.8 ± 0.4 ml per minute) were studied. All received the same diet (Na, 10 mEq; K, 100 mEq; protein, 1.5 g per kg, daily). ¶ No increase in $T^c_{H_2O}$ was apparent during saline diuresis in the cirrhotic patients (mannitol, 2.1 ± 0.4; NaCl, 2.3 ± 0.3 ml per minute, at C_{osm} 12 ml per minute) despite a 97% increase in the estimated absolute amount of sodium delivered distally. ¶ During saline diuresis estimated fractional delivery of sodium to the distal tubule was higher ($p < 0.05$) in cirrhotics than in "controls" at comparable C_{osm}. Fractional sodium excretion was higher ($p < 0.001$) in cirrhotics (10.2 ± 0.4%) than in "controls" (6.5 ± 0.3%). ¶ It is concluded that the defect in $T^c_{H_2O}$ formation exhibited by some patients with cirrhosis is not determined by a decreased delivery of sodium to the distal concentrating site or by a diminished concentration of sodium in the tubular urine.

The Effects of Ethyl Palmitate-induced Splenic Destruction on Rodent Hemolytic Anemias. LEONARD R. PROSNITZ, SHO KAWASAKI, JAMES J. DINEEN, IRWIN M. BRAVERMAN, PASQUALE E. PERILLIE, AND STUART C. FINCH,* New Haven, Conn.

The objectives of these studies were to produce functional and anatomical splenic ablation in rodents by means of either single or multiple intravenous injections of ethyl palmitate (EP). This procedure of "chemical splenectomy" was evaluated in normal Sprague-Dawley (S/D) rats, normal mice (C57Bl/CJ, ICR, and BALB/C strains), methyl cellulose-induced hypersplenic S/D rats, NZB mice with acquired hemolytic anemia, and deer mice with hereditary spherocytosis. An EP emulsion (300 mg per ml) with a particle size of about 1 μ was prepared by sonication of a mixture of EP, Tween 20, and 5% dextrose. All normal rats and mice developed focal splenic necrosis, maximal at 48 hours after a single injection of 3 g per kg of EP. No significant pathological changes were observed in other organs. EP-^{14}C studies demonstrated high preferential splenic localization at 24 and 48 hours. Biweekly injections of EP (3 g per kg) to normal S/D rats over a period of 4 weeks resulted in virtually complete splenic atrophy when the animals were examined 4 weeks after the last injection. Mortality was less than 5%. Similar treatment of mice with either acquired or hereditary hemolytic disease resulted in amelioration of hemolysis comparable to that obtained with surgical splenectomy. The S/D rats with methyl cellulose-induced hypersplenism tolerated EP injections poorly, and there was little evidence of improvement of functional hypersplenism. ¶ Splenic destruction appears to be the result of highly selective localization of the particulate aggregates of EP resulting in multiple areas of necrosis. Repeated injections produce extensive necrosis followed by fibrosis and atrophy. The end result is severe functional impairment of the spleen.

Effect of O_2 at 2 Atmospheres upon the Human Pulmonary Capillary Bed. R. J. M. PUY, R. W. HYDE, A. B. FISHER, J. M. CLARK, J DICKSON, AND C. J. LAMBERTSEN,† Philadelphia, Pa.

Despite the clinical use of hyperbaric O_2 (OHP), few studies of the pulmonary circulation have been made in humans who have developed respiratory symptoms due to OHP. To evaluate the effect of OHP upon the pulmonary capillary bed, we exposed 6 subjects to O_2 at 2 atmospheres for 6 to 11 hours by which time chest pain, cough, and dyspnea had appeared. The single-breath CO diffusing capacity (D_{LCO}) was determined at two different alveolar P_{O_2} (P_{AO_2}), which permitted the calculation of the capillary blood volume (Vc) and the membrane diffusing capacity (D_M). Capillary blood flow ($\dot{Q}c$) and pulmonary tissue volume (V_T) were determined by using the acetylene single-breath technique. Compared to control values, average changes obtained 0.5 to 5 hours after termination of OHP were as follows: D_{LCO} (measured at P_{AO_2} of 200 mm Hg) −8%, Vc −21%, D_M +20%, V_T −7%, and $\dot{Q}c$ +4%. Twelve to 20 hours after discontinuing OHP, the changes were these: D_{LCO} −15%, Vc −29%, D_M +5%, V_T −1%, and $\dot{Q}c$ −5%. Only changes in D_{LCO} and Vc were statistically significant ($p < 0.05$). ¶ The lack of significant changes in D_M and V_T associated with a decrease in Vc suggests that pulmonary congestion, such as seen in animals dying during exposure to OHP, had not yet occurred in our subjects. The fall in Vc might have been secondary to pulmonary vasoconstriction such as has been reported to occur in rats and guinea pigs poisoned by O_2. Mock experiments performed in 2 of the subjects suggest that approximately one-half of the change in D_{LCO} was due to OHP rather than

factors related to the duration of the experiments. Comparison of measurements of D_{LCO}, vital capacity, and lung compliance, performed in these subjects ½ to 8 hours after termination of OHP, showed that D_{LCO} offered no advantage over the other two parameters as an index for the detection of pulmonary O_2 toxicity.

Fluorescence Studies on Ribonucleic Acid in Chromosomes. L. M. RAZAVI, Boston, Mass. (introduced by Edward H. Kass†).

The distribution and role of ribonucleic acid (RNA) in mitosis are uncertain. It is thought that in mitosis RNA of the interphase nucleus is distributed among nucleolar-associated parts of the chromosomes. However, in human chromosomes, RNA is difficult to detect by conventional staining methods, because RNA is either in low concentration or changes its configurations. ¶ It was observed that at pH 4.75 and ionic strength 0.025, the electrostatic interaction between a serum protein (probably prealbumen) and RNA is amplified so that fluorescinated normal serum is seen to attach specifically to RNA. The reaction is abolished *a*) by absorption with RNA but not with DNA, and *b*) by digestion with ribonuclease but not DNAse. By this means, RNA in interphase nuclei is seen either diffusely distributed throughout the nucleus, or in large particles solely around the nuclear membrane. This pattern correlates with the distribution of basophilia. In mitosis during prophase, RNA forms sharply discrete zones within chromatids frequently at sites of secondary constriction. In metaphase, RNA coats the entire chromatid. ¶ RNA therefore is an invariant chemical property of chromosomes, but its form depends on the dynamics of the chromosome. The findings suggest that RNA is involved in chromosomal contraction and, perhaps, in protection of DNA and other components of the chromosome from cytoplasmic enzymes after the nuclear membrane has disappeared. Steric variability of other nuclear components may also occur during the cell cycle, rendering them difficult to detect by the usual measurements or by stains whose stoichiometry depends upon a static substrate. Generally, reactions between cationized proteins and polyanionic electrolytes (such as nucleic acids) can be used to predict pH and ionic strength at which proteins of a given isoelectric point will react stoichiometrically with a nucleic acid.

Sustained Antiarrhythmic and Inotropic Action of Norepinephrine during Myocardial Ischemia. T. J. REGAN,* W. M. BURKE, H. A. OLDEWURTEL, AND S. K. ASOKAN, Jersey City, N. J.

The substantial change in cardiac function that occurs during myocardial ischemia has suggested a need for examining the effects of the major regulatory hormone of the heart on the course of this event. Coronary thrombosis was induced with an electrode catheter in the left anterior descending coronary artery of intact anesthetized dogs, ventilated to maintain normal pH. ^{85}Kr in saline injected distal to the thrombus permitted serial regional coronary blood flow (CBF) measurements. Paired sampling of arterial and great cardiac venous blood at 5-minute intervals was performed to assess the relation of net ion and substrate transport to the development and control of ventricular tachycardia. Sustained systemic infusion of *l*-norepinephrine in a nonpressor dose, 0.1 μg per kg per minute, in 8 normal animals effects a 10% increment in left ventricular dp/dt and myocardial O_2 consumption. There are enhanced myocardial uptake of K^+, diminished glucose uptake, and no lactate production. ¶ Reduction of CBF in 10 thrombus controls to 25% of normal increased LVEDP 5.3 mm Hg and decreased LV dp/dt 30%. A net K^+ loss of 1.06 μEq per g of ischemic tissue anteceded ventricular ectopic beats, persisting through tachycardia. Fibrillation occurred in 90% within 4 hours. Norepinephrine was infused in 13 animals, 15 minutes after onset of a similar degree of ischemia and ion loss. Five had developed early ventricular tachycardia, which was corrected by hormone infusion, and sinus rhythm was maintained through 4 hours of infusion in these and the remaining 8, without increasing CBF to the ischemic area. K^+ egress was uniformly reversed ($p < 0.001$), uptake usually ensued, and glucose uptake remained diminished. Myocardial production of lactate was interrupted. The LVEDP rise was significantly reduced and dp/dt rose, whereas heart rate, arterial pressure, and the injury potential were unchanged. Participation of the ischemic area in the contractility response was evidenced in another group during local hormone infusion distal to thrombus. ¶ Thus, early use of norepinephrine in nonpressor doses significantly affects ventricular ectopic activity during ischemia, associated with regulation of ion transport. Unlike classical antiarrhythmic agents, the hormone enhances ventricular contractility, despite marked reduction of oxygen consumption.

Erythrocyte Transport Defect in Experimental Magnesium Deficiency. J. RENN, J. W. BALFE, C. COLE, AND L. G. WELT,† Chapel Hill, N. C.

Magnesium deficiency in rats has been consistently demonstrated to be accompanied by a loss of potassium from skeletal muscle. It has also been reported that intact rat diaphragm muscle will lose potassium *in vitro* in a magnesium-free medium, in contrast to a medium that contains 10^{-3} M magnesium. These and other data suggest that this loss of muscle potassium may represent a defect in transport. Rats maintained on a magnesium depletion regimen for 6 weeks or longer have consistently demonstrated an increase in the concentration of sodium in erythrocytes, which implies a transport defect in these cells as well. This phenomenon was evaluated by conventional methods of studying the active component of sodium efflux from erythrocytes in magnesium-depleted rats and their pair-fed controls. It is clear that the magnesium-deficient animals develop an increase in the concentration of erythrocyte sodium and a diminution in the scillaren-sensitive (pump I) component of the rate constant for sodium efflux from erythrocytes; in addition, erythrocytes from magnesium-deficient rats demon-

strate a significant reduction in the rate constant of the pump II of Hoffman and Kregenow (which is inhibited by the addition of ethacrynic acid to glycoside). The steady state levels of ATP in the erythrocytes cannot be implicated in these defects. The manner in which magnesium depletion induces these alterations in sodium transport in the erythrocyte is unknown. There is presumably some direct influence of magnesium deficiency per se, or one of its consequences, on the mechanisms involved in the active phases of ion transport in erythrocytes.

Partition of Calcium, Phosphate, and Protein in the Fluid Phase Aspirated at Calcifying Sites in Enchondral Cartilage. L. RICCA, D. S. HOWELL,* AND J. PITA, Miami, Fla.

A method developed previously in this laboratory from renal micropuncture techniques permits reproducible sampling of 20 mμl of a clear viscous fluid from epiphyseal cartilage of rats *in vivo*. The fluid has been demonstrated to originate from hypertrophic cell cartilage rather than adjacent metaphysis, epiphysis, articular, or resting cell cartilage. The calcium and phosphate in this fluid were fractionated by means of ultracentrifugation (105,000 × *g* for 8 hours at 12° C). The study was conducted with rats from 42 to 47 days old and was complemented with pH and protein determinations. The proportion of the total calcium that was "free" was greater in the specimens from normal and healing rachitic rats than in the rachitic animals. Values for pH in fluid from normal, calcifying, epiphyseal plates ranged from 7.6 to 7.8 when collected and measured in oil equilibrated with 5% CO_2. Bicarbonate was estimated from the pH slope at different measured CO_2 tensions to which the microscopic samples were exposed. Differences in pH and "free" calcium times phosphate product between rachitic and normal cartilage fluids were in the direction that would favor calcification in the normal plates. The calcium times phosphate product in several different preparations for healing rickets roughly correlated with histologic evidence of mineral deposition. The largest effect on calcium times phosphate product was short term starvation.

Tubular Secretion of Urate per Unit GFR in Gout. RICHARD E. RIESELBACH, LEIF B. SORENSEN,* WELDON D. SHELP, AND THOMAS H. STEELE, Madison, Wis., and Chicago, Ill.

A renal secretory defect for urate has been implicated in the pathogenesis of gout, in that C_{urate}/C_{inulin} is difficult to interpret because urate transport is bidirectional; also, purine loading may introduce variables. In the present studies, a technique was employed that estimates minimal tubular secretory rate of urate proportionate to nephron population (TS_{Ur}). This technique measures decrement in $U_{urate}V/C_{inulin}$ after pyrazinamide, a potent inhibitor of urate secretion. TS_{Ur} is a direct function of plasma urate (P_{Ur}). Variance of TS_{Ur} is greater with hyperuricemia. Therefore, 18 gouty patients were studied after being rendered normouricemic with allopurinol. Ten patients had essentially normal renal function (C_{inulin} > 85 ml per minute); 8 patients had chronic renal disease (CRD) with C_{inulin} 15 to 50 (mean 34). Uric acid-^{14}C pool and turnover studies were performed on 15 patients; 2 had excessive urate production. ¶ Mean TS_{Ur} of gouty patients without CRD was lower than normal (2.2 μg per minute per C_{inulin} at P_{Ur} 4.8 mg per 100 ml vs. 4.0 at P_{Ur} 5.4 in 10 normal subjects previously studied); however, only 4 had a TS_{Ur} below 2 SD from the normal mean. Mean TS_{Ur} in gouty patients with CRD was above normal (4.5 at P_{Ur} 5.0); 7 patients were above the normal mean. Before allopurinol, gouty patients without CRD had a lower TS_{Ur} than gouty CRD patients (5.1 at P_{Ur} 9.1 vs. 10.1 at P_{Ur} 9.0). Hyperuricemia of gouty CRD patients was not totally explicable by renal disease per se, in that 8 nongouty CRD patients with C_{inulin} 15 to 50 (mean 33) had a significantly lower P_{Ur} of 6.71 ($p < 0.01$). ¶ Thus, some gouty patients exhibited a secretory defect for urate. However, the majority had a normal TS_{Ur} and rate of production.

Enhancement of Apparent Excretory Maximum of Sulphobromophthalein Sodium (BSP) by Taurocholate and Dehydrocholate. DONALD J. RITT AND BURTON COMBES,* Dallas, Texas.

The present studies were carried out to test the validity of the concept that excretion of BSP into bile is limited by a transport maximum. Anesthetized (pentobarbital) dogs, after common duct cannulation and cystic duct ligation, were infused with BSP at a rate estimated to saturate the hepatic excretory mechanism. An apparent maximal rate of BSP excretion into bile was observed despite continuously rising blood levels of BSP, suggesting the operation of a limited transport system. However, BSP excretion could be enhanced significantly above the apparent maximal control rate (mean maximal increase above control value was 1.9 ± SD 0.3) when bile flow was stimulated at the canalicular level by infusions of taurocholate or dehydrocholate. Of the increased BSP excreted, proportionately more was free than conjugated. Reversible depression of BSP excretion ensued at very high rates of taurocholate excretion. Secretin stimulation of bile flow did not enhance BSP excretion. It is not yet possible to decide whether enhanced BSP excretion is the direct consequence of increased bile salt excretion per se or is indirectly related to enhanced canalicular bile flow induced by increased bile salt excretion. If increased bile flow is the important factor, the following explanation would appear to account for the present findings. BSP collected in common duct bile represents the net between BSP transported into canaliculi and that which back diffuses into hepatic cells along favorable concentration gradients. Increased canalicular flow by decreasing intracanalicular concentration of BSP would diminish back diffusion and result in increased net biliary excretion of dye. This possibility is supported by the observation that excretion of the relatively more diffusible free BSP molecule is preferentially enhanced. Ir-

respective of mechanism, these studies demonstrate that the apparent transport maximum for BSP is not a fixed value since it may be significantly enhanced by bile salts.

Glucose Metabolic Pathways of Rheumatoid and Nonrheumatoid Synovial Membrane. J. E. ROBERTS, B. D. MCLEES, AND G. P. KERBY,† Durham, N. C.

Using surgical specimens and a system of plane dissection to be described, we obtained tissue for study consisting of synovial lining cells (and proliferating villi in rheumatoid synovia) with some subsynovial connective tissue and capillaries. Dense connective tissue and adipose tissue were excluded. Factors derived secondary to hemoglobin determinations and lysozyme assays were introduced to correct in part for metabolic activity due to infiltrating erythrocytes and leukocytes. Cofactors were present in optimal concentration and substrates in excess in incubation mixtures. Glucose-1- and 6-^{14}C were incorporated variously, and $^{14}CO_2$ activity was determined. Lactates were measured enzymatically. A total of 14 human synovia were used in the studies of this report. ¶ Markedly increased metabolic activity of rheumatoid villous tissue was confirmed, with the over-all pattern otherwise resembling that of nonrheumatoid synovial tissue. A Pasteur effect was demonstrable with nonrheumatoid tissue. The Embden-Meyerhof cycle was again shown to be a major energy pathway for all synovial tissue studied, in contrast to the tricarboxylic acid cycle, which appeared to be of no major importance. Significant pentose cycle activity was found, however, raising the indirect question of possible importance of lipid metabolism as an energy source for this tissue. Exploration of this possibility is scheduled. ¶ An attempt was made to demonstrate metabolic changes reflecting phagocytic activity, by adding to incubation mixtures various proteins including rheumatoid euglobulin, aggregated human plasma γ-globulin, and complexes thereof. Results were inconclusive.

Serum Opsonins and Bactericidins for Group B Meningococci. RICHARD B. ROBERTS, New York, N. Y. (introduced by James G. Hirsch*).

Studies have been made on the interaction *in vitro* between meningococci and rabbit polymorphonuclear leukocytes in the presence of normal or immune rabbit serum. In the system containing normal serum there was no phagocytosis, nor was there extracellular bactericidal activity on most of the strains tested. Antiserum collected 12 to 21 days after subcutaneous inoculation of living log phase meningococci exhibited opsonic activity with apparent type specificity. The opsonic action depended on both heat labile and heat stable components; immune serum heated at 56° C for 30 minutes lost its phagocytosis-promoting property, but this property could be restored by the addition of unheated normal rabbit serum. After ingestion by granulocytes meningococci were rapidly killed. These studies thus indicate that some meningococcal strains contain specific antiphagocytic surface factors of an as yet unknown chemical nature. ¶ Antisera obtained 4 or more weeks after immunization or after more intensive antigenic stimulation showed bactericidal activity with apparent type specificity. This bactericidal activity was also lost after heating and restored by the addition of normal serum. It was therefore impossible to test bactericidal serum specimens for opsonic activity. ¶ Further study of opsonizing and bactericidal antibodies to meningococci may shed light on virulence factors of these bacteria, and also may prove to be useful for typing and epidemiologic studies.

Reticulocyte Death: A Source of Early Labeled Bile Pigment. STEPHEN H. ROBINSON, Boston, Mass. (introduced by A. Stone Freedberg†).

Production of early labeled bile pigment (ELP), which is derived from sources other than senescent erythrocytes, is increased by erythroid stimulation. Several mechanisms have been proposed for this erythropoietic component of ELP, including ineffective erythropoiesis, degradation of hemoglobin attached to extruded normoblast nuclei, and destruction of circulating reticulocytes, but there has been no direct evidence for any of these hypotheses. ¶ Erythropoiesis was stimulated in rats by hemorrhage. Animals were then given glycine-2-^{14}C, and 1 day later were exsanguinated to obtain reticulocyte-rich blood. Rats not previously hemorrhaged were given glycine-^{14}C 1 day before exsanguination to obtain labeled normal reticulocytes, and others were given glycine-^{14}C 10 days beforehand to obtain labeled mature erythrocytes. After removal of most plasma and dilution of residual labeled precursor with "cold" glycine, donor cells were transfused intravenously into recipient rats with external bile drainage. Disappearance of labeled erythrocyte hemoglobin-heme and excretion of bilirubin-^{14}C in bile were measured in the recipients over the ensuing 3 to 5 days. ¶ Bilirubin-^{14}C production was expressed as per cent conversion of labeled erythrocyte heme, and mean values over the first 3 days were as follows: *1*) from reticulocytes produced in response to hemorrhage 7.6% (SE 0.5), *2*) from normal reticulocytes 1.6% (SE 0.2), and *3*) from adult erythrocytes 2.1% (SE 0.3). This fivefold difference between transfusion of labeled reticulocytes produced after hemorrhage and normal reticulocytes was sufficient to account for more than one-third the increase in ELP production that was measured directly in hemorrhaged rats. ¶ The findings support the observation that there is early destruction of reticulocytes produced with erythroid stimulation. Further, for the first time they directly define a mechanism for the increase in ELP formation that is associated with accelerated erythropoiesis.

The Influence of Saline Loading on the Kinetics of Glucose Reabsorption: A New Perspective in the Interpretation of Third Factor Activity. ALAN M. ROBSON, PREM L. SRIVASTAVA, AND NEAL S. BRICKER,* St. Louis, Mo.

As the nephron population is reduced, sodium excretion per remaining nephron increases. This occurs inde-

pendently of hyperfiltration and mineralocorticoid insufficiency and appears to be due to a significant degree to enhanced third factor activity. As the nephron population decreases, there also occurs a change in the kinetics of glucose reabsorption. This is characterized by increased splay in the titration curve and a decreased Tm_g/GFR ratio. Because of the known dependency of glucose transport on sodium transport, the question arose as to whether third factor also influences glucose transport. If so, insight might be gained into the mechanism of third factor activity, for although sodium transport can be studied over a very limited range of the total potential transport capacity, glucose may be studied over an extensive range and the kinetics of transport defined precisely. Glucose titration studies were performed in normal unanesthetized female rats both in the hydropenic state and during saline loading. In hydropenia there was little or no splay. During saline loading, splay consistently increased, often to a striking degree, and the patterns mirrored those observed in animals with a drastic reduction in nephron population. Glomerular filtration rate increased by an average of 17.0%, yet despite this (and the probable associated increase in tubular diameter) $Tm_{glucose}$ values were diminished by 19.5%, and Tm_g/GFR decreased consistently. Identical changes in the kinetics of bicarbonate reabsorption have been observed after nephron reduction. Because substrate (i.e., glucose) concentrations were very high, an effect of third factor on luminal permeability seems unlikely. The data are consistent with inhibition of some step in the coupled energy production-transport system, and they indicate that whatever the step is, it influences sodium, glucose, and bicarbonate. From a kinetic analysis, third factor could act as a noncompetitive inhibitor.

Imino-Glycinuria: An Inborn Error of Renal Transport. LEON E. ROSENBERG AND JOSEPH L. DURANT, New Haven, Conn. (introduced by Howard Levitin*).

Previous renal clearance studies in normal subjects and patients with familial hyperprolinemia showed that the imino acids, proline and hydroxyproline, share a renal tubular reabsorptive mechanism with glycine. The present findings indicate that this shared transport system is under specific genetic control. The propositus, a 6-year-old boy with congenital nerve deafness, excreted markedly increased quantities of glycine, proline, and hydroxyproline in several 24-hour and random urine samples. Renal function was otherwise normal as were plasma amino acid concentrations. The child's parents each demonstrated hyperglycinuria without iminoaciduria, and his twin siblings had normal urinary amino acid patterns. Endogenous renal clearance studies showed that the propositus reabsorbed only 60% of filtered glycine compared to 85 to 87% in his parents and more than 92% in eight control adults and children. Furthermore, in contrast to normal subjects, proline infusion failed to alter glycine reabsorption significantly in the propositus. A gut transport defect was excluded in the propositus by the following results: absence of proline in stool extracts after an oral proline load, a normal oral glycine tolerance curve, and normal uptake of glycine by jejunal mucosa *in vitro*. These data suggest that the product of a single gene, which controls a significant fraction of glycine and imino acid reabsorption in the proximal tubule, is defective in the family under investigation. The disparity in urine pattern and clearance data between the propositus and his parents implies that the former is a homozygote for this recessively inherited transport defect and that the latter are each heterozygotes for the abnormality. The absence of a gut defect contrasts sharply with findings in cystinuria and Hartnup disease and indicates that intestinal and renal transport of the imino acids and glycine is not controlled by a single, common genetic mechanism.

A Biochemical Relationship between an Abnormality of Purine Metabolism and Central Nervous System Function. FREDERICK M. ROSENBLOOM, WILLIAM N. KELLEY, JOHN M. MILLER, J. FRANK HENDERSON, AND J. EDWIN SEEGMILLER,† Bethesda, Md.

In their original description of a familial syndrome characterized by choreoathetosis, spasticity, mental retardation, self-mutilation, and hyperuricemia, Lesch and Nyhan suggested that the neurological symptoms might be related to the marked overproduction of uric acid present in these children. The normal concentrations of uric acid in cerebrospinal fluid of these patients argue against uric acid being a direct cause of the neurological problem. However, in five patients the concentration in cerebrospinal fluid of uric acid precursors, the oxypurines hypoxanthine and xanthine, averaged nearly five times the concentration found in control subjects who did not have the syndrome (0.58 ± 0.07 vs. 0.13 ± 0.06 mg per 100 ml) despite the fact that the plasma concentrations of oxypurines were the same. In these patients the oxypurine concentration of cerebrospinal fluid was three to four times that of plasma, suggesting that the oxypurines were not derived from plasma. Treatment of affected children with allopurinol to control their hyperuricemia and uric acid nephrolithiasis resulted in a two- to threefold increase in concentration of oxypurines in cerebrospinal fluid with a ten- to twelvefold increase in concentration of oxypurines in the plasma. ¶ If a high concentration of oxypurines in the cerebrospinal fluid causes abnormal function of the central nervous system, then treatment with allopurinol may be detrimental. ¶ Our recent demonstration of the absence in this disorder of the enzyme hypoxanthine-guanine phosphoribosyltransferase, which is concerned with the reutilization of the free bases hypoxanthine and guanine, provides a reason for the increased concentrations of oxypurines in cerebrospinal fluid. ¶ A self-mutilation induced in rats by administration of a methylated xanthine may provide an animal model of this syndrome.

Reaction of Cold Agglutinin Antibodies with I^+ and I^- Red Blood Cells. WENDELL F. ROSSE, TIBOR BORSOS, AND HERBERT J. RAPP, Durham, N. C., and Bethesda, Md. (introduced by R. Wayne Rundles†).

The reaction of autoimmune cold agglutinin antibodies with human red cells was measured by the C'1a fixation and transfer test of Borsos and Rapp. Since these antibodies are IgM in type, one molecule of antibody was sufficient and necessary for the fixation of one molecule of C'1a. The number of C'1a molecules fixed was used as a direct measure of the amount of antibody bound to the red cells. ¶ Cold agglutinin antibodies from different patients vary markedly in their affinity for red cell antigens (I antigens). Some antibodies are tightly bound by adult (I^+) red cells, whereas others are very loosely bound. The fixation of C'1a appears to enhance the binding of antibody to antigen. This enhancement is more marked for loosely bound antibodies. ¶ Cold agglutinin antibody is bound by all human red cells tested, but in general, so-called I^- red cells (umbilical cord cells and genetic I^- cells) fixed less antibody than I^+ adult cells. The difference in the amount of antibody fixed by I^+ and I^- cells was larger for those antibodies that are highly dissociable. If the dissociability of the antigen-antibody reaction was increased by increasing the temperature at which the reaction took place, the difference in amount of antibody bound by I^+ and I^- cells became larger. If the dissociability of the reaction was decreased by treatment of the cells with papain, the amount of antibody bound by I^+ and I^- cells became more nearly the same. ¶ These findings suggest that all human red cells contain antigens that react with cold agglutinin antibodies. The dissociability of this reaction varies with different cells and with different antibodies, and the degree of dissociability may play a role in determining the denomination of the cell as I^+ or I^-.

A Radioenzymatic Assay for Folic Acid in Serum. SHELDON P. ROTHENBERG, New York, N. Y. (introduced by Solomon A. Berson†).

In an enzymic reaction system containing 0.25 mμg of tritium-labeled folic acid (folic acid-^{3}H), TPNH, and a rate-limiting quantity of folic acid reductase, the addition of stable folic acid will competitively inhibit the conversion of the folate-^{3}H to tetrahydrofolic acid (FH_4). At the end of the reaction the folate-^{3}H is precipitated with $ZnSO_4$ and TCA after the addition of excess stable folate. The FH_4-^{3}H in the supernate is assayed in a liquid scintillation counter. The reciprocal of the fraction of folate-^{3}H converted to FH_4 after 24 hours' incubation at 4° C is plotted as a function of stable folate to yield a nearly linear standard curve. The radioassay can detect approximately 2 mμg of folic acid in 0.2 ml serum. At this concentration or greater the assay can be performed in whole serum without extraction, but folate-deficient and normal sera cannot be distinguished. However, the clearance of folic acid from the blood can be measured, and 20 minutes after a small intravenous dose (30 μg per kg b. w.) deficient patients retained 0.1% or less and nondeficient subjects 5% or more per L plasma. FH_4 and 5-formyl FH_4 were less inhibitory on the reaction than equimolar concentrations of stable folate. Significantly, however, 5-methyl FH_4 was not inhibitory at all. Since 5-methyl FH_4 may be the "circulating folate," lack of inhibition by this compound on the enzymic reduction of folate may, in part, explain why this radioassay could not distinguish folate-deficient serums from normal serums.

The Colloid Osmotic Regulation of Albumin Synthesis Demonstrated in the Isolated Perfused Rabbit Liver. MARCUS A. ROTHSCHILD,* MURRAY ORATZ, JOSEPH MONGELLI, AND SIDNEY S. SCHREIBER, New York, N. Y.

Albumin synthesis, *in vivo,* has been shown to be depressed in the presence of hypergammaglobulinemia and of added colloid, and an osmotic regulatory system effecting control over albumin synthesis has been proposed. To study this problem further, we used the isolated perfused rabbit liver and measured albumin synthesis employing isosmotic and hyperosmotic perfusion mixtures. Carbonate-^{14}C was used to label the guanidine carbon of arginine. This labeled carbon enters urea and as arginine enters albumin. The mean specific activity of the urea produced during the 2.5-hour perfusion periods was used as an index of the specific activity of the intracellular arginine incorporated into albumin. Albumin from the perfusion was isolated by alcohol-trichloroacetic fractionation and checked for purity by immunoelectrophoresis. The acid hydrolysate of the protein was treated with arginase and the released urea determined by the method of Conway. $^{14}CO_2$ was trapped in phenethylamine for assay in a liquid scintillation counter. Livers were perfused with 2 parts heparinized rabbit blood to 1 part Ringer's solution with added amino acids, and the solution was gassed with 95% O_2–5% CO_2. pH was maintained between 7.3 and 7.4. The final albumin concentration was 3% in six control studies, and albumin synthesis averaged 40.6 ± 3.1 mg per 100 g liver. When the albumin level was increased to 9%, albumin synthesis decreased 29% to 28.7 ± 3.2 mg per 100 g liver ($p < 0.05$) in eight studies. Perfusion with 3% albumin–1% sucrose resulted in a 39% decrease in albumin synthesis to 23.4 ± 3.4 mg per 100 g liver ($p < 0.01$) in five studies. There was no difference in urea production, which averaged 5.5 to 6.7 mg urea C per hour per 100 g liver weight. Bile flow was continuous in all studies, and the specimens showed no histologic abnormality. These results are in accord with the concept that albumin synthesis can be regulated by changes in colloid or osmotic concentration within the liver.

Heavier DNA Labeling from Thymidine-^{3}H. JOSEPH R. RUBINI, Miami, Fla. (introduced by Eugene P. Cronkite†).

Although thymidine-^{3}H (TDR) has been effectively employed as a tracer for cell proliferation studies, it is only minimally utilized as a precursor for new DNA

synthesis. Heavier labeled DNA from TDR-^{3}H should be of potential therapeutic and investigative value. The present experiments tested normal dog bone marrow cell suspensions for their ability to utilize TDR-^{3}H for DNA-^{3}H synthesis under standardized conditions *in vitro* and in the presence of alterations in TDR metabolism selected to increase label uptake. *1*) Degradation of TDR-^{3}H by TDR phosphorylase was blocked by adding unlabeled thymine. *2*) Endogenous high pressure thymidylate synthesis was impaired by adding aminopterin or FU. *3*) More TDR-^{3}H was initially presented to the cells for uptake by raising SA from 2 to 15 c per mmole and by adding more microcuries. *4*) Prolonged contact of the cells with intact TDR-^{3}H was then carried out by lengthening the incubation period. *5*) Even further uptake was obtained by intermittent cell washing. Each alteration contributed to increasing DNA radioactivity. After prolonged incubation (3 hours) and hourly cell washing, extracted DNA-^{3}H of 6 million cpm was achieved, about 120 times greater than obtained with usual methods. Calculations showed, however, only little improvement in TDR-^{3}H utilization for DNA synthesis. Considerable radioactivity remained in the supernatants, and the persistence of intact but nonutilized TDR-^{3}H after labeling was demonstrated. Although heavier labeling of DNA by TDR-^{3}H was obtained, DNA-^{3}H synthesis still appears limited.

The Role of Adipose Cell Enlargement in the Carbohydrate Intolerance of Human Obesity. LESTER B. SALANS, JEROME L. KNITTLE, AND JULES HIRSCH,* New York, N. Y.

Human obesity is often accompanied by abnormal glucose tolerance and elevated levels of plasma insulin; indeed, the high plasma insulin concentration of some diabetic patients may be related to the presence of obesity. The relationship between these metabolic abnormalities and adipose depot size was studied in 21 nonobese and 5 obese hospitalized subjects. ¶ Subcutaneous adipose tissue was removed by needle aspiration and the rate of glucose-^{14}C incorporation into CO_2 and triglyceride measured with and without insulin in the medium. The number and size (micrograms lipid per cell) of adipose cells in the tissue fragments were determined. In obese individuals, plasma insulin response to oral glucose was measured immunochemically. ¶ These studies indicate that the basal rate of glucose metabolism is independent of cell size when data are expressed on a "per cell" basis. In contrast, the effect of insulin is highly dependent upon the size of adipose cells. Thus, the small cells of children (0.428 μg) show a 150% increase in glucose oxidation when insulin is added to the medium, compared to nonobese adults (0.665 μg) and obese patients (0.903 μg), who show only 110% and 50% increases, respectively. Moreover, when cells of obese patients are reduced in size (0.531 μg) by prolonged weight reduction, they become "normally" responsive to insulin, with a 153% increase in CO_2 production. ¶ Plasma insulin concentration in obese patients, 2 hours after oral glucose, was markedly elevated (12 mμg per ml) when compared with nonobese subjects (4 mμg per ml). Furthermore, the insulin level returned to normal (3 mμg per ml) with weight reduction and the return of adipose tissue sensitivity to insulin. ¶ Thus, the impairment of carbohydrate metabolism found in obese subjects is closely related to decreased insulin sensitivity of adipose tissue, which accompanies adipose cell enlargement.

A Cross-Reaction between Streptococcal Hyaluronate and Proteinpolysaccharides from Human Connective Tissue. JOHN SANDSON* AND DAVID HAMERMAN,* New York, N. Y.

Recently, the proteinpolysaccharides (PP) of both normal human synovial fluid hyaluronateprotein (NHP) and cartilage (CPP) have been shown to be antigenic. Antiserum in rabbits to human CPP formed two precipitin lines on double diffusion in 0.6% agarose (0.025 M barbital, pH 8.6) with hyaluronidase-digested human CPP. One line is due to a determinant that is species specific. The other line is due to a determinant that is common (F) to CPP obtained from humans as well as from the cow and pig. F has also been shown to be present in NHP. Studies were performed to determine whether F was also present in hyaluronate (SH) produced by streptococcus type X (obtained from M. Heidelberger) or by streptococcus type XI (obtained from A. Cifonelli). SH produced by both types of streptococci formed a precipitin line with the antiserum to CPP, which fused completely with the precipitin line formed by F in hyaluronidase-digested CPP or NHP. After absorption with either SH or CPP, the antiserum to CPP no longer formed this precipitin line with SH. After digestion of SH with testicular hyaluronidase, the precipitin line was broader and less intense. The linkage of protein to polysaccharide appears to be similar in CPP and NHP: serine (or threonine) on the protein is linked by a glycosidic bond to either galactose or xylose, which is in turn linked to the polysaccharide. Analyses of a glycopeptide isolated from SH revealed serine and threonine to be the predominant amino acids, and galactose to be present. These chemical similarities suggest that F, which is present in CPP, NHP, and SH, may be located at or near the site of linkage of protein to polysaccharide. This appears to be the first cross-reaction demonstrated between a bacterial polysaccharide and the proteinpolysaccharides of human connective tissue.

***In Vitro* Production of Alpha-Methyl Dihydroxyphenylalanine Melanin.** RICHARD J. SASSETTI, San Francisco, Calif. (introduced by H. Hugh Fudenberg*).

The occurrence of positive antiglobulin tests in individuals receiving α-methyldopa suggests that it or its polymeric oxidative product is involved in the mechanism of this reaction. Investigation of this mechanism requires control of oxidation and polymerization in order to obtain a stable, soluble, reproducible, macromolecular species. ¶ Since in the case of dihydroxyphenylalanine (DOPA) the initial step in the oxidative polymerization is the con-

version of the hydroxyl group to a quinone, reversible modification suggests itself as a method of control. Borate ion was chosen as the modifying agent because of its well-known chelation to dihydrols in the *cis* configuration and its antioxidant effect on epinephrine. ¶ Comparison of the ultraviolet and visible spectra of DOPA and α-methyldopa at intervals in the course of their autooxidation suggests that the α-methyl group does not affect the oxidative pathway, and that methods for following the reaction of DOPA were valid for α-methyldopa. With these spectrophotometric methods, the effect of borate on the oxidative polymerization was studied. Although the rate of the reaction is pH dependent, borate is inhibitory proportional to its concentration above a minimum of 0.03 mole per L. In all cases the inhibition is almost complete at 0.125 mole per L and is complete at 0.25 mole per L. ¶ Addition of borate ions to a reaction already underway stops the formation of intermediates but does not prevent the incorporation of those already formed into the polymer. By a method based on these observations, a polymer of α-methyldopa has been produced that is soluble, not ultrafilterable, and stable in light and air at room temperature.

Variation in Proliferation of Human Leukemic Cells. E. F. SAUNDERS, BEATRICE C. LAMPKIN, AND ALVIN M. MAUER, Cincinnati, Ohio (introduced by Edward L. Pratt†).

Proliferative activity of marrow samples from children with acute leukemia was assessed by means of mitotic index and tritiated thymidine labeling index. The marrows were more than 90% replaced with leukemic cells. In 43 studies on 31 patients labeling indexes ranged from 1.8 to 63%. Mitotic indexes in 15 studies ranged from 1.0 to 11.1. Labeling indexes at diagnosis ranged from 1.8 to 15.5 (mean 6.7) and in relapse from 6.0 to 63 (mean 17.3). Ten patients with multiple studies all showed marked changes in labeling from diagnosis to subsequent relapses. The leukemic cell population is heterogenous, consisting of large, dividing and small, nondividing cells. In 4 patients with multiple studies, changes in labeling index were directly related to changes in the proportion of large blasts in the marrow. ¶ Diurnal variation of proliferation was investigated in 6 patients by obtaining mitotic and labeling indexes at 6-hour intervals. Four patients had greatest mitotic indexes at midnight, as do normal subjects. Four patients had small but significant variations in labeling indexes, without, however, any consistent pattern. ¶ The appearance of labeled mitotic figures in serial marrow samples after a single injection of tritiated thymidine was followed in 1 patient at diagnosis and 2 patients in relapse. From the time course DNA synthesis times of 20 hours and minimal generation times of 60 hours were deduced. ¶ Observed variations in labeling, therefore, are related to changes in the proportion of dividing cells, not drastic alterations in generation time. The observation of high labeling indexes in relapse, and short duration of symptoms in patients with the greatest labeling indexes at diagnosis, is consistent with progressive accumulation of nondividing malignant cells as found in some animal tumors.

Human Serum High Density Lipoprotein (HDL). A Study of the Molecular Weight of Its Polypeptide Chains. A. SCANU,* Chicago, Ill.

Previous studies from this and other laboratories have provided evidence for a multichain structure of the human serum HDL apoprotein (apo HDL). As a part of a study aimed at the elucidation of the physical and chemical properties of these subunits, molecular weight determinations were conducted on apo HDL, suitably deprived of its lipid complement, under conditions favoring maximal subunit dissociation. For this purpose, apo HDL was modified by treatment with succinic anhydride and then studied in the analytical ultracentrifuge in high dilutions (0.01 to 0.02%) by a special multichannel system with the aid of interference optics. Under these conditions, the succinylated products behaved as a homogeneous entity with a mol wt of 25,800 ± 1,000. No further dissociation was obtained either by increasing the net negative charge of the subunits in high alkaline media (pH 11 to 12.5) or after reduction or alkylation. Values of molecular weight of the same order of magnitude were obtained by *1*) quantitative *N*-terminal amino acid analysis in the presence of sodium dodecylsulfate or guanidine hydrochloride, *2*) peptide maps after digestion of apo HDL by trypsin freed of any chymotryptic activity, and *3*) quantitative amino acid analysis. ¶ The results indicate that the subunits of HDL have a similar molecular weight and interact, after delipidation, probably through noncovalent linkages. The same conclusions were derived from studies with the two HDL subclasses, HDL_2 (1.063 to 1.125) and HDL_3 (1.125 to 1.21). The present findings and the knowledge of the molecular weight and chemical composition of these two HDL species clearly support the multipeptide structure of these lipoproteins and also the previously postulated concept that the difference between the light and heavy classes of HDL is in both lipid content and number of peptide subunits.

Molecular Basis for Vitamin D Action in the Small Intestine. DAVID SCHACHTER,* SZLOMA KOWARSKI, AND PHYLLIS REID, New York, N. Y.

Prior studies in rats indicate that vitamin D maintains a mechanism that transfers calcium against concentration and electrical gradients from mucosa to serosa of everted gut sacs *in vitro*. Present studies with a calcium activity electrode demonstrate that the transfer is an active cation transport yielding activity gradients of calcium ion serosal/mucosal of approximately 3.0. In vitamin D deficiency the gradients do not exceed 1.0. Increased activity gradients serosal/mucosal account for enhanced absorption observed with gut sacs from young, growing rats, pregnant animals, and rats on low calcium diets. Na^+ is required for the active transport *in vitro*. ¶ In deficient rats restoration of the active transport by

vitamin D accompanies the appearance in mucosal homogenates of a soluble protein or proteins that bind Ca with high affinity, similar to that in chickens described by Wasserman and Taylor. In rats the calcium binding activity and the active transport correlate closely with respect to distribution in the small intestine and variation with age, pregnancy, and diet. The binding protein may be a transport carrier for Ca. (In the hamster, however, active calcium transport is maximal in ileum, whereas calcium binding activity is greatest in duodenum.) ¶ The following model incorporates the known features of the transport mechanism. Vitamin D acts in the mucosal epithelial cell to induce the biosynthesis of the transport carriers, which may include the cytoplasmic calcium binding protein. Uptake into the epithelial cell at the mucosal surface is carrier mediated and along activity gradients. Subsequent transfer out of the cell at the serosal surface occurs against activity gradients and could result from dissociation of carrier-calcium complexes in the membrane by the relatively high concentrations of extracellular Na^+.

Myocardial Ischemia. High Energy Phosphate Levels and Electrocardiographic Changes. JAMES SCHEUER AND WILLIAM STEZOSKI, Pittsburgh, Pa. (introduced by Jack D. Myers†).

To study the relation of high energy compounds to the electrocardiogram (ECG), we compared rat hearts perfused for 30 minutes at control rates (C) with hearts perfused for 25 minutes at control rates and then at reduced, ischemic rates. The mean reduction in qO_2 was 60% ($p < 0.001$). Perfused hearts were stopped by crushing with clamps cooled in liquid nitrogen. Two types of ischemic experiments were performed. Hearts were frozen in group R1 as soon as the ECG changed (mean duration of ischemia 1.8 minutes) and in R2 after 5 minutes of ischemia. ECG changes occurred in all ischemic experiments. In 10 out of 18 hearts ischemic ECG changes were found in the presence of normal levels of creatine phosphate (CP) and adenosine triphosphate (ATP). These are treated statistically as a separate group in R3. Ischemia caused decreased heart rate and left ventricular pressure ($p < 0.01$). ECG changes included diminished R wave amplitude, deepened Q waves, and ST and T wave alterations. Results are in μmoles per g dry heart. Only in R2 were there significant changes in mean levels of CP (C 22.4 ± 1.1, R2 17.2 ± 1.7), ATP (C 22.3 ± 0.3, R2 20.0 ± 0.7), and adenosine monophosphate (C 1.04 ± 0.08, R2 1.46 ± 0.13). Adenosine diphosphate was not significantly elevated. All ischemic groups showed significant elevations in inorganic phosphate (P_i) (C 38.0 ± 0.6, R1 44.0 ± 1.3, R2 43.4 ± 1.3, R3 43.6 ± 1.3). Myocardial and perfusion medium lactate and lactate to pyruvate ratios were consistently elevated in ischemia. P_i and myocardial and perfusion medium lactate were the most sensitive indicators of hypoxia. These findings demonstrate that in isolated hearts, ischemic ECG alterations can occur with normal myocardial high energy stores, but suggest that more subtle changes in high energy phosphate balance or distribution may occur.

On the Mechanism of Delayed Hypersensitivity Reactions. STUART F. SCHLOSSMAN, Boston, Mass. (introduced by Howard H. Hiatt*).

Previous work from our laboratory led us to postulate that the initial event in delayed hypersensitivity reactions is an active process analogous to a secondary response wherein an immunogenic molecule triggers immunologically committed lymphoid cells to produce antibody locally. This concept would explain the delay in appearance of the skin response, the requirement for lymphoid cells to effect transfer of the delayed response, and the inability of polysaccharides, which are incapable of causing an anamnestic response, to provoke delayed reactions. It contrasts with the suggestion of others that the exquisite immunologic specificity of the delayed response reflects the specificity or affinity of a preformed "delayed" antibody. ¶ Recent studies with the α,DNP-oligo-L-lysine system were undertaken to explore further immunochemical aspects of antigen recognition. The chemical properties of antigen necessary to induce the immune response and cause an anamnestic response are precisely the same as those required to elicit or desensitize to the delayed response. For example, addition of a single lysyl residue to α,DNP-hexa-L-lysine converts this compound, which is neither immunogenic nor capable of provoking or desensitizing to the delayed response, to one that is both. Further, the substitution of a single D-lysine in α,DNP-nona-L-lysine converts this immunogenic compound to one that is neither immunogenic nor capable of eliciting a delayed response. In contrast, in this system, immediate hypersensitivity reactions are mediated by preformed circulating antibody with dinitrophenyl specificity. Thus, nonimmunogenic α,DNP-oligo-lysines, dinitrophenylated proteins, or polypeptides, although incapable of triggering a delayed response, can readily react with circulating antibody to elicit immediate reactions. These findings provide strong evidence for the concept that the initial event in the delayed reaction is an active process triggered by antigen rather than the result of passive interaction of preformed cell-fixed or circulating antibody with antigen.

Effect of Oral Contraceptives on Vitamin K-dependent Clotting Factor Activity. JOHN J. SCHROGIE, HARVEY M. SOLOMON, AND PHILIP D. ZIEVE, Baltimore, Md. (introduced by Louis Lasagna*).

Estrogens and napthoquinones (vitamin K) can produce similar physiological effects. Rats treated with estrogens have a diminished hypoprothrombinemic response to anticoagulants; conversely, treatment with vitamin K produces an estrogenic effect on the uterus of the rat. After treatment with estrogen-progestin combinations, the anticoagulant response to Dicumarol in female volunteers was diminished even though the metabolism of the anticoagulant was unchanged. ¶ A marked decrease in prothrombin time (measured by Thrombotest)

was observed in plasma of women treated for 2 months or longer with a variety of oral contraceptives. The decrease was demonstrated only after storage of the patients' plasmas for 16 hours or more at 6° C. The prothrombin times of fresh plasmas from treated subjects and controls were similar. After storage, the vitamin K-dependent clotting activity of pooled treated plasma was five times that of stored pooled control plasma. This increased activity was present in the majority of plasmas from women treated with estrogen-progestin combinations for 2 months or more. The effect disappeared within 1 month after therapy had been discontinued. Assays of specific factors demonstrated an increase in the activity of Factor X in stored plasmas from treated subjects. ¶ Recent studies have suggested that vitamin K acts late in the synthesis of Factors VII and X, perhaps by activating a precursor of these proteins. Oral contraceptives may increase the synthesis of a precursor of Factor X, which becomes activated on storage.

Hepatic Cholesterol Ester Storage Disease. WILLIAM K. SCHUBERT, LEON SCHIFF,† A. JAMES MCADAMS, EARL L. SPIEGEL, AND JAMES F. O'DONNELL, Cincinnati, Ohio.

This rare disease has been studied in a boy age 15 years, who was found to have an enlarged liver at age 2. At age 13½, in addition to hepatomegaly his spleen was found to be enlarged and liver function tests were normal. A needle specimen of the liver floated in formalin and was intensely orange colored. Frozen sections showed an abundance of oil red O positive material filling all parenchymal cells and, on polarization, a myriad of needle-shaped birefringent crystals. A high intensity brief (10-second) autofluorescence suggested vitamin A concentration. H and E stained sections revealed septal cirrhosis and diffusely vacuolated histiocytes with a bluish-brown pigmented cytoplasm attributable to chromolipid material in the portal areas. Bone marrow was normal. ¶ At laparotomy the liver was intensely orange colored, smooth, and soft. The gall bladder appeared normal. The spleen had a normal color, but was about five times the usual size. A total of 4 g of liver tissue was removed. ¶ Chemical analysis of the liver tissue revealed a lipid content of approximately 22%, the major portion of which showed a migration on a thin layer chromatographic plate similar to that of cholesterol ester. Upon hydrolysis the migration was similar to that of free cholesterol. Serum lipoprotein electrophoresis revealed a slight decrease in alpha lipoprotein and a slight increase in beta lipoprotein. The total serum cholesterol and cholesterol ester were slightly increased. The serum bile acids were elevated, with a disproportionate increase in chenodeoxycholic over cholic acid. ¶ Studies are in progress to determine any enzymatic defect that may be responsible for the excess cholesterol ester deposition in the liver. Currently under investigation is the occurrence of the disease in other members of the family; one has hepatosplenomegaly and others have abnormal serum bile acid patterns.

Peripheral Metabolic Antagonism of Estrogen and Human Growth Hormone. ERNEST SCHWARTZ, ELSA ECHEMENDIA, AND MARTIN SCHIFFER, New York, N. Y. (introduced by Stanley Ulick*).

Reports in the older literature have described beneficial effects of estrogen upon the hypercalciuria and also upon the clinical manifestations of acromegaly. In our metabolic balance studies, human growth hormone administration to three nonacromegalic patients produced nitrogen retention, elevation of urinary calcium and hydroxyproline, and elevation of the serum phosphorus. Estrogen administration during continuance of growth hormone therapy resulted in partial or complete reversal of these growth hormone effects. Estrogen administration to four active acromegalic patients sharply reduced elevated urinary calcium and altered other chemical parameters of disease activity. Radioimmunoassays completed from the first two of these patients showed no change of plasma growth hormone levels in one patient, and a questionable slight decrease of growth hormone levels in the other. The data suggest that the favorable effects of estrogen therapy in acromegaly are largely due to antagonism of growth hormone action at a peripheral level. Inhibition by estrogen of growth hormone secretion cannot yet be ruled out, but does not appear to be the most significant mechanism of estrogenic action.

Accelerated Plasma Clearance and Reduced Eosinopenic Activity of Cortisol in "Steroid Resistant" Asthma. HOWARD J. SCHWARTZ, FRANCIS C. LOWELL,† AND JAMES C. MELBY,* Boston, Mass.

Four asthmatic patients (group A) were judged on clinical grounds to be relatively resistant to corticosteroid therapy. These, and 19 unselected asthmatics (group B), were given a single intravenous injection of 40 mg cortisol, no steroid having been taken previously on the test day. Total eosinophil counts (TEC) were done at 2, 4, and 6 hours and revealed, in group A, mean/median per cent TEC changes of −10/−7, −36/−40, and +5/+6, whereas group B showed changes of −35/−36, −77/−78, and −73/−76 at the above intervals. The changes were independent of the initial TEC; the difference was highly significant ($p \leqq 0.01$). ¶ The above studies were repeated in conjunction with the giving of a tracer dose of tritium-labeled cortisol. Plasma levels were measured at 30, 60, 90, and 120 minutes; 6-hour urine specimens were collected. There were again four patients in group A and five in group B. ¶ The TEC changes were as before. Cortisol half-life values ($t_{\frac{1}{2}}$) in group A were 75, 87, 92, and 93 minutes. In group B they were 114, 130, 135, 135, and 206. With the exception that the last high value, obtained by the Porter-Silber method, has not yet been checked by the isotope method, chemical and isotope findings were in close agreement. Urinary 17-hydroxycorticoid excretions were similar in both groups. One additional patient with untreated severe hyperthyroidism, an illness associated with rapid plasma cortisol removal, showed a prompt, intense eosinopenia despite a $t_{\frac{1}{2}}$ of 95 minutes and increased urinary 17-hydroxycorticoid

excretion. It is concluded that *1*) most asthmatics possess normal eosinopenic responsiveness to cortisol; *2*) asthma requiring unusually large steroid doses for control may be associated with a corresponding diminution in eosinopenic response to cortisol associated with accelerated plasma cortisol clearance. This is one of the few reproducible observations in man relating clearance rate to cortisol's biologic effect.

The Dynamic State of Leukocyte Glycogen. ROBERT B. SCOTT AND LAVERNE W. COOPER, Richmond, Va. (introduced by G. Watson James III†).

Leukocytes incubated *in vitro* in the absence of glucose degrade their glycogen stores, and addition of adequate glucose allows glycogen stores to be replenished. This phenomenon was used as a system for study of glycogen degradation and the utilization of glucose for synthesis of macromolecular glycogen. ¶ Leukocyte suspensions containing 80 to 90% neutrophiles were prepared from normal blood by sedimentation of erythrocytes with dextran and removal of platelets of repeated low speed centrifugation. During a 2-hour incubation in glucose-free Hanks solution the cells lost 3.07 ± 0.40 (SE) mg glycogen per 10^9 neutrophiles (38%). At a glucose load of 5 mg or more per 10^9 neutrophiles per hour (cell density 25 to 50×10^6 per ml), the balance of glycogen synthesis and degradation favors glycogen synthesis. Glycogen synthesis approaches a maximum when the glucose load exceeds 50 mg per 10^9 neutrophiles per hour. With a pulse label of glucose-^{14}C followed by a "chase" of unlabeled glucose, the specific activity of isolated glycogen fell, although the total glycogen content remained stable under the conditions employed. When leukocytes previously incubated in glucose-^{14}C were placed in glucose-free medium to stimulate glycogenolysis, the radioactivity of the isolated glycogen decreased faster than the total content of glycogen. ¶ Leukocyte glycogen from crude cell lysates sedimented in sucrose density gradients as a single peak ahead of glycogen purified by alkali extraction. Both sedimented ahead of a rat liver ribosome marker. ¶ These data indicate that leukocyte glycogen is in constant turnover, that the most recently added glucose molecules are likewise the first to be removed from glycogen, and that a major macromolecular form of leukocyte glycogen is a group of similar size particles, in contrast to the spectrum of molecular sizes reported for liver glycogen.

Mutations in L Forms of *Staphylococcus aureus*. STEPHEN J. SELIGMAN, Los Angeles, Calif. (introduced by A. F. Rasmussen†).

Varying dilutions of an overnight culture of *S. aureus* 209 P were inoculated onto trypticase soy agar plates containing 5% NaCl and 10 μg per ml benzylpenicillin. After 2 to 4 days' incubation, typical L colonies with a "fried egg" appearance were observed. More prolonged incubation and subculture revealed the presence of genetic variants, presumably originating by mutation. The mutations affected changes in colonial morphology, differences in ability to revert to the normal bacterial (vegetative) form, and differences in ability to grow on media supplemented with 10% horse serum. ¶ With more prolonged incubation, diffuse sectors appeared in the "white of the egg" periphery of some colonies. The diffuse appearance resulted from growth of the peripheral surface into the medium. Subculture from the diffuse sectors resulted in colonies that had entirely diffuse peripheries. Those that reverted from diffuse L colonies to the vegetative form were reinduced to the L form and produced diffuse L colonies. Thus, a mutation had occurred in the L form that persisted after reversion to the vegetative form. ¶ Mutants with diminished ability to revert to the vegetative form could be divided into two types. The first type could readily mutate back to the vegetative form in the absence of penicillin and the second could not. If passage of the L form were done by agar block push instead of by isolated colony, then reversion from back mutation would be confused with simple reversion of the genetically unaltered parent L form. The second type of mutant was more stable and did not revert when growth was attempted on solid or liquid media. In addition, the second type of mutant produced approximately equal numbers of colonies on plates with or without horse serum. L forms of the parent strain were suppressed by horse serum. This finding is in contrast with the stimulatory effect usually reported with serum. Differentiation from the parent strain of mutants with diminished reversion ability is important, since such variants have presumably developed a biochemical defect resulting in loss of ability to synthesize peptidoglycan, the component of the cell wall responsible for rigidity. Recognition that horse serum may suppress the unstable parent strain offers a systematic method for isolating stable mutants.

Mechanism of Acetophenetidine-induced Hemolysis in Glucose 6-Phosphate Dehydrogenase Deficiency. N. T. SHAHIDI AND I. TREICHEL, Madison, Wis. (introduced by R. F. Schilling†).

To investigate hemolysis in G-6-PD deficiency most experimenters have evaluated the effects of drugs *in vitro*. Whereas some drugs such as primaquine may exhibit oxidative properties both *in vitro* and *in vivo*, others such as acetophenetidine cause significant red cell damage only *in vivo*. Although it is believed that causative agents are products of drug biotransformation, their nature and mechanism of action remain unknown. Our studies on biotransformation of acetophenetidine have uncovered a highly oxidant metabolite, 2-hydroxyphenetidine (*p*-ethoxy-o-hydroxy aniline), which was excreted as sulfate in all 15 normal individuals tested. Some individuals excrete small amounts in free and glucuronide form. A method for measuring total 2-hydroxyphenetidine in the urine was developed. Normal individuals excreted as much as 15% of a 2-g dose of acetophenetidine as 2-hydroxyphenetidine sulfate. ¶ Crystallized 2-hydroxyphenetidine was incubated with hemoglobin (as intact red cells) in equimolar concentrations. After 1 hour, normal cells revealed 32% methemoglobin with no significant lysis, whereas G-6-PD–deficient erythrocytes

showed 56% methemoglobin and 30% lysis. Sulfhemoglobin was less than 1%. After 2 hours' incubation red cell lysis and methemoglobin increased but sulfhemoglobin did not. Acetylphenylhydrazine and nitrosophenotol incubated in the same concentration produced, in G-6-PD–deficient erythrocytes, 10 and 16% methemoglobin and 1 and 2% sulfhemoglobin, respectively, without hemolysis. o-Aminophenol produced higher methemoglobin concentrations in both normal and G-6-PD–deficient red cells but no sulfhemoglobin or hemolysis. ¶ The extensive hemolysis and lack of significant sulfhemoglobin formation suggest that 2-hydroxyphenetidine (or its quinone imine) may significantly damage the erythrocyte membrane.

Production of Hormone by Human Parathyroid Tissue *In Vitro*. L. M. SHERWOOD, I. HERRMANN, G. M. AGOSTO, AND C. A. BASSETT, New York, N. Y. (introduced by P. A. Marks*).

Since physiological studies by radioimmunoassay have indicated that the secretion of parathyroid hormone (PTH) is controlled by the serum calcium, human parathyroid adenomas were grown in tissue culture in an attempt to study their secretion pattern *in vitro*. Explants of six adenomas obtained at surgery were grown on stainless steel grids using Eagle's medium and 10% human placental serum. Hormone released into the medium was detected by a modified radioimmunoassay using dextran-coated charcoal to separate bound and free PTH-^{131}I. The immunologically reactive substance in the medium behaved exactly like PTH in the assay, and there was good agreement in the estimated concentration over a wide range of dilutions. Specialized function of the tissue has been maintained in continuous culture for periods as long as 3 months. Although decreases in hormone concentration were occasionally noted during the first 2 weeks of culture, PTH production generally rose to a plateau (between 10 and 400 mμg per ml for different cultures) as high as that observed during the first 2 days of incubation. Tissue explants grown in the presence of L-leucine-^{14}C produced labeled protein that could be bound to antibodies specific for PTH and displaced by unlabeled bovine hormone. Changes in the concentration of calcium in the medium produced no significant variations in the apparent production of hormone by the tissue. No evidence of functional suppression was observed, even at levels of calcium (13 mg per 100 ml) sufficient to cause precipitation of calcium salts in the explant or suppression of PTH secretion *in vivo*. The failure of changes in environmental calcium to affect significantly the production of hormone by adenoma explants suggests that this tissue functions autonomously in producing clinical hypercalcemia.

Dynamics of Metabolism of Plasma Unesterified Fatty Acids by Erythrocytes. STEPHEN B. SHOHET, DAVID G. NATHAN,* AND MANFRED L. KARNOVSKY, Boston, Mass.

Incorporation of plasma unesterified fatty acids into membrane lipids of erythrocytes has been described by Oliveira and Vaughan and by Van Deenen and associates. We have investigated the different stages in the transfer of albumin-bound fatty acids to red cells and their eventual incorporation into membrane phosphatides. The experimental stratagems included separation of different lipid pools by extraction of cells with fatty acid–poor albumin and solvents after pulse labeling. Lipid classes were separated by thin-layer chromatography. The stages of incorporation encountered were the following: *1*) Equilibration of albumin-bound fatty acids with a "superficial" red cell pool (F1). This pool is easily removed by washing with albumin depleted of fatty acids. This rapid equilibration is independent of red cell metabolism. *2*) Passage of the fatty acids of the F1 pool into a second "deeper" red cell membrane pool (F2), which is not extractable from the cells with lipid-poor albumin. *3*) Incorporation of F2 fatty acids into phosphatides, mainly lecithin (PL). The data are consistent with precursor-product relationships between F1 and F2, and F2 and PL pools. Processes 2 and 3 above require metabolic energy. About 40 nmoles of palmitic acid enters each of F1 and F2 per hour and milliliter packed cells. Conversion of F2 fatty acids to phosphatides is 25 nmoles per hour and milliliter cells under the conditions studied. The renewal rate of phospholipid fatty acids is 2% per hour, a value comparable to that recently found by Tarlov by entirely different methods. If the mechanism of this final incorporation is acylation of lysophosphatides as described by Lands, approximately 1.5% of the energy available from glycolysis is used in this lecithin synthesis. Preliminary studies indicate that young and certain abnormal red cells may markedly increase in these processes.

Abnormal Vascular Tone as the Basis for the Hyperdynamic State in Septic Shock and Portal Hypertension. JOHN H. SIEGEL, MARK GREENSPAN, JOSEPH D. COHN, AND LOUIS R. M. DEL GUERCIO, New York, N. Y. (introduced by Stanley M. Levenson†).

The classic cardiovascular problem complicating surgical management is the development of a low cardiac output state. However, a circulatory imbalance common both to patients in septic shock and cirrhotics with severe portal hypertension is the development of a hyperdynamic syndrome. Study of 35 patients in septic shock, 51 patients with cirrhosis and portal hypertension, 14 patients in nonseptic shock, and 13 normal subjects has revealed that this hyperdynamic state is characterized by a decrease in net vascular tone, diminished oxygen transport in the face of increased cardiac output, increased pulmonary veno-arterial admixture, and the development of moderate to severe myocardial depression. Evidence is presented which suggests that the increased but ineffective circulation is related to the development of arteriovenous shunts in both the systemic and pulmonary circulations and that this shunting imposes limits on peripheral oxygen consumption. Comparison of ventricular function relationships with the patient's clinical course demonstrated that decreased cardiac function is a factor in nearly all septic

patients and in many cirrhotics manifesting the hyperdynamic syndrome, and that the development of a high output cardiac failure is common in both conditions. These data suggest that the high cardiac output reflects a compensatory increased cardiac sympathetic response to an abnormal vascular tone and that both disease entities may be manifestations of a generalized pathologic process affecting the microcirculation.

Metabolism of Maltosyloligosaccharides in the Rat. JACK SIMON, MATTHEW FRIEDMAN, MARVIN H. SLEISENGER,* AND ELLIOT WESER, New York, N. Y.

Maltosyloligosaccharides have been isolated from rat liver, but their metabolic role is unknown. It has been suggested that they are derived from glycogen via the action of amylase and subsequently hydrolyzed to glucose by tissue maltases. Assay of several rat organs other than small bowel mucosa revealed that kidney had significant maltase activity, with cortex four to five times more active than medulla. ¶ Alcohol extracts of pooled kidney homogenates were subjected to descending paper chromatography and stained with silver nitrate reagent. Sugars were separated with R_g values corresponding to standard maltose, maltotriose, maltotetrose, and higher maltosyloligosaccharides. Kidney slices were incubated for 1 hour at 37° C in 2.5 ml Krebs-bicarbonate buffer, pH 7.4, containing 0.5 μc of either maltotriose-U-^{14}C, maltose-U-^{14}C, or glucose-U-^{14}C. The $^{14}CO_2$ produced during the incubation with maltotriose-U-^{14}C as well as with maltose-U-^{14}C was 50% of $^{14}CO_2$ after glucose-U-^{14}C, indicating active metabolism. ¶ To determine whether maltosyloligosaccharides may be metabolized *in vivo*, we injected intravenously 0.5 μc of maltotriose-U-^{14}C into rats and measured the expired $^{14}CO_2$. After maltotriose injection, 64% of the dose was recovered as $^{14}CO_2$ and 12% was excreted in the urine. Similar findings were noted after the injection of maltose-U-^{14}C and glucose-U-^{14}C. ¶ The results of these studies suggest that maltosyloligosaccharides are metabolized in the rat. Since rat serum has maltase activity, circulating maltosyloligosaccharides may undergo intravascular hydrolysis to glucose and subsequent oxidation. Tissue maltosyloligosaccharides may be oxidized to CO_2 after hydrolysis by tissue maltases or entry into the circulation.

Transplantation of Mouse Tumors into Immunologically Tolerant Rabbits. YEHOUDITH SINAI, PHIL GOLD, AND SAMUEL O. FREEDMAN,* Montreal, Quebec.

Neonatal rabbits were made immunologically tolerant to mouse Ehrlich ascites cell (EAC) tumors by the injection of 5×10^7 EAC cells intraperitoneally within 12 hours of birth. Daily intraperitoneal and subcutaneous injections of EAC cells were given for the next 20 days. At first the recipient rabbits developed typical ascitic tumors, which contained mouse antigens and grew on back transplantation into adult mice. These ascitic tumors disappeared spontaneously within 6 to 8 days after birth. However, at approximately 21 days, 80% of the rabbits began to develop solid fibrosarcomas of the abdominal wall that had none of the antigenic characteristics of mouse tumors, did not grow on back transplantation into adult mice, but grew slowly on two successive transplantations into adult rabbits until they were finally rejected. The immunologically tolerant rabbits bearing solid fibrosarcomas of the abdominal wall died within 8 weeks after the initial tumor transplantation. Similar results were obtained with mouse sarcoma-37 (S-37) transplantations. ¶ Adult rabbits which received 550 roentgens of total body irradiation were injected with 1×10^9 S-37 cells on the first and seventh days after irradiation. Small solid tumors appeared in the abdominal wall in 50% of the animals but were fully rejected within 6 weeks. ¶ Treatment of living EAC cells and S-37 cells with 2-mercaptoethanol before injection markedly enhanced the development of transplanted tumors in both neonatal and irradiated adult animals. The changes in morphology, the lack of mouse antigens, and the failure to grow on back transplantation into mice would suggest that the solid tumors originated in rabbit cells.

Myocardial Contractile State in Hypertrophy and Failure, Studied in the Intact Heart and the Isolated Muscle. JAMES F. SPANN, JR., JAMES W. COVELL, DWAIN L. ECKBERG, EDMUND H. SONNENBLICK,* JOHN ROSS, JR.,* AND EUGENE BRAUNWALD,* Bethesda, Md.

Little is known of the intrinsic mechanical properties of the chronically hypertrophied and failing ventricle. Chronic right ventricular (RV) hypertrophy with heart failure was produced in seven cats by pulmonary arterial constriction. The contractile properties of the intact RV and isolated RV papillary muscles of these cats were then compared quantitatively with those of normal cats. In intact failing hearts, average RV weight increased 132% to 1.2 ± 0.1 g per kg, peak isovolumic pressure 89% to 111 ± 16 mm Hg, and RV end-diastolic pressure from 3 ± 0.5 to 13 ± 3 cm H_2O. RV end-diastolic volume (EDV) increased 95% to 1.9 ± 0.1 ml per kg, and maximal isometric tension (P_0) 32% to 2.3 ± 0.3 g per mm^2. However, the maximal extrapolated contractile element velocity (V_{max}) was markedly reduced from 2.31 ± 0.12 (normal) to 1.39 ± 0.09 muscle lengths per second. In the papillary muscles from the same ventricles, P_0 at the apex of the length-tension curve was greatly reduced, from 8.3 ± 1.4 (normal) to 3.1 ± 0.6 g per mm^2, and V_{max} was decreased from 0.95 ± 0.08 to 0.32 ± 0.08 muscle lengths per second. Thus, in failure, both force and velocity were depressed in the isolated muscle, whereas increased fiber length maintained force developed per unit of muscle in the intact ventricle. This increased preload and the greater muscle mass provided augmented total ventricular force and pressure development. However, V_{max}, which is unaltered by muscle length or mass, provided an accurate reflection of the depressed ventricular contractile state. These findings provide a quantitative analysis of depressed intrinsic contractile state in the intact, chronically hypertrophied, and failing myocardium, indicating that the rate of interaction of contractile sites

is reduced. They further demonstrate the manner in which augmentation of EDV and total muscle mass compensate for this intrinsic defect.

The Importance of ACTH in the Activation of Aldosterone Secretion. RICHARD F. SPARK, ROGER B. HICKLER, AND JAMES C. MELBY,* Boston, Mass.

The effect of dexamethasone suppression on aldosterone secretory rates before and after angiotensin infusions as well as the effect of dexamethasone suppression on diurnal tetrahydroaldosterone (THA) excretion was studied to clarify the role of the pituitary in the regulation of aldosterone secretion. ¶Dexamethasone suppression (2 mg per day × 2 days) resulted in an average 38% decrease in aldosterone secretory rate (ASR) in 22 individuals. Angiotensin infusion at subpressor doses for 6 hours produced a 27% increase in ASR before dexamethasone suppression. A comparable per cent increase in ASR was obtained with angiotensin stimulation and dexamethasone suppression if the ASR during dexamethasone suppression was used as the reference point. However, in 12 of 13 cases the ASR during dexamethasone suppression and angiotensin stimulation was still 29% less than the control ASR. ¶ Sodium restriction was associated with a progressive rise in aldosterone secretion and plasma renin activity, and, during dexamethasone suppression while plasma renin activity continued to rise, aldosterone secretion decreased below control levels. In normal volunteers on a low salt diet, the normal diurnal THA excretion was blunted during dexamethasone suppression. This was largely due to a decrease in the upright (daytime) THA excretion. ¶ Two patients who had received renal transplants and thus had denervated kidneys exhibited normal diurnal variations in THA excretion and plasma renin activity. Dexamethasone suppression abolished the diurnal excretion of THA, although diurnal variation in plasma renin persisted. Again, the loss of diurnal variation was due to a marked decrease in the upright (daytime) THA excretion with only minimal change noted in the recumbent (nighttime) THA excretion. ¶ The results of these studies suggest that ACTH plays a significant role in the regulation of aldosterone secretion.

The Direct Action of Vitamin D and Lactose on Bone in Osteomalacia. MARTHA STAUFFER AND CLAYTON RICH,* Seattle, Wash.

Weanling rats were maintained in the dark on diets containing 0 to 1.2% calcium, 0.4% phosphorus, and either no vitamin D (D−) or a replacement dose (D+) for 3 weeks. Intestinal absorption was measured by whole body counting after oral ^{47}Ca mixed with the food and intraperitoneal ^{47}Ca. From this and the known food intake, the milligrams calcium absorbed (mg abs Ca) was calculated. The average width of osteoid seams in the midfemoral diaphysis was used as a quantitative measure of the severity of osteomalacia. D− animals all had osteomalacia (osteoid seams 3.0 to 5.8 μ), irrespective of the mg abs Ca, which ranged from 0 to 90 per day. D+ animals did not (osteoid seams $< 2.0\,\mu$), even when mg abs Ca = 0. Blood Ca was linearly related to mg abs Ca in both groups but lower for any value of mg abs Ca in the D− than D+ group. Thus, in addition to its action to increase calcium absorption, vitamin D prevents osteomalacia and maintains the blood Ca by a direct action on bone. No over-all correlation was found between the severity of osteomalacia and the fractional absorption of calcium, the Ca × P ratio of the diet, or the serum phosphate concentration. ¶ Findings almost identical to those described above for the D+ group (including the prevention of osteomalacia when mg abs Ca = 0) occurred when D− animals were given 20% lactose instead of sucrose in the diet. Thus, lactose or some factor in it has a direct vitamin D-like action on bone as well as on gut. Efforts to extract a substance with the chemical properties of vitamin D have been unsuccessful so far.

Thromboembolic Bronchoconstriction: The Role of Chemical Substances and Platelets. MYRON STEIN, SUSUMU SUETSUGU, AND ISRAEL ALKALAY, Providence, R. I. (introduced by Milton W. Hamolsky*).

The changes in lung mechanics observed in patients with pulmonary thromboembolism are believed to be related to release of amines from platelets. Experimentally, massive autologous thromboemboli in animals produce apnea followed by tachypnea and bronchoconstriction of small airways as demonstrated by decreases in lung compliance (C_L) and increases in total lung flow resistance (R_L). Microthrombi of platelets induced by intravenous endotoxin injections result in similar changes in respiratory frequency, C_L, and R_L. The alterations of R_L and C_L produced by autologous thromboemboli as well as platelet emboli were prevented by heparinization, antiserotonin agents, or rendering the animal thrombocytopenic, indicating that the observed changes in lung mechanics were due to thrombin-induced release of serotonin from circulating blood platelets. The following experiments were performed to investigate the role of certain nucleosides and nucleotides possibly released during cellular injury. Intravenous injection of adenosine (8 to 15 mg per kg) failed to produce changes in C_L, R_L, and blood platelet or leukocyte counts, but increases in respiratory frequency did occur. After intravenous adenosine-5′-diphosphate (8 to 15 mg per kg), rapid decreases in C_L and platelet and leukocyte counts and increases in R_L and breathing frequency occurred within 30 seconds and lasted 10 minutes. If, before ADP injection, the animals were made thrombocytopenic or given adenosine, a potent *in vitro* inhibitor of ADP platelet aggregation, the fall in C_L and rise in R_L were completely abolished. In the presence of adenosine, post-ADP decreases in platelet and leukocyte counts were transient and returned to control values within 40 seconds. Light and electron microscopic examinations of autologous thromboemboli removed from pulmonary arteries have shown a surface layer of degranulated platelets. After

endotoxin and ADP injections, aggregates of degranulated platelets and leukocytes were observed in pulmonary arterioles. Thus, our experiments indicate that ADP, a physiological substance, can induce *in vivo* platelet aggregation and lead to release of amines capable of producing significant constrictions of small airways in lungs.

The Enzymatic Defect in Refsum's Disease. DANIEL STEINBERG,* J. H. HERNDON, JR., B. W. UHLENDORF, JOEL AVIGAN, C. E. MIZE, AND H. M. FALES, Bethesda, Md.

Our previous studies showed that the phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) that accumulates in patients with Refsum's disease is of exogenous origin and that the inherited metabolic defect lies on the catabolic pathway. We have recently shown that a major pathway for phytanic acid degradation in the rat and in the mouse involves an unusual initial α oxidation, which yields the (n-1) fatty acid, pristanic acid (2,6,10,14-tetramethylpentadecanoic acid). Pristanic acid is degraded by successive β oxidations, and three lower intermediates have been identified. These new findings on the degradative pathway provided a basis for seeking to identify the site of the enzyme defect in Refsum's disease. ¶ Two affected siblings given phytanic acid-U-^{14}C intravenously converted it to $^{14}CO_2$ at a rate less than 5% that seen in two normal subjects. Cultures of skin fibroblasts from the patients retained the enzyme defect—the rate of oxidation of phytanic acid-U-^{14}C to $^{14}CO_2$ in their cultures was less than 10% that in control cultures. In contrast, oxidation of pristanic acid-U-^{14}C was comparable in normal and in Refsum cultures, as was oxidation of palmitic acid-1-^{14}C. After incubation with phytanate-^{14}C, pristanate-^{14}C was found in control cultures but not in Refsum cultures. A specific impairment in ability to release phytanic acid from esters might explain these results but seems unlikely, since phytanic acid is present in all major classes of lipid esters. It is concluded that the genetic defect most probably lies in the very first step in phytanic acid oxidation, i.e., in its conversion to pristanic acid. This conclusion is supported by the absence of accumulation in patients with Refsum's disease of pristanic acid or any of the lower degradation products.

Correlation of Pulmonary Blood Flow Distribution with Cardiovascular Dynamics. SHELDON H. STEINER AND JAMES M. QUINN III, Chicago, Ill. (introduced by David P. Earle†).

Gravitational effects combined with intrapulmonary and cardiac pressures are such that in the upright position little pulmonary arterial blood normally flows to the lung apexes. ^{131}I-labeled macroalbumin (MAA) was injected into seated patients with normal hemodynamics, with pulmonary hypertension, and with rheumatic heart disease. The ratios of counts obtained from the right upper and right midlung fields were compared to supine pressures and flows obtained during cardiac catheterization. The correlation of upper/middle MAA-^{131}I ratios with pulmonary arterial mean pressure was +0.84 ($p < 0.001$). The correlation with left atrial pressure was +0.78 ($p < 0.001$). There was an inverse correlation of −0.49 ($p < 0.05$) with cardiac output. When the patients with normal hemodynamics and with predominant mitral stenosis were grouped, the correlation was +0.85 ($p < 0.001$) with left atrial pressure and +0.95 ($p < 0.001$) with pulmonary arterial mean pressure. When the patients with normal hemodynamics and primary pulmonary hypertension were grouped, the correlation coefficient with pulmonary arterial mean pressure was +0.85 ($p < 0.001$). The upper/middle ratio was increased in pulmonary hypertension, but when an approximately uniform distribution pattern occurred, the gradient was apparently independent of further elevation in pulmonary arterial pressure. An upper/middle ratio of greater than 0.6 indicated pulmonary hypertension or, concomitantly, pulmonary hypertension and left atrial pressure elevation with mitral stenosis. The left atrial and pulmonary pressures may be predicted with considerable accuracy from the macroalbumin-^{131}I distribution when mitral stenosis is present.

Independence of Hydrogen Ion and Sodium Transport in Turtle Bladder. PHILIP R. STEINMETZ AND RODNEY S. OMACHI, Boston, Mass. (introduced by Irving H. Goldberg*).

Acidification of the solution bathing the mucosal surface of the isolated turtle bladder is associated with alkalinization of the serosal solution and depends on active transport of either H^+ or alkali. We examined the active step in acidification by analyzing the stoichiometry of acid and alkali secretion on the two sides of the epithelium and by exploring its relation to Na^+ transport. In CO_2 and HCO_3^-–free Ringer's buffered with 1 μmole Na_2HPO_4 on each side of the short-circuited bladder, acidification of the mucosal solution (M) was greater (> 2 pH U) than alkalinization (< 1 pH U) of the serosal solution (S). As measured by a pH stat technique, acid secretion into M exceeded alkali secretion into S by 15%. Acute changes in cellular P_{CO_2} induced by deoxygenation were reflected in pH changes in S but not in M. ¶ These results are consistent with a location of the H^+ pump at the mucosal barrier of the epithelium; alkali generated in back of the pump is buffered within the cell and moves passively across the serosal surface of the epithelium into S. ¶ Removal of ambient Na^+ had little effect on H^+ secretion and caused reversal of the potential difference. The reversed short circuit current had the same magnitude as H^+ secretion. Exposure to 1.10^{-5} M dinitrophenol had no effect on H^+ secretion, whereas Na^+ transport was reduced. Exposure to 1.10^{-5} M ouabain virtually abolished Na^+ transport, although H^+ secretion continued at half the initial rate. ¶ These results indicate that H^+ transport in the turtle bladder is not coupled to Na^+ transport by a common carrier or metabolic reaction and that the mucosal membrane is the probable site of the H^+ pump.

The Prevention of Clinical Wilson's Disease. IRMIN STERNLIEB* AND I. HERBERT SCHEINBERG,† New York, N. Y.

The diagnosis of Wilson's disease (hepatolenticular degeneration) was established biochemically in 49 apparently healthy individuals, all without a symptom or sign of this disorder, and all siblings or children of patients with this illness. The illness was based on a combination of hypoceruloplasminemia of less than 20 mg per 100 ml and an increased hepatic copper concentration of more than 250 μg per g dry liver in 36 of the subjects. In 8, the diagnosis was made after the demonstration of two or more of the following symptoms: hypercupriuria, abnormal liver function or morphology, and/or a characteristic result of a test of incorporation of ^{64}copper into ceruloplasmin. In the remaining 5 subjects, the diagnosis, suggested by hypoceruloplasminemia, was not initially confirmed; none of these 5 received treatment, and frank Wilson's disease subsequently developed in all of them. These 49 individuals have been examined for as long as 10 years, for a total of more than 150 patient years. ¶ Thirty-nine of these patients have regularly followed a "decopperizing" regimen based on the administration of D-penicillamine. In none of these 39 has any significant manifestation of the disease appeared. ¶ Of the 10 untreated patients, 5 have died, 2 are severely ill with Wilson's disease, and the diagnosis was made only recently in the remaining 3. ¶ Together with additional chemical and histological findings these results sharply differentiate clinical laboratory analyses that are useful in the diagnosis of Wilson's disease from those that are of little value or misleading. More important, they also indicate that Wilson's disease is preventable if it is diagnosed while the patient is still asymptomatic.

Absorption of Polyglutamic Folic Acid. RICHARD R. STREIFF AND IRWIN H. ROSENBERG, Boston, Mass. (introduced by W. B. Castle†).

Much of folic acid in dietary sources occurs as polyglutamates of folic acid (PFA). It is presumed that the action of a tissue enzyme (folic acid conjugase), which splits off glutamates to produce a monoglutamate folic acid (MFA), is a required step in the conversion of PFA to metabolically active forms. To investigate the participation of the intestine in this process we employed a PFA preparation, isolated from Difco yeast extract by DEAE chromatography. This PFA preparation was essentially free of MFA and yeast conjugase inhibitor, which may have complicated interpretation of previous absorption studies. In 13 fasting normal subjects oral administration of MFA (10 μg) and PFA (of equivalent *Lactobacillus casei* activity after deconjugation *in vitro*) resulted in 20 to 50% and 10 to 35% increases, respectively, in serum folate (*L. casei*) activity at 1 or 2 hours. By contrast, 8 of 11 patients with malabsorption but with normal serum folate levels showed no increase in serum folate activity after administration of PFA, whereas response to MFA was similar to that of normal subjects. Evidence that the intestinal epithelium is itself capable of converting PFA to MFA was obtained from studies of rat intestine *in vitro*. Upon incubation of PFA with everted sacs in buffer, MFA (*L. casei* activity) appeared in the bath as well as in the serosal-lined interior of the sacs. Within a concentration range projected from the clinical studies, incubation of PFA with everted rings of intestine produced MFA in excess of the rate of cellular MFA uptake. It appears that the process of intestinal absorption of natural folate involves an enzymatic deconjugation, presumably at the luminal border of the intestinal cell, a reaction that may become rate limiting in malabsorption syndromes.

Mechanism of the Effect of Thiazide Diuretics on Calcium and Uric Acid. W. N. SUKI, A. R. HULL, F. C. RECTOR, JR.,* AND D. W. SELDIN,† Dallas, Texas.

During saline diuresis excretion of both sodium and calcium rises, suggesting a direct link between Na^+ and Ca^{++} reabsorption in the kidney. Chronic administration of thiazide diuretics (TD), however, increases Na^+ excretion but decreases Ca^{++} excretion and uric acid (UA) clearance. To investigate the possibility that the latter findings are the result of increased proximal tubular reabsorption secondary to shrinkage of extracellular fluid volume (ECF), we performed studies on volunteers given a TD while on a low (2-g) and a high (15-g) salt diet. Ca^{++}, Na^+, creatinine (Cr), and uric acid were measured in serum and urine. During TD and low salt diet there was a shrinkage of ECF, evidenced by loss of weight and rise in hematocrit (Hct) accompanied by a sustained decrease in Ca^{++} excretion and UA clearance and hyperuricemia. Despite continuation of the TD, addition of salt to the diet re-expanded ECF back to normal as evidenced by weight gain and fall in Hct; this was associated with a rise in Ca^{++} excretion and a return of UA clearance and serum uric acid towards control values. Low salt diet alone, without TD, also resulted in a decreased Ca^{++} excretion and UA clearance and hyperuricemia; refeeding of salt corrected these alterations. We concluded from these studies that the reduction in Ca^{++} excretion and the hyperuricemia due to TD administration are the results of shrinkage of ECF and a nonspecific stimulation of proximal tubular reabsorption.

Serum Vitamin B_{12}-binding Capacity in Pregnancy. L. W. SULLIVAN, R. J. GREENE, AND Y. K. LIU, Boston, Mass., and Jersey City, N. J. (introduced by C. P. Emerson†).

A number of serum transport proteins have been found to be increased in pregnancy, including thyroxin-binding globulin, corticosteroid-binding globulin, ceruloplasmin, and transferrin. The present study was undertaken to ascertain whether similar increases occurred in serum vitamin B_{12}-binding capacity in pregnancy. Serum vitamin B_{12} was determined by assay with *Euglena gracilis*. The capacity of serum to bind added radioactive vitamin B_{12} *in vitro* (serum B_{12}-binding capacity) was determined by the method of Gottlieb and co-workers. Subjects included

8 normal female volunteers and 29 nonanemic pregnant patients. Serum B_{12} levels averaged 532 pg per ml in nonpregnant subjects and 589 in pregnant patients. There was no significant difference noted in serum B_{12} levels in any trimester of pregnancy. The ability of serum to bind added vitamin B_{12} averaged 966 pg per ml in the normal females and 1,360 in all pregnant patients. Increased serum B_{12}-binding capacity was observed as early as the fourth week of pregnancy in the subject observed at this time. Serum B_{12}-binding capacity averaged 1,485 pg per ml in the 7 pregnant patients studied in weeks 35 to 38 of pregnancy. In 2 patients, the serum B_{12}-binding capacity was 1,419 and 1,746 pg per ml at term. These values declined to 754 and 1,052 by 37 and 44 days postpartum, respectively. Studies from several laboratories have shown that endogenous serum vitamin B_{12} is bound primarily to an alpha globulin, whereas vitamin B_{12} added to serum *in vitro* is bound primarily by a beta globulin. The rise of serum vitamin B_{12}-binding capacity early in pregnancy and its return to normal by the sixth week postpartum suggest an intimate association between the beta globulin vitamin B_{12} binder in serum and the physiologic changes accompanying pregnancy.

Australia Antigen, Down's Syndrome, and Hepatitis. ALTON I. SUTNICK, W. THOMAS LONDON, AND BARUCH S. BLUMBERG,* Philadelphia, Pa.

"Australia antigen" [Au (1)] is a serum antigen that has not been found in the normal U. S. population but is relatively common in patients with acute myelogenous and chronic lymphocytic leukemia and Down's syndrome. It is also present in apparently normal populations from several tropical and subtropical areas (e.g., Australian aborigines, Filipinos, Indians, and so forth). It has generally persisted for years in people in whom it has been repeatedly tested. Recently, it has been found that approximately 10% of patients with virus hepatitis have Australia antigen. It has not been found in more than 1,500 patients with other diseases. ¶ Twenty-eight of 100 Down's syndrome patients from a New Jersey institution had Au (1) as detected by the micro-Ouchterlony double diffusion technique. The median serum glutamic pyruvic transaminase (SGPT) level for those with Au (1) was 42, and it was 15 for those without the antigen. The median SGPT of 17 Au (0) mentally retarded controls without Down's syndrome from the same institution was also 15. Two mentally retarded children with Au (1) had evidence of hepatic damage on biopsy. Au (1) has been detected before the development of laboratory evidence of hepatitis and in one instance disappeared as the disease subsided. The relation of these findings to the pathogenesis of leukemia, Down's syndrome, and virus hepatitis will be discussed.

The First Step in ACTH Action: Binding to Tissue. O. DAVID TAUNTON, JESSE ROTH, AND IRA PASTAN, Bethesda, Md. (introduced by J. E. Rall†).

Adrenal and fat cells exposed briefly to ACTH and washed thoroughly show a persistent hormone effect that is largely abolished by subsequent exposure of the tissue to trypsin or anti-ACTH antibody. Rat adrenal slices, exposed to ACTH at 2° and washed, show increased steroid production at 37°. The persistent ACTH effect, which cannot be washed off, is *1*) proportional to ACTH concentration up to 1,000 mU per ml without further increase up to 50,000 mU per ml, and *2*) proportional to duration of ACTH exposure up to about 5 minutes, when the response reaches a plateau. When adrenal slices are incubated with ACTH for 5 minutes or less and washed, trypsin (3 mg per ml, 2°, 15 minutes) largely obliterates the persistent effect. With prolongation of ACTH exposure, the effect becomes progressively less reversible by trypsin; by 30 minutes it is completely irreversible. Since trypsin-treated adrenals are fully responsive to ACTH, reversal cannot be ascribed to tissue damage. ¶ Isolated fat cells, incubated with ACTH and washed, show increased FFA release that is *1*) proportional to ACTH concentration up to 20 mU per ml without further increase up to 5,000 mU per ml, and *2*) proportional to duration of ACTH exposure up to about 3 minutes, when the response reaches a plateau. When fat cells are exposed to ACTH for 5 minutes or less and washed, anti-ACTH antibody completely reverses the increased FFA release. Since the lipolytic effect of epinephrine on these cells is unaltered by ACTH antibody, reversal cannot be ascribed to cell damage. ¶ We conclude that the first step in ACTH action is rapid equilibration between ACTH in solution and a finite number of receptors on the external surface of hormone-sensitive cells. The K for the interaction of ACTH with sites on fat cells is tentatively estimated to be 10^8 and for adrenal cell sites about 20-fold less.

A Qualitative Description of Factors Involved in Lysis of Diluted Whole Blood Clots. FLETCHER B. TAYLOR, JR., AND HANS J. MÜLLER-EBERHARD, Philadelphia, Pa. (introduced by Earl S. Barker†).

A preliminary qualitative study was undertaken to determine the requirements for lysis of clots formed by the addition of 1 U of thrombin to whole blood diluted 1:10 in phosphate buffer (μ 0.082, pH 7.4, 4° C). Removal or destruction of platelets before addition of thrombin by differential centrifugation or sonication inhibited normal clot retraction and lysis. Addition of antisera specifically directed against purified γM-globulin and β_{1E}- and β_{1C}-globulins (complement components C'4, C'3) inhibited normal clot retraction and lysis. Antisera to plasminogen inhibited normal clot lysis. Antisera to γ-globulin and albumin did not inhibit clot retraction or lysis. Platelets washed three times were preferentially agglutinated by antisera to the above proteins. Photomicrographs of clots formed in the presence of γM antisera had fewer platelets and less platelet aggregation and rhexis than the control samples. ¶ These findings suggest that *1*) platelets, γM-globulin, and β_{1E}- and β_{1C}-globulins may participate in a series of reactions leading to lysis of the clot under these conditions, *2*) the γM-globulin and β_{1E}- and β_{1C}-globulins are preferentially adsorbed onto the platelet surface under the conditions of

the assay, and *3*) these platelets with their attached proteins in turn are adsorbed onto the fibrin network. The resulting physical chemical milieu is postulated to be favorable for γM activation of complement which return is postulated to activate plasminogen either directly at the platelet-fibrin-serum interface or indirectly by aggregation and lysis of platelets and release of plasminogen-activating substance.

The Renal Handling of Hydroxyproline. GEORGE B. THEIL, Iowa City, Iowa (introduced by George N. Bedell*).

Recent investigations have indicated that the urinary excretion rate of bound hydroxyproline (peptide) after oral gelatin loading may exceed the filtered load. The endogenous clearance, however, is usually between 26 and 66 ml per minute, and these values do not exceed 60% of the glomerular filtration rate. ¶ The measurements of total (OHprT) and free (OHprF) hydroxyproline were made on renal arterial and venous plasma in an attempt to study further the renal handling of the nonprotein forms of this imino acid. Determinations were made of either OHprT (eight subjects), OHprF (seven subjects), or both (four subjects) of these forms of hydroxyproline. Peptide hydroxyproline was estimated in the latter group by subtracting OHprF from OHprT. ¶ The mean venous concentration of OHprT was 18% less than the arterial level, and the mean venous OHprF was 12% less than the arterial concentration. When both values were determined in the same subject, the following results were obtained: Mean arterial and venous OHprT was 10.41 and 9.09 μmoles per L, respectively. The mean arterial OHprF was 8.21 and the venous level was 7.04 μmoles per L. The calculated mean renal arteriovenous fall in peptide concentration was, therefore, 0.15 μmole per L. This amount represents 6.7% of the derived arterial peptide level, which was significantly less than the simultaneously measured transrenal fall in creatinine concentration (21.3% ± 2.9). ¶ These results do not show the tubular secretion of hydroxyproline peptides in the basal state. They emphasize the limitation inherent in estimating these peptides by the method of OHprT-OHprF difference.

Quantitative Determination of Catecholamines by Isometric Technique. GURDARSHAN S. THIND, LYSLE H. PETERSON,† AND HARRY F. ZINSSER,† Philadelphia, Pa.

There is increasing evidence that the sympathetic nervous system plays a significant role in the etiology of essential hypertension and in the chronic phase of renal hypertension. Therefore, it is important to have feasible and reliable methods to quantitate the behavior of the sympathetic nervous and renal-adrenal systems. Unlike most of the fluorometric techniques, bioassay methods specifically measure the biologically active catecholamines. The new technique developed by us to bioassay angiotensin II is now being extended to measure catecholamines. The results of this investigation show that this isometric aorta strip method is sensitive to epinephrine (EP) and norepinephrine (NEP) in concentrations as low as 0.0001 μg per ml. By operating the aortic strips within an optimal strain (131 ± 9% for EP and 108 ± 13% for NEP), the tension developed to the same dose can be potentiated up to fourfold compared to other ranges of strain. Dose-response curves relating the concentration of EP to NEP to the tension developed by the tissue are linear. The concentration of EP and NEP in an unknown sample may be calculated by a Michaelis-Menten-type equation. Conclusions: *1*) A new isometric technique combining a high degree of sensitivity, reproducibility, and an easy means of quantitating catecholamines has been developed and evaluated; *2*) Mechanical responses of rabbit aorta to angiotensin (AS) and catecholamines are qualitatively similar, but the quantitative difference can be determined by the difference in the dissociation constant (K_A) of the pharmacon-receptor complex (K_A AS 0.0053 μg, K_A EP 0.0013 μg, and K_A NEP 0.0015 μg).

Effect of Micellar Lipid on Transport of Vitamin D into Lymph. GILBERT R. THOMPSON, ROBERT K. OCKNER, MATTHEW A. BUDD, AND KURT J. ISSELBACHER,* Boston, Mass.

The intestinal absorption of vitamin D has been shown to occur in two phases, rapid uptake followed by a prolonged exit from the mucosa into lymph chylomicrons. The purpose of the present study was to investigate the nature of the uptake step and the effect of concomitant lipid absorption on the exit of vitamin D into lymph. ¶ Vitamin D uptake by rat intestinal mucosa was measured *in vitro* by incubating vitamin D_3-1,2-^{3}H with "purified" preparations of brush borders. This process was shown to be very rapid (50% of the uptake occurring within 1 minute), temperature-dependent, but energy-independent. Uptake was rapidly reversible if the brush borders were reincubated in a vitamin D-free medium. ¶ The exit of vitamin D_3-1,2-^{3}H from mucosa into lymph was studied in rats with cannulated intestinal lymphatics. Fasted rats were given an intraduodenal dose of a mixed micellar solution (mono-olein, oleic acid, and taurocholate) 1 hour before vitamin D. This resulted in a 40% increase in 24-hour lymph radioactivity as compared to control rats given taurocholate without lipid. The enhancement of vitamin D transport by mixed micelles was independent of any changes in lymph flow but, rather, was associated with increased amounts of triglyceride in lymph. Additional studies with both isolated loops and brush borders showed that vitamin D uptake was not affected by the simultaneous presence of mixed micelles. ¶ These results suggest that administration of micellar lipid results in an increased absorption of vitamin D. This effect is not primarily on mucosal uptake but appears to result from an increased rate of exit into lymph.

The Pressure Response in the Left Side of the Human Heart during Coughing, Vomiting, and Cardiac Arrest. JAMES H. THOMSEN, ROGER R. STENLUND, AND GEORGE G. ROWE,* Madison, Wis.

Intracardiac pressures have been recorded systematically in a considerable number of human subjects

during diagnostic cineangiocardiography. As a result, pressure recordings have been made in the left side of the heart during a number of events such as coughing, vomiting, direct current defibrillation, cardiac arrest, and closed-chest cardiac massage. Neither intrapleural nor esophageal pressures were recorded, so we cannot correct for the increase in intrathoracic pressure; nevertheless, the data are interesting. ¶ The elevations that occur in left side intracardiac pressures are striking. During vomiting, for example, left atrial pressure over 100 mm Hg has been recorded. During closed-chest massage, left atrial and aortic pressures are very nearly the same. (Average left atrial mean in seven subjects was approximately 60 mm Hg.) As cardiac activity begins, the left atrial pressure decreases and the aortic pressure rises. Such data may explain the development of pulmonary edema during prolonged chest massage. They also suggest that an emergency state exists so long as there is no effective cardiac contraction and that immediate electrical defibrillation is indicated, even in the presence of satisfactory systemic arterial blood pressure during closed-chest massage.

The Relationship between Tissue Oxygen Tension and Hematocrit. E. B. THORLING AND A. J. ERSLEV,† Philadelphia, Pa.

It is generally assumed that the high hematocrit found in secondary erythrocytosis is a compensatory device designed to improve tissue oxygenation. However, an increase in hematocrit is associated with an increase in viscosity, and the resulting increase in peripheral resistance could, at least in theory, nullify any gain from the increased oxygen-carrying capacity. In an attempt to solve this paradox, studies were made of the relationship between "tissue" oxygen tension and the hematocrit. A subcutaneous air pocket was used as a model tissue in rats, pneumoperitoneum in mice. Oxygen tension of air in the pocket or pneumoperitoneum was determined at different hematocrits after 20 hours of equilibration. ¶ A curvilinear correlation between hematocrit and P_{O_2} was found in both systems. Decreases in hematocrit induced by bleeding or phenylhydrazine resulted in an almost linear decrease in the P_{O_2}. Increases in hematocrit induced by transfusions resulted first in an increase in P_{O_2}, then in a broad P_{O_2} maximum at hematocrits of 60 to 65%, and finally at hematocrits above 65%, in a decrease in P_{O_2} toward normal values. The maximal tissue P_{O_2} was consequently found at polycythemic hematocrit values in contradistinction to *in vitro* hematocrit-oxygen flow curves, which show a maximum at normal hematocrits. Since this difference could have been caused by the increase in blood volume found in the transfused animals, normovolemic erythrocytosis was induced in groups of rats by exchange transfusion. In these groups, there was no increase in P_{O_2} at hematocrits above 45%. ¶ These studies suggest the following: *1*) A moderate degree of hypervolemic erythrocytosis is advantageous for the oxygenation of tissues. *2*) This advantage is probably not due to the increased oxygen-carrying capacity but to an increase in total blood volume with a resulting increase in organ vascularity and cardiac output.

Stimulation of Aromatization in Human Placenta by Human Placental Lactogen. TOSHIRO TOMINAGA AND PHILIP TROEN,* Pittsburgh, Pa.

The possible role of trophic hormones in controlling steroid biosynthesis in the human placenta has not been fully explored. The discovery of human placental lactogen (HPL) as a protein hormone produced by the human placenta provided an opportunity to test further the hypothesis that trophic hormones produced by the human placenta control various aspects of placental steroid biosynthesis. ¶ Human placental tissue slices were incubated for 4 hours with androstenedione-^{14}C (Δ^4) and dehydroepiandrosterone-^{3}H (DHA). After the incubation, radioactive estrone, estradiol, Δ^4, and DHA were separated, measured, and identified. The recovery of radioactivity in all experiments was uniform, ranging from 75 to 85%. Replicate incubations were performed. The average control conversion to estrogens was 33% for a 12-week placenta (I), 3% for a 23-week placenta (II), and 49% for a term placenta (III). Thicker slices of tissue were used in placenta II. The effect of a partially purified preparation (Lederle) of HPL was determined at three dose levels. With added HPL an increased conversion to estrogens was noted, twofold for placenta I, tenfold for placenta II, and 1.6 times for placenta III. For placentas I and II, the response appeared dose related. ¶ Control incubations with human serum albumin showed no increased conversion of radioactive precursors to estrogens. HPL heated to 55° C for 30 minutes gave a reduced response; HPL heated to 55° C for 60 minutes caused no increased estrogen formation. ¶ It appears that an HPL preparation increases the aromatization activity *in vitro* of the human placenta. Such a self-regulating control mechanism for placental steroid metabolism could have significant implications concerning maintenance of normal pregnancy and could provide another rationale for detection of abnormal placental function.

Pharmacology of Renal Carbonic Anhydrase Inhibition by Benzolamide (CL 11,366) in Man. DAVID M. TRAVIS, Gainesville, Fla. (introduced by Leighton E. Cluff*).

A new enzyme inhibitor, 2-benzenesulfonamido-1,3,4-thiadiazole-5-sulfonamide, which is highly concentrated in kidney by tubular secretion and largely excluded from action at other sites by its physicochemical characteristics, was studied in man to explore its general human pharmacology and its effects on electrolyte balance and respiration in patients with existing or potential ventilatory failure. ¶ Two healthy subjects and eight emphasematous patients, three of whom were hypercapneic, were given 1.5 to 5 mg per kg orally in single doses or every 12 hours for 2 to 6 days. Absorption of the drug was 25 to 35%. Peak plasma inhibitor concentrations of 3 to 10 μmoles

per L (95% protein-bound) at 1 to 2 hours were less than required for physiological effects in tissues not accumulating drug. Plasma half-life was 2 to 4 hours. Red cell inhibitor concentrations of 10 to 40 μmoles per L after single doses and 80 to 140 after continued dosage were below saturation values for red cell enzyme. On the first drug day, urinary HCO_3^- output was 100 to 120 mEq and was maximal at 3 mg per kg; Na^+ and K^+ output doubled. Urinary Cl^-, Mg^{++}, and Ca^{++} losses did not change. Acidemia developed with average fall in arterial blood H^+ of 60 nmoles and plasma HCO_3^- of 4 mmoles and with rise in plasma Cl^- of 6 mmoles. There was an associated mild hyperventilation as arterial, alveolar, and mixed venous CO_2 tensions fell 2 to 3 mm Hg and arterial P_{O_2} rose 4 mm Hg. Performance of routine ventilatory tests remained stable or improved slightly. Changes were similar in patients with and without CO_2 retention. Recovery from acidemia required 3 to 5 days. The drug was well tolerated. ¶ In summary, benzolamide inhibits carbonic anhydrase predominantly in kidney, and other undesirable effects may be excluded in man.

Reproduction of Fulminant Hepatic Necrosis in Rhesus Monkeys and the Reversal of Hepatic Coma by Exchange Transfusion. CHARLES TREY, NORVAL W. KING, AND FÉLIX G. GARCIA, Boston, Mass. (introduced by Charles S. Davidson†).

Evaluation of therapy in fulminant hepatic necrosis requires a reproducible model. Carbon tetrachloride, 0.18 ml per kg intraportally, in rhesus monkeys produced a similar lesion and a reproducible clinical and pathological syndrome. Acute hepatic necrosis was thus induced in six pairs of monkeys. The animals were given identical supportive treatment, including antibiotics and intravenous glucose. After the development of deep coma, the blood of the animal that appeared the more moribund was "exchanged" one blood volume with previously collected citrated or heparinized rhesus blood. ¶ The initial clinical and laboratory course was similar to that described for hepatectomized animals. Hypoglycemia was corrected, but despite continual glucose and electrolyte control, coma supervened 10 to 12 hours later. Death occurred 1 to 1½ hours after onset of coma in the six animals not given exchange transfusion. ¶ Exchange transfusion was performed 1 to 1½ hours after onset of coma in the remaining six animals. Each regained consciousness and remained alert for 16 to 24 hours thereafter. Coma again supervened, but subsequent exchanges again reversed this. No untoward effects were observed. The serum bilirubin concentration decreased after each exchange by about 50% but subsequently rose again. These animals survived an average of 80 hours (range 50 to 120 hours), whereas the unexchanged animals lived from 10 to 14 hours after carbon tetrachloride. Liver lesions were similar in all and included large confluent zones of infarction at the hilar region of each lobe and smaller foci of necrosis in the periphery. The peripheral portions of the livers of the exchanged animals contained abundant fat. In the three exchanged animals that survived 72 hours, the hepatocytes in the periphery showed regeneration, as evidenced by mitotic nuclei and multinucleated cells. ¶ Thus, in these reproducible animal models, exchange transfusion reversed the coma, prolonged survival time, and permitted some liver regeneration.

Hemophilia B_M: A New Type of Christmas Disease. JEREMIAH J. TWOMEY AND CECIL HOUGIE, El Paso, Texas, and Seattle, Wash. (introduced by E. D. Thomas†).

Two brothers (P.M. and M.M.) who have severe Christmas disease had prolonged prothrombin times that could not be attributed to Factor IX deficiency. All other factor assays were normal. This one-stage defect was not demonstrable in four unrelated Factor IX-deficient patients. The brothers' one-stage defect, which on mixing experiments proved to be an inhibitor, showed species specificity. It was most evident with bovine brain and thrombotest, which is partly derived from bovine brain, but not with human brain thromboplastic extracts. The inhibitor was removed from the siblings' plasma by $Al(OH)_3$ adsorption, and in this respect it resembles Factor IX. It was found in the fractions of sucrose gradient ultracentrifugation and Sephadex gel filtration, where Factor IX activity would be expected, and was slightly less heat stable than Factor IX. The siblings' mother and maternal grandmother had intermediate levels of both Factor IX (50 to 60%) and titers of inhibitor, both of which are consistent with heterozygous expression. These findings suggest that the brothers have inherited an abnormal inactive form of Factor IX in sex-linked fashion that interferes with the normal reaction between Factor VII and animal brain. These observations also explain why the thrombotest may be abnormal in Christmas disease. Christmas disease may no longer be considered a single entity. Experience with the thrombotest suggests the M brothers represent only a minority of Factor IX-deficient patients.

Mechanisms of Response of Plasma "Glucagons" to Ingested Nutrients: Evidence for Enteric Control of Islet Hormone Secretion. R. H. UNGER,* A. OHNEDA, I. VALVERDE, AND A. M. EISENTRAUT, Dallas, Texas.

Gastrointestinal influence upon islet response to ingested nutrients was studied by comparing the effects of intraduodenal and intravenous administration of amino acids and glucose upon immunoassayable plasma glucagon (G) and insulin (I) in conscious dogs with indwelling vena caval (VC), pancreaticoduodenal (PV), and mesenteric venous (MV) catheters. ¶ Amino acids given intravenously (1 g per kg per hour) raised mean plasma amino nitrogen 11 mg per 100 ml (7.8 to 14.3); PVI rose 550 μU per ml (SD ± 285) and PVG 1.2 mμg per ml (SD ± 1.5). This dose given intraduodenally raised amino nitrogen only 6.7 mg per 100 ml (3.6 to 12.1), but PVI rose 686 μU per ml (SD ± 900) and PVG 4.4 mμg per ml (SD ± 3.8). Glucagon/amino nitrogen was sig-

nificantly greater ($p < 0.001$) after intraduodenal administration. Intravenous glucose (2 g per kg per 90 minutes) raised mean plasma glucose 218 mg per 100 ml (87 to 380); PVI rose 1,277 μU per ml (SD $\pm$ 1,080), but VCG did not change and PVG often declined. This dose given intraduodenally raised glucose only 119 mg per 100 ml (69 to 167), but PVI rose 2,182 μU per ml (SD $\pm$ 2,841) and VCG 1.8 mμg per ml (SD $\pm$ 0.8), a significant ($p < 0.005$) difference from intravenous administration. ¶ To determine whether hyperglucagonemia induced by intraduodenally administered nutrients involves an enteric alpha-cytotropin or release of enteric "glucagon-like immunoreactivity" (GLI), VC, PV, and MV glucagon were compared. After amino acids were given intraduodenally, PVG > VCG > MVG, indicating pancreatogenous hyperglucagonemia compatible with enteric alpha-cytotropism; 10 U per minute of pancreozymin, an amino acid-responsive duodenal hormone, raised PVG 1.0 mμg per ml. After glucose intraduodenally, MVG > VCG > PVG, indicating enterogenous hyper-"glucagon"-emia. GLI of jejunum differed from pancreatic glucagon immunologically (shallower dilution slopes) and was larger by Sephadex chromatography (mol wt > 6,000); partially purified GLI (1 "μg equivalent") caused no hyperglycemia but stimulated insulin release in four of six experiments. ¶ Conclusions: *1*) Hyperglycemia depresses pancreatic glucagon secretion. *2*) Hyperglucagonemia occurs during intestinal absorption of glucose, but is enterogenous, perhaps representing cross-reacting enteric GLI, which differs immunologically, physically, and biologically from pancreatic glucagon, though it may be insulinogenic. *3*) Aminogenic stimulation of pancreatic glucagon secretion is potentiated during amino acid absorption, perhaps by pancreozymin, the only known alpha-cytotropin.

Alterations in Myocardial Mechanics Produced by Valvular Regurgitation: The Concept of Instantaneous Impedance as a Determinant of Performance of the Intact Heart. CHARLES W. URSCHEL, JAMES W. COVELL, EDMUND H. SONNENBLICK,* JOHN ROSS, JR.,* AND EUGENE BRAUNWALD,* Bethesda, Md.

Although the alterations in cardiovascular dynamics produced by valvular regurgitation have been well characterized, the effects of such lesions on myocardial performance have not been described. Accordingly, in 17 dogs, controlled, dynamically measured aortic (AI) and/or mitral (MI) valvular regurgitation was induced. With either lesion there were immediate increases in total stroke volume (+65%), left ventricular end-diastolic pressure (+1.6 mm Hg), and peak ejection velocity (+32%), although contractility remained unchanged, judged by identical length-active tension curves in the presence and absence of regurgitation. Despite unchanged contractility, the ejection fraction (SV/EDV) rose from 0.43 (control) to 0.55 (AI) ($p < 0.01$) and to 0.59 (MI) ($p < 0.01$). With both AI and MI the rate of tension decline during ejection was increased above control because of the more rapid diminution of ventricular size during ejection. Consequently, contractile element (CE) velocity was increased (control 10.4, AI 13.6, and MI 14.4 cm per second) as were CE work and peak CE power. When regurgitant beats were compared with control beats with end-diastolic volume held constant, ventricular stroke volume, work, power, and SV/EDV as well as CE velocity, work, and power consistently increased. Thus, reduction of instantaneous impedance to ejection allowed the ventricular muscle to shorten faster and further, increasing external energy output despite unchanged contractility and diastolic fiber length. It is concluded that, since loading during ejection governs the velocity and extent of CE shortening and hence affects stroke volume, peak aortic flow rate, and SV/EDV, the alterations of ventricular function accompanying AI and MI can be explained by the effects of these lesions on the instantaneous impedance to left ventricular ejection.

Determination of Angiotensin II and Renin Activity in Human Plasma by Radioimmunoassay. M. VALLOTTON, L. PAGE, E. HABER,* AND S. LAGG, Boston, Mass.

We previously showed that copolymers of angiotensin II (Hypertensin, Ciba) and poly-L-lysine elicit specific antibodies to angiotensin in the rabbit. The quantitative displacement of ^{125}I-labeled angiotensin II from antibody by unlabeled angiotensin II has permitted the development of a radioimmunoassay. This technique has been adapted to the assay of angiotensin and renin in human plasma. ¶ Cold samples of undialyzed plasma are analyzed directly for angiotensin or after 3 hours' incubation at 37° C for renin. In either case, angiotensin is extracted from plasma on Dowex 50, eluted, dried, and suspended in buffer. Equilibration at 4° C with ^{125}I-labeled angiotensin II and antiserum follows for 14 hours. Antibody-bound angiotensin is separated from free hormone on Sephadex G-25 columns and the eluent fractions are counted. Synthetic asp(NH_2)^1-val^5-angiotensin II and asp^1-ileu^5-angiotensin II give identical displacement curves, whereas a 20-fold excess of angiotensin I is necessary to achieve the same displacement. Present sensitivity is 0.1 ng per ml plasma, with virtually 100% recovery of added angiotensin. Samples treated with chymotrypsin to destroy angiotensin give results similar to the buffer blank. ¶ In 2 ml of plasma 0.001 U of human renin (Haas preparation) generates 1 ng angiotensin II. Subjects on 110 and 10 mEq Na diets show circulating levels of 10 to 30 ng per 100 ml. The former generate little additional angiotensin during renin determination, whereas the latter generate 10 to 40 ng per 100 ml. ¶ Plasmas from patients in various disease states have been examined. Highest values for both circulating and generated angiotensin are found in patients with cirrhosis and ascites (up to 220 ng per 100 ml) and in renal venous blood of patients with renal arterial stenosis (up to 2,500 ng per 100 ml). These values are considerably lower than those reported by bioassay. It is suggested that other pressor materials in addition to angiotensin are generated during incubation in renin bioassay methods.

The Regulation of Heme Synthesis: Studies on Erythrocyte Aminolevulinic Acid Synthetase. JOHN D. VAVRA, St. Louis, Mo. (introduced by Carl V. Moore†).

Aminolevulinic acid (ALA) synthetase catalyzes the initial and rate-limiting step in heme biosynthesis. Its localization to mitochondria together with the final two steps of heme biosynthesis suggests that it is the important step of a feedback control mechanism, whereby heme synthesis is inhibited and integrated with the synthesis of the rest of the hemoglobin molecule. Kinetic studies of a mitochondrial preparation of this enzyme obtained from chicken reticulocytes have been performed to elucidate both the mechanism of ALA synthesis and the influence of components of the hemoglobin molecule upon it. The rate of ALA synthesis in the presence of optimal concentrations of cofactors is described by Michaelis-Menten kinetics for a two-substrate reaction. Michaelis constants obtained by $1/V_{max}$ plots include: glycine, 12 mmoles; α-ketoglutarate (αKG), 0.20 mmole [reaction synthesizing succinyl coenzyme A (CoA)]; and succinate, 1.7 mmoles (succinate and CoA as substrates). Activities were optimal in the presence of an iron concentration of 0.01 mg per ml. Mixed competitive inhibition of ALA synthesis was produced by chicken and human hemoglobin, human methemoglobin, partially degraded human globin, and human α and β globin chains. The inhibition in each instance was produced by the globin part of the molecule and was a hundredfold greater for the isolated peptide chains than for intact globin; bovine serum albumin produced no inhibition. Heme inhibited ALA synthesis from the substrates succinate and CoA much more strongly than from αKG, indicating an inhibitory effect upon succinyl CoA synthesis rather than upon its condensation with glycine to form ALA. These studies indicate that globin peptide chains and heme inhibit ALA synthesis at different steps and that these sites of inhibition may be important in the over-all control of the rate of heme synthesis.

Purification of a Low Molecular Weight Type 12 Streptococcal M-Protein. KENNETH L. VOSTI AND RUDOLPH H. JOHNSON, Palo Alto, Calif. (introduced by Halsted R. Holman*).

Protein is probably the most important of the streptococcal antigens. It correlates with virulence, stimulates immune antibodies, and provides specificity to different strains of group A hemolytic streptococci. In spite of continued interest for nearly 40 years, little progress has been made in the purification and characterization of this protein. This study reports the separation and characterization of two different molecular weight species of type 12 M-protein. ¶ A crude acid extract of a single strain (Boatman) of group A, type 12 streptococcus was neutralized and subjected to differential fractionation by repeated ammonium sulfate precipitation. A highly M-active fraction was obtained that was not significantly contaminated with nucleic acids or rhamnose; however, polyacrylamide disc gel electrophoresis revealed six bands. Column chromatographic fractionation allowed the individual recovery of the two bands that migrated slowest in polyacrylamide gel. Type-specific precipitating and hemagglutinin-inhibiting activity were associated with each of these bands but not with the other four. Analysis in the analytical ultracentrifuge revealed that one of the antigenically active bands had a molecular weight of approximately 40,000 and the other of about 10 to 12,000. ¶ These data represent the first separation of a species of M-protein with a molecular weight as small as 10 to 12,000 that retains antigenic activity, and suggest the possibility that M-protein may consist of a number of repeating small units.

The Effect of Secretin and Acetazolamide on the Volume and Electrolyte Composition of Hepatic Bile in Man. ALBERT M. WAITMAN AND HENRY D. JANOWITZ,† New York, N. Y.

This study was undertaken to determine the effect of secretin and of acetazolamide on the electrolyte composition of human bile. Five patients with T-tubes implanted in the common bile duct after cholecystectomy were studied. Samples were collected for six consecutive 10-minute periods in the basal state and at 10-minute intervals after the intravenous injection of secretin (Boots, 1 U per kg). The effect of acetazolamide (50 mg per kg iv) on spontaneous and secretin-stimulated bile flow was also determined. Volume, Na, K, Cl, HCO_3, and taurocholate concentrations were determined for each sample. ¶ Spontaneous bile flow and electrolyte composition varied within a narrow range for each patient, although greater variation was noted among patients. Secretin increased flow and HCO_3 concentration to peak levels within the first 10 minutes; the effect was over in 30 minutes. During this period, there was an average increase of 130% in volume rate, 413% in HCO_3 output, 131% in Na output, 119% in K output, and 23% in the taurocholate output compared to the basal state. Bicarbonate concentration rose markedly in all patients (mean peak = 51 mEq per L), whereas chloride concentration fell in three patients. Taurocholate concentration fell markedly, whereas Na and K concentrations were only slightly depressed. ¶ Acetazolamide reduced the spontaneous rate of flow and output of all components. It reduced secretin-stimulated outputs by the following percentages: volume, 18%; HCO_3, 38%; Cl, 9%; Na, 17%; K, 39%; and taurocholate, 37%. ¶ These studies indicate that secretin stimulates the secretion of bile in man by augmenting the output of HCO_3-containing solution, which may be inhibited by acetazolamide.

Thymoma, Hypogammaglobulinemia, and Absence of Eosinophils. THOMAS A. WALDMANN,* WARREN STROBER, R. MICHAEL BLAESE, AND ARTHUR J. L. STRAUSS, Bethesda, Md.

Patients with thymoma have been shown to have a variety of hematological, neuromuscular, and immunological disorders, including hypogammaglobulinemia. In the present study, the immunoglobulins of 85 patients with

thymoma were quantitated by radial diffusion in agar. Ten had IgG concentrations at least **2 SD** below the mean of 50 control subjects. Six of these 10 also had markedly reduced IgA and IgM levels. Two of the 10 had dysgammaglobulinemia with reduced IgG and IgA levels but markedly elevated IgM levels. Seven of the 10 had an increased incidence of infections. ¶ Immunoglobulin metabolism was studied in 3 patients with thymoma and hypogammaglobulinemia using purified radioiodinated proteins. Each of the patients had a markedly reduced synthetic rate of IgG, IgA, and IgM. In 2 the survival of IgM and IgA was normal, whereas that of IgG was prolonged. One patient with mild gastrointestinal protein loss demonstrated with albumin-^{51}Cr had normal IgG and short IgM survival. Antibody responses to Vi and tularemia antigens were markedly reduced or absent in the 3 patients studied. Two of them had normal lymphocyte counts and positive delayed skin tests. The third had lymphocytopenia and was unable to reject a skin homograft. ¶ Four of the 10 patients with thymoma and hypogammaglobulinemia had aplastic anemia or pure red cell aplasia. Each of the 5 patients with thymoma and hypogammaglobulinemia studied had a complete absence of marrow and circulating eosinophils. Ten patients with thymoma and normal immunoglobulins and 13 patients with agammaglobulinemia without thymoma did not have eosinopenia. Thus, patients with thymoma have a significant incidence of hypogammaglobulinemia. This defect is synthetic in nature and need not be associated with evidences of cellular immunologic deficiency. The absence of eosinophils is uniquely associated with this category of hypogammaglobulinemia.

Multiple Cation Transport by the Toad Bladder. MACKENZIE WALSER,* RAJA N. KHURI, AND BENNETT MACHANIC, Baltimore, Md.

Bufo marinus bladders were soaked for 1 hour, everted empty, cannulated, and then suspended in Tris-buffered oxygenated Ringer's solution containing glucose, penicillin, aldosterone, and vasopressin. The sacs gained 60 to 400 mg in 20 hours at 25°. Leakage and contamination with interstitial or adherent fluid were excluded by adding blue dextran to soaking and bathing media. Uneverted sacs failed to gain. The elaborated serosal fluid contained sodium at a concentration $20 \pm 9\%$ (SD, $n = 25$) higher than the bath. Mean sodium transport rate (T_{Na}) was 25 μEq per mg dry weight per hour; early rates were higher. Mean serosal/mucosal ratios (S/M) for other cations were magnesium, 0.52; calcium, 0.57; ^{85}strontium, 0.14; ^{86}rubidium, 0.28; ^{137}cesium, 0.27. Such movement up electrochemical gradients could reflect solvent drag, multiple pumps, or a common pump. To examine this problem, we replaced bath sodium by calcium in varying proportions in 75 sacs. Fluid transport was not significantly affected until mucosal sodium (M_{Na}) was reduced below 1 mmole per L, despite M_{Ca} up to 70 mmoles per L. At zero sodium it ceased. In any given medium, T_{Ca}/M_{Ca} was proportional to T_{Na}/M_{Na}, but the clearance ratio $T_{Ca}/M_{Ca} \div T_{Na}/M_{Na}$ varied widely in different media. At $M_{Ca} > 50$ mmoles per L, T_{Ca} regularly exceeded T_{Na}; maximal T_{Ca}/T_{Na} was 35. Maximal S_{Na}/M_{Na} was 5, maximal S_{Ca}/M_{Ca}, 2. In other experiments, replacement of most of the sodium with strontium or lithium also led to the elaboration of fluid containing predominantly these cations. These results suggest a common cation pump whose selectivity among cations varies with medium composition, but which cannot transport one cation without transporting all. Sodium is the most readily transported but is required in only minute amounts.

Chemotactic Factors Elaborated by Bacteria. PETER A. WARD AND IRWIN H. LEPOW,* Washington, D. C., and Cleveland, Ohio.

Recent techniques for measuring chemotactic attraction of polymorphonuclear leukocytes (PMN) and separating biologically active molecules make possible renewed attack on mechanisms of PMN accumulation in bacterial infections. Modified Boyden chambers were employed in which rabbit PMN were placed in an upper compartment separated from the test sample by a Millipore filter. Chemotactic activity was expressed as the number of PMN that migrated from the upper to the lower surface, observed microscopically after staining and clearing the filter. ¶ Filtrates of 18- to 48-hour cultures of the following organisms had marked chemotactic properties: *Pneumococcus,* types 1, 2, 3, 7, 8, 12, 18, and 29; *Staphylococcus albus* and *aureus; Streptococcus faecalis; Escherichia coli;* two species of *Proteus;* and *Pseudomonas aeruginosa.* Activity was not affected by the medium employed (Todd-Hewitt broth, medium 119, or mixtures of both). Possible influence of medium was indicated, however, by results with β-hemolytic *Streptococcus,* which failed to exhibit chemotactic activity in partially defined media but gave positive results in Todd-Hewitt broth. Filtrates of the following organisms have thus far been negative: three strains of *Meningococcus,* type B, sulfadiazine-resistant (Frantz or Mueller-Hinton broth, 18 to 48 hours growth), and *Mycobacterium tuberculosis* $H_{37}Ra$ (Proskauer-Beck medium, 7 weeks growth). ¶ The pneumococcal chemotactic factor is not demonstrable in intact or sonically disrupted organisms. In culture filtrates, it is relatively heat stable (56°, 60 minutes), dialyzable, and appears to be unaffected by type-specific antiserum. It is more highly retarded on G25 Sephadex columns than cytochrome c, indicating a molecular weight below 12,500. Isolation and characterization of chemotactic factors are in progress for comparisons within and among bacterial species and for studies on mechanisms of inflammation in infectious processes.

Granuloma Formation as a Manifestation of Delayed Hypersensitivity. KENNETH S. WARREN, ERNESTO O. DOMINGO, AND RICHARD B. T. COWAN, Cleveland, Ohio (introduced by John H. Dingle†).

Granulomas are a major pathological component of many diseases such as tuberculosis, sarcoidosis, and schistosomiasis. Although an association between granuloma formation and delayed hypersensitivity has been

suggested, the relationship between these phenomena is not known. In order to establish such a relation it would be necessary to show that sensitization (an accelerated and augmented granulomatous reaction) occurs, that it is specific, and that it can be transferred by cells and not by serum. In the present study the isolated living schistosome eggs used to elicit granuloma formation were injected via a tail vein into the lungs of mice. One, 4, 8, 16, and 32 days after injection, groups of mice were sacrificed and their lungs sectioned. The average diameter of approximately 100 lesions (eggs and granulomatous reactions around them) was measured at each time interval with an image-splitting eyepiece. No reaction was observed 1 day after injection; the peak reaction (mononuclear, epithelioid, and giant cells) occurred at 16 days. Sensitization induced by prior intraperitoneal injection of schistosome eggs resulted in granulomatous reactions at day 1 and peak reactions at day 8 much larger than those which had occurred at 16 days in unsensitized animals. This sensitization did not occur after intraperitoneal injection of *Ascaris suis* eggs or vice versa (schistosome eggs intraperitoneally followed by ascaris eggs intravenously). Finally, sensitization was elicited by intraperitoneal injections of spleen and lymph node cells from mice infected with *Schistosoma mansoni,* but not by serum from infected mice or by cells or serum from control mice. These results indicate that the granuloma, which is a prime aspect of the pathology of many diseases, may be a manifestation of delayed hypersensitivity, thus providing an understanding of the pathogenesis of these diseases and suggesting approaches to their control.

Differentiation of Immunologic from Nonimmunologic Forms of Idiopathic Thrombocytopenic Purpura. STANLEY P. WATKINS, JR., DALE H. COWAN, AND N. RAPHAEL SHULMAN,* Bethesda, Md.

The antiplatelet γ-globulin present in ITP plasma can be measured reliably only by its thrombocytopenic effect when transfused into normal recipients. Since not all patients have a detectable ITP factor by this test, decreased platelet production has been suspected as the basis for some cases of ITP. Differentiation of a platelet production defect from the immunologic form of ITP is not always clear-cut, as evidenced by the following observations. Of 24 patients whose plasma was titered, 8 had ITP factor measurable at *in vivo* dilutions of 1/40 to 1/3. This group responded to therapy; the lower the titer, the better the response. Of 16 that had no measurable ITP factor at dilutions less than 1/3, 11 had shortened platelet survival, indicating that ITP factor was present at a level too low to measure by passive transfer. All 11 had excellent therapeutic results. The remaining 5 had no detectable ITP factor by passive transfer and platelet survival longer than expected. In these 5, diminished production or selective destruction of newly formed platelets could account for the thrombocytopenia. Two of the 5 patients had complete remissions after adrenocorticosteroid therapy or splenectomy, whereas 3 were totally refractory to treatment, including immunosuppressive agents. The patients that responded to therapy may represent an immunologic form of ITP with low titer ITP factor that selectively destroys newly formed platelets, whereas those that did not respond to therapy appear to represent a production defect. Newer forms of therapy for refractory ITP cases, particularly immunosuppressive agents, can be better evaluated by establishing the basis for thrombocytopenia.

Abnormalities of Carbohydrate Metabolism in Acute Intermittent Porphyria. ALAN D. WAXMAN, DON S. SCHALCH, WILLIAM D. ODELL,* AND DONALD P. TSCHUDY,* Bethesda, Md., and Rochester, N. Y.

Carbohydrate metabolism was studied in acute intermittent porphyria (AIP) because *1*) carbohydrate administration blocks the induction of hepatic δ-aminolevulinic acid synthetase known to occur in this disease, and *2*) inappropriate release of pituitary hormones (antidiuretic hormone, growth hormone) also occurs in AIP. ¶ Twelve patients with well-documented AIP were given 100 g glucose orally after 3 days of a 250 g per day carbohydrate intake. Glucose tolerance was decreased and blood pyruvate and lactate levels after glucose administration were abnormally high in all 9 patients studied during symptomatic periods. These parameters were either normal or borderline in the 3 who had never had significant symptoms of AIP. ¶ A paradoxical rise of blood levels of growth hormone in response to a glucose load was seen in 5 of 11 studies, including a patient who had a normal glucose tolerance and had never had symptoms of AIP. In 4 studies where glucose tolerance was abnormal, no abnormalities of growth hormone were observed, demonstrating that the decreased glucose tolerance in AIP is not primarily caused by excessive release of growth hormone. In 4 of 11 studies, excess insulin release occurred after a glucose load. In all 4 the glucose tolerance was diminished. In some patients, excess growth hormone release occurred with a normal insulin response and vice versa. ¶ These studies demonstrate that decreased glucose tolerance is frequently seen in AIP, but differs from that of diabetes in the high rather than low levels of pyruvate and lactate seen in the latter disease after a glucose load. Despite these differences of abnormal carbohydrate metabolism in AIP and diabetes, exogenous insulin reverses the abnormalities of glucose, lactate, and pyruvate after a glucose load in AIP. The above findings suggest defective intracellular carbohydrate utilization in AIP.

An Acquired Reversible Abnormality of Erythrocyte Lipids Associated with Liver Disease and Hemolytic Anemia. PETER WAYS, Seattle, Wash. (introduced by Clement A. Finch†).

Genetically determined abnormalities of human erythrocyte phospholipids have been clearly documented. Elevations of the erythrocyte lipids occur in some patients with liver disease, but serial studies of such abnormalities

have not been reported. In the present case, gross abnormalities of erythrocyte cholesterol, phospholipids, and phospholipid fatty acids were found in conjunction with brisk hemolysis. The plasma lipids were similarly deranged. As the patient's clinical picture and liver function tests improved, both the erythrocyte and plasma lipids returned to normal or near normal values. ¶ An enlarged nontender liver, transient serum lactescence, hyperbilirubinemia (5.9 mg per 100 ml), elevated alkaline phosphatase (100 King-Armstrong U) and serum glutamic oxaloacetic transaminase (SGOT) (141 U), anemia [hematocrit (Hct) 33%], and reticulocytosis (7 to 10%) were found in a 58-year-old male after 2 weeks of heavy alcohol ingestion. Three and one-half weeks after cessation of alcohol, the liver was not palpable; bilirubin was 0.8 mg per 100 ml; cholesterol, 238 mg per 100 ml; alkaline phosphatase, 23; SGOT, 24; Hct, 35%; and reticulocytes, 3.0%. ¶ Erythrocyte and plasma lipids were analyzed at 0, 0.5, 1.5, 3.5, and 5.5 weeks. Initially, the total lipid/erythrocyte (6.73×10^{-10} mg, normal 4.88×10^{-10} mg), phospholipid/erythrocyte (0.168×10^{-10} mg, normal 0.127×10^{-10} mg), and cholesterol/erythrocyte (1.67×10^{-10} mg, normal 1.26×10^{-10} mg) were 30% above the normal means despite mean corpuscular volumes of 99 to 106 (normal 100). Plasma lipid phosphorus and cholesterol were 29 and 400 mg per 100 ml, respectively. The abnormal increment in both erythrocyte and plasma phospholipids was 85% lecithin. Erythrocyte fatty acids were high in oleic (22%, normal 16.3) and low in stearic (10.8%, normal 19.0) and arachidonic (8.8%, normal 16.1) acids. After 5.5 weeks the major erythrocyte and plasma lipids were normal and the erythrocyte fatty acids nearly so. Simultaneously, hemolysis was subsiding (hematocrit 38%, reticulocytes 2.4% after 5.5 weeks). ¶ Thus, abnormalities in circulating lipoproteins arising from deranged liver function can influence erythrocyte membrane lipid composition and *in vivo* survival. In the present instance all abnormalities were reversible.

Erythrocyte ATP, Calcium Transport, and Cellular Viscosity. ROBERT I. WEED,* Rochester, N. Y.

The contribution of the metabolic state of erythrocytes to the non-Newtonian viscosity of blood was studied during and after metabolic depletion of defibrinated blood *in vitro* for periods up to 48 hours by simultaneous measurement of erythrocyte ATP, Ca^{++}, viscosity, nonhemoglobin protein content of ghosts, and cell shape. Viscosity of washed red cell suspensions was measured with a cone-plate viscosimeter. ¶ No changes in red cell viscosity or Ca^{++} content were observed over the initial 10 hours of incubation while ATP levels decreased to approximately 10% of their initial value. After ATP depletion, cellular Ca^{++} and viscosity increased linearly with time. The Ca^{++} content of fresh red cells (5.2×10^{-18} mole per cell) rose 200 to 400% over 24 hours of incubation and continued to rise thereafter to approach equilibrium with the suspending serum. Viscosity of red cell suspensions begins to increase at 10 hours and progresses throughout the incubation with the greatest increases apparent at the lowest shear rates (300% at 2.3 $second^{-1}$, hematocrit 60). Addition of adenosine at 24 hours produces a 30% restoration of ATP with a concomitant extrusion of Ca^{++} against the concentration gradient and a marked reversal of viscosity to near preincubation values. The ATP-dependent reversible disc-sphere transformation parallels the viscosity changes. The viscosity of soluble hemoglobin separated from lysed cells at various times displays no increase in viscosity, but the nonhemoglobin protein content of ghosts increases 5% and 17% at 24 and 48 hours, respectively. ¶ These results suggest that erythrocyte Ca^{++} and ATP levels contribute to the shear dependence of blood viscosity by control of a sol-gel transformation involving nonhemoglobin protein. Intracellular ATP may be of importance both as a Ca^{++} chelator and as an energy source for the ouabain-insensitive Ca^{++} extruding pump described by Schatzmann.

Stimulation of Plasma Renin Activity without Increased Aldosterone Production after Administration of Chlorothiazide to Hypertensive Patients. M. H. WEINBERGER, A. J. DOWDY, G. W. NOKES, AND J. A. LUETSCHER,† Palo Alto, Calif.

Small groups of normal volunteers and hypertensive patients have been studied. A general survey, radiography, and renal function studies had revealed no specific etiology in the hypertensive patients. Each subject was studied on a high sodium intake, followed by a week of sodium deprivation. At the end of this period, the patients were given chlorothiazide for 3 days. During sodium loading, both groups showed aldosterone secretion (AS) below 100 μg and excretion (AE) below 10 μg, as well as low plasma renin activity (PRA, Boucher's method). Sodium deprivation was followed by increased AS and AE, and PRA was further increased after the patients stood upright. ¶When thiazide was given to the hypertensive patients, PRA was trebled, compared to the recumbent, low sodium values, and there was a further, large increase in PRA on their assuming the upright posture. There was no comparable increase in AS or AE, which in a few cases declined as PRA rose. This failure of AS to follow rising PRA is attributed to the significant urinary loss of potassium and to the fall in plasma potassium concentration. Under the conditions of this study, the effects of chlorothiazide on body fluid volume and on the circulation appeared to take precedence over increased sodium in renal tubules, resulting in increased PRA; but the anticipated increase in aldosterone secretion did not occur in the presence of significant potassium depletion.

Hepatic Glucuronyl Transferase: Submicrosomal Distribution and Role in Intracellular Transport of Bilirubin. JULIUS WEISS AND IRWIN M. ARIAS,* New York, N. Y.

Previous studies revealed that approximately 60% of intracellular unconjugated bilirubin (UCB) in rat liver is bound to cytoplasmic protein or proteins and 25% is associated with the microsomal fraction that contains 70%

of hepatic glucuronyl transferase activity. The microsomal fraction consists of smooth and rough endoplasmic reticula (SER, RER), which were separated by centrifugation in sucrose density gradients. Glucuronyl transferase activity, with O-aminophenol as a glucuronide acceptor, and side chain oxidation of hexobarbital were found primarily in the SER fraction (3.5- to 5.6-fold enrichment in specific activity from original microsomal fraction), whereas microsomal RNA was primarily found in the RER fraction (4.7-fold enrichment over original microsomal fraction). ¶ The role of glucuronyl transferase in intracellular transport of UCB was studied in normal and homozygous glucuronyl transferase deficient (Gunn) rats treated with sodium phenobarbital (100 mg per kg per day for 4 days), 3,4-benzpyrene (1 mg per day for 5 days), or isotonic saline (control). Phenobarbital treatment of normal and Gunn rats and benzpyrene treatment of Gunn rats enhanced barbiturate oxidation (3.4-fold increase) but not glucuronyl transferase activity in liver homogenates and microsomal and SER fractions. Benzpyrene-treated normal rats had 1.5-fold increase in hepatic glucuronyl transferase activity and 3.9-fold increase in barbiturate oxidation. ¶ Drug-treated and control rats were injected intravenously with UCB-^{3}H (80 μg containing 2×10^7 dpm). The hepatic submicrosomal distribution of radioactivity was estimated 10 minutes later and correlated with the above enzyme activities. In each experiment, UCB-^{3}H distribution in the total microsomal and SER fractions correlated with glucuronyl transferase activity ($p < 0.01$) but not with RNA, protein, or barbiturate oxidation activity. Approximately 4×10^{-3} μmole UCB-^{3}H was bound per μmole of O-aminophenol glucuronide formed per g of liver. ¶ These studies indicate that glucuronyl transferase is primarily associated with the SER fraction, provides an acceptor site for UCB, and plays an important role in intracellular transport of UCB.

A New Action of Tumor-promoting Agents: Lysis of Cell and Intracellular Membranes. GERALD WEISSMANN,* WALTER TROLL, BENJ. VAN DUUREN, AND GRAZIA SESSA, New York, N. Y.

Tumor-promoting agents, such as phorbol esters of *Croton* oil, induce few if any tumors when applied to mouse skin. However, repeated applications of these agents induce multiple tumors when preceded by a single nontumorigenic dose of carcinogens such as 7,12-dimethylbenz(a)anthracene (DMBA). Potent tumor-promoting agents have been isolated from *Croton* oil; however, their mechanism of action has remained unexplained. The lipophilic-hydrophilic structures of these, and of weaker promoting agents such as Tween 60 and 80, suggest that they might alter the permeability of cells and organelles. Therefore we tested the action of tumor-promoting agents on lysosomes, mitochondria, and erythrocytes. Tumor-promoting activity increases with increasing purification of the active principles: *Croton* oil < *Croton* resin < phorbol ester fraction A; the last fraction's activity is enhanced 100- to 1,000-fold over *Croton* oil. In direct ratio to tumor promotion *in vivo*, the Tweens and *Croton* oil fractions (>50 μg per ml) released up to 81% of lysosomal beta-glucuronidase, acid beta-glycerophosphatase, and aryl sulfatase activity from large granule fractions of rabbit tissues in 0.25 M sucrose. They released only 35% and 25%, respectively, of malate dehydrogenase and monoamine oxidase from mitochondria. At higher concentrations (>500 μg per ml), the promoting agents were hemolytic for rabbit and human erythrocytes, but their tumor-promoting activity did not correlate as closely with hemolytic activity as with their preferential effects on lysosomes; nor did the promoting agents release ions from artificial phospholipid/cholesterol spherules, an effect common to other hemolytic agents such as polyene antibiotics, steroids, or streptolysins. No tumor-initiating agent (e.g., DMBA, beta-propriolactone, 3-methylcholanthrene) was membrane lytic. These studies suggest that tumor-promoting agents act on biological membranes by a mechanism qualitatively distinct from other lytic agents. Furthermore, since their action on lysosomes parallels their *in vivo* activity, tumor-promoting agents may induce changes in living cells by releasing previously inactive hydrolases to interact with nuclear structures.

Inhibition of Human Lymphocyte Transformation in Uremia. MARC E. WEKSLER, F. PAUL ALEPA, AND G. E. SCHREINER,* Washington, D. C.

Uremic patients show abnormal responses to antigenic stimuli. Reagenic reactions, development of delayed hypersensitivity, and homograft rejection are all suppressed. To further investigate the immunologic defect in uremia, we made cultures of peripheral leukocytes from 15 patients with uremia (BUN greater than 100 mg per 100 ml) secondary to nephrosclerosis (7), chronic glomerulonephritis (3), and chronic pyelonephritis (5) and from 10 normal volunteers. Equal numbers of lymphocytes were incubated for 72 hours in the presence and absence of phytohemagglutinin (PHA). Incorporation of tritiated thymidine and L-amino acids-^{14}C was then used as an index of lymphocyte transformation. Lymphocytes from normal volunteers in the presence of PHA incorporated 300 times more thymidine and amino acid than in the absence of PHA. Without PHA both normal and uremic lymphocytes incorporate equal amounts of thymidine and amino acid. However, when PHA was present, uremic lymphocytes incorporated less than one-half the amount of thymidine and amino acid incorporated by normal lymphocytes. This defect in uremic lymphocyte transformation could not be corrected by adding normal serum to uremic lymphocytes or by washing them with culture medium. This suggests the presence of a defect in uremic lymphocytes. The addition of uremic serum to normal lymphocytes significantly inhibited the stimulatory effect of PHA. Such inhibition could not be reproduced by adding urea to normal lymphocytes. Lymphocytes obtained after hemodialysis incorporated significantly less thymidine than did lymphocytes obtained from the same patient before the dialysis. Moreover, serum

obtained after dialysis inhibited the transformation of normal lymphocytes more than serum obtained before dialysis. These studies indicate that uremic patients possess both a cellular and serum defect that suppresses lymphocyte tranformation. Hemodialysis may worsen lymphocyte transformation despite amelioration of uremia.

Defoaming Agent, Polymethylsiloxane, as Cause of Hemolysis in Extracorporeal Circulation. Roe Wells, Ali Shahriari, and M. Stellan Bygdeman, Boston, Mass. (introduced by Clifford L. Derick†).

Erythrocyte hemolysis is a major complication of cardiopulmonary bypass. Plasma foaming occurring with both disc and bubble oxygenators, and especially with high vacuum aspirators, has been controlled by passing blood through stainless steel mesh coated with polymethylsiloxane (PMS). Studies of extracorporeal pump oxygenator components (Kay-Cross disc) revealed that the major hemolytic injury occurred in the defoaming chamber. Whole blood, plasma, albumin, and saline were oxygenated (95% O_2, 5% CO_2) with and without PMS-coated mesh. Hemoglobin values were determined by spectrophotometry. Oxygenation at 25° C, with PMS mesh, caused profound hemolysis in whole blood and also of red cells added to previously oxygenated plasma, albumin, or saline. Oxygenation of plasma or saline without the PMS mesh or with PMS-free mesh did not cause hemolysis. Oxygenation of whole blood or red cells in albumin or saline caused slight hemolysis. At 4° C no hemolysis was noted, but defoaming action did not occur. PMS mesh in nonoxygenated plasma also hemolyzed added red cells, but less than oxygenated samples. Electrophoresis of plasma lipids and proteins, before and after oxygenation with PMS added, showed no significant changes. Ultracentrifugation revealed slight but significant increases in α- and β-globulins and decreased albumin; there was no change in controls. Thin layer and gas chromatography showed no significant lipid changes. Free fatty acids (FFA) were decreased in oxygenated PMS plasma and returned to control levels after mixing with washed erythrocytes. FFA levels of packed cells decreased after mixing with oxygenated PMS plasma. Polyoxyethylene-propylene, a lipoprotein stabilizing agent, was added to red cells before and after exposure to oxygenated PMS plasma. It prevented the hemolytic action of oxygenated PMS plasma and halted hemolysis when subsequently added to the plasma PMS red cell mixture. PMS added to lung extracts in a Wilhelmy surface balance decreased surface area hysteresis loops by 50%.

Prolonged Survival of Mice with Established Friend Virus Leukemia after Sendai Virus Inoculation. E. Frederick Wheelock,* Cleveland, Ohio.

The virus etiology of certain murine leukemias makes it reasonable to consider treatment of these diseases in terms of virus inhibition, perhaps through interference by nontumor viruses. ¶ Sendai virus (parainfluenza 1) inoculated into DBA/2 mice as early as 6 weeks before Friend leukemia virus markedly inhibited both Friend virus replication and the splenomegalic response to Friend virus. Mice inoculated with Sendai virus before Friend virus also had prolonged survival times, and 10% did not develop evidence of leukemia, whereas 100% of Friend virus control mice died of leukemia. Deaths in Friend virus control mice began at 26 days after inoculation, and all mice were dead at 66 days. ¶ When mice with established Friend virus were inoculated with Sendai virus 30 days after Friend virus, their survival time was prolonged 14 to 21 days. Sendai virus produced a severe but nonlethal infection and induced four to eight times as much interferon in leukemic mice as in normal mice. Statolon, an anionic polysaccharide that induces large amounts of interferon in leukemic mice, prolonged survival of mice 14 to 21 days when injected 30 days after Friend virus. Leukemic mice whose survival was prolonged by Sendai virus inoculated 30 days after Friend virus were further protected by Statolon injected 21 days after Sendai virus. Livers in leukemic mice enlarge progressively until death, but injections of Sendai virus or Statolon arrested liver enlargement for 2 to 3 weeks, coinciding with the diminution in death rate. ¶ The protective effects of inoculation of a nontumor virus into mice with established virus-induced leukemia may correspond to those effects observed in a previously reported study of a patient with acute myelogenous leukemia. In that study inoculations of high titered viruses were repeatedly followed by transient clinical and hematologic remissions resulting in an unexpectedly prolonged survival.

The Influence of Nonionic Diffusion on Renal Concentration and Excretion of Antibiotics. A. Whelton, W. G. Walker,* and G. G. Carter, Baltimore, Md., and Washington, D. C.

The importance of nonionic diffusion, judged by pH and volume dependent clearance, in the renal excretion of many weak acids and bases stimulated the present study of its role in renal transport of antibiotics that are either weak acids or bases. Dogs were made acidotic, and antibiotic clearance was followed as urine pH was increased from 4.8 to 8.0 by $NaHCO_3$ administration. Antibiotic concentrations were measured by a microbiological assay so that values reflect antibacterial activity. In 38 clearance periods in 10 dogs, cephalothin (pKa 2.25) clearance was correlated with pH ($r = +0.41$, $p < 0.01$). Penicillin (pKa 2.8) (21 clearances, 10 dogs) exhibited a similar correlation ($r = +0.61$, $p < 0.005$). For both antibiotics, clearance values exceeded glomerular filtration rate at pH above 7.0. Penicillin exhibited a fivefold variation in clearance over the pH range 4.8 to 8.0, and cephalothin clearance varied fourfold over this range. Oxytetracyline, a more complex molecule (pKa_1 3.27, pKa_2 7.32, pKa_3 9.11), yielded clearance values that were correlated negatively with urine pH ($r = -0.56$, $p < 0.01$), indicating that the higher pKa was the signifi-

cant one under these circumstances. ¶ Antibiotic concentrations in renal tissue water were influenced by urine flow and probably by pH. Cephalothin increased from an average of 25.9 μg per g tissue water in the cortex to a high of 71 μg per g in the papilla during hydropenia but decreased from 33 μg per g of cortical water to 19 μg per g of papillary water during water diuresis. Papilla/plasma antibiotic ratio decreased from 6.1 in hydropenia to 1.3 in hydrated animals. Data were similar for penicillin but reversed for oxytetracycline with papillary concentrations always less than cortical values. In acidosis there was no difference between cortical and papillary concentrations of cephalothin, but in alkalosis papillary concentrations were nearly four times greater than cortical concentrations. These studies indicate that urinary pH and state of hydration are important determinants of plasma and renal concentrations of antibiotics and may be important considerations in planning managements of patients with pyelonephritis.

L-Glyceric Aciduria and Glycolic Aciduria: Two Genetic Variants of Primary Hyperoxaluria. HIBBARD E. WILLIAMS, JURGEN KOCH, AND LLOYD H. SMITH, JR.,* San Francisco, Calif.

Excessive urinary excretion of oxalate may result from increased ingestion of oxalate or one of its precursors (glyoxylate, glycolate, ethylene glycol), from pyridoxine deficiency (a cofactor in the transamination of glyoxylate), or from a genetic block in the metabolism of glyoxylate. The genetic disorder, associated with early onset of nephrolithiasis, nephrocalcinosis, and metastatic calcium oxalate deposits, has been termed primary hyperoxyluria. Two distinct genetic diseases resulting in excessive urinary oxalate have been delineated in patients with primary hyperoxaluria. Fifteen patients have been found with glycolic aciduria and hyperoxaluria. They exhibit a marked reduction in the metabolism of glyoxylate-^{14}C or glycolate-^{14}C to CO_2 *in vivo* and increased conversion of glyoxylate-^{14}C into urinary glycolate as previously reported from this laboratory. The enzyme defect has been found in biopsies of liver, spleen, and kidney to be that of soluble 2-oxo-glutarate: glyoxylate carboligase. The mitochondrial carboligase, presumably an isozyme of the soluble enzyme, was present in normal activity. Transamination of glyoxylate to glycine was normal. ¶ Three patients (2 siblings) with primary hyperoxaluria excreted normal amounts of glycolic acid but large amounts (178 to 645 mg per 24 hours) of another organic acid, absent from normal urine. By chromatography, synthesis, derivative analysis, and substrate specificity (lactic dehydrogenase, D-glyceric dehydrogenase) the organic acid was found to be L-glyceric acid, a compound not previously demonstrated in biological material. Hydroxypyruvic acid-^{14}C was a precursor of urinary L-glyceric acid, but not of oxalate. Glyoxylate-^{14}C *in vivo* was incorporated excessively into urinary oxalate, but showed reduced conversion to glycolate, and no conversion to L-glyceric acid. Current studies are consistent with a defect in D-glyceric dehydrogenase (glyoxylate reductase), an enzyme in both hydroxypyruvate and glyoxylate metabolism.

Cell Proliferation in Intestinalized Gastric Mucosa. SIDNEY J. WINAWER AND MARTIN LIPKIN, New York, N. Y. (introduced by Thomas P. Almy†).

Proliferation of gastric and intestinal cells in intestinalized atrophic gastric mucosa was studied in two subjects after injection of tritiated thymidine (TdR-^{3}H) and preparation of microradioautographs from mucosal biopsies. Both subjects had carcinomas of the stomach. TdR-^{3}H was incorporated into DNA of gastric mucous cells and intestinal principal, goblet, and Paneth cells. The appearance of TdR-^{3}H-labeled mitoses indicated rapid proliferation of each of the cell types. Labeling and mitotic indexes of gastric cells were comparable to the indexes observed in normal stomachs. Indexes of intestinal principal and goblet cells approximated those seen in crypt cell populations of normal jejunum, whereas Paneth cell indexes were higher. Per cent labeled mitosis curves of the gastric and intestinal cells revealed proliferative cell cycle phases comparable to normal gastric and intestinal cells: T_{G_2} 1 to 8 hours, T_S 15 to 18 hours, $T_{G_2} + 0.5$ M 4 hours, and T_C 30 hours. An abnormal spatial distribution of DNA synthesizing cells was also observed in some areas of the atrophic intestinalized mucosa, characterized by an extension of the immature cells to the surface. In contrast, normal gastric mucosa had a surface zone of mature nonproliferative cells. In addition, the reduction of TdR-^{3}H grains caused by cell division, normally seen in the leading edge of migrating cells of jejunum, was not observed in migrating cells of the atrophic stomach. ¶ Intestinalized atrophic gastric mucosa therefore contained independent populations of rapidly proliferating gastric and intestinal cells, with normal cell cycle phases, each cell type capable of self-renewal; migration to the surface of epithelial cells that appear to have an increased complement of DNA; and DNA synthesizing epithelial cells on the surface of the gastric mucosa, an abnormality also seen in gastrointestinal carcinomas and the precancerous tissues of familial polyposis and villous papillomas.

The Genetic Definition of a Newly Described Disease due to Leukocyte Malfunction. DOROTHY WINDHORST, PAUL QUIE, BEULAH HOLMES, ARTHUR R. PAGE, AND ROBERT A. GOOD,* Minneapolis, Minn.

In 1957, we defined a new disease characterized by progressive and fatal granulomatosus in boys, and last year at this meeting we first reported that this disease is associated with a failure of normal leukocyte function. Since that time, we have determined that the defective leukocytes, though capable of adequate uptake of particulate matter, do not increase their utilization of oxygen in a normal manner, nor do they properly utilize glucose labeled in the first or third and fourth positions. In addition, they do not oxidize formate as do normal cells. ¶ From the earliest studies, the disease has appeared to be familial and possibly transmitted as a sex-linked recessive

trait. We have now studied, by a battery of analyses, 11 of 14 affected boys in 8 families as well as the primary family members. These include 2 sets of maternal cousins and 2 affected boys who had the same mother but different fathers. We find that the *in vitro* leukocyte bactericidal test will demonstrate a defect of function less severe than that of the boys in the mothers, maternal grandmothers, and 6 of 13 female siblings. The fathers, normal brothers, and remaining female siblings are consistently normal in this test. In addition, the females who are abnormal in the function test are, as a group, intermediate between controls and the affected boys in the metabolic tests. Thus the carrier state of fatal granulomatosus can be identified. More specifically, a combined phagocytosis-histochemical test developed by Baehner and Nathan permits the direct demonstration of 2 populations of cells in the females intermediate in the functional test. The significance of this newly defined sex-linked disease for evaluation of certain aspects of the Lyon hypothesis of random inactivation of the female X chromosome will be discussed.

Cellular Sites of Immunosuppression by Cortisol and 6-Mercaptopurine. ALAN WINKELSTEIN AND CHARLES G. CRADDOCK,* Los Angeles, Calif.

The effects of hydrocortisone (cortisol) and 6-mercaptopurine (6-MP) on lymphoid histology, proliferative activity, and production of hemolytic plaque-forming cells (PFU) were serially assessed in rats after antigenic stimulation with sheep red cells (SRBC). Proliferative activity of components of the lymphoreticular system was estimated by H3T radioautography and quantitative radiochemical incorporation. The immunosuppressive effects were correlated with alterations occurring in spleens of animals challenged with SRBC. Each agent induced distinctive alterations. Cortisol (5 to 10 mg per 100 g) caused lympholysis with rapid reduction in lymphoid mass; proliferation by residual cells in lymph nodes and spleen was not suppressed. Restoration of peripheral blood lymphocytes and lymph node and spleen cellularity occurred rapidly after drug withdrawal. Sites of short-lived lymphocyte production (thymus, bone marrow, germinal centers) were most seriously depleted by cortisol and showed delayed restoration. Cortisol was effective in suppressing hemolysin formation when administered before SRBC, suggesting that a major effect was inhibition of antigen phagocytosis and processing. The dramatic destruction of short-lived lymphocytes suggested that they may be functionally involved in antigen processing. ¶ In contrast to cortisol, 6-MP (7.5 mg per 100 g) is not overtly toxic to small lymphocytes. However, cellular proliferation is reduced by 60 to 70% within 24 hours after a single dose. Cessation of 6-MP is followed by prompt resumption of proliferation. Continued treatment results in a gradual decline in peripheral lymphatic tissue mass and a more marked reduction in short-lived lymphocytes. These changes reflect a block in proliferation that prevents normal compensation for cell attrition. 6-MP inhibits logarithmic increase in PFU by arresting cell replication, hence was effective in immunosuppression after antigen stimulation. Pretreatment with 6-MP did not suppress formation of PFU, suggesting that cell proliferation is not essential for antigen processing. Neither agent inhibited antibody production once initiated. The major effect of 6-MP appears to be inhibition of immunoblast proliferation.

A New Test for Primary Hypertension: The Apparent Norepinephrine Secretion Rate in Normotensive and Hypertensive Man. ROBERT L. WOLF, MILTON MENDLOWITZ,† AND JULIA ROBOZ, New York, N. Y.

To detect a possible alteration in norepinephrine (NE) metabolism in patients with primary benign hypertension, we studied *in vivo* apparent norepinephrine secretion rates (NESR) in 12 normotensive subjects and 9 untreated subjects with primary benign hypertension. The technique depends on the intravenous administration of a dose of DL-norepinephrine-7-^{3}H (NE-^{3}H) and measurement of the *in vivo* dilution of the tracer by endogenous NE through isolation of the specific metabolite, normetanephrine (NM) from the urine. NM, the urinary metabolite of NE, was separately assayed by high voltage paper electrophoresis. The apparent NESR (milligrams per 24 hours) is equal to the amount of radioactivity (NE-^{3}H) injected divided by the specific activity of the urinary NM in the 24-hour urine. ¶ Apparent NESR values (milligrams per 24 hours) for the mean, standard deviation, and range in the subjects on unselected diets were as follows: normotensive subjects, 42.5, 14.6, 26.3 to 77.7; and untreated primary benign hypertensive subjects, 14.0, 7.3, 3.3 to 26.8. One patient with a pheochromocytoma had an apparent NESR of 234.0 mg per 24 hours before removal of the tumor and 36.2 mg per 24 hours after removal of the tumor. One patient with chronic glomerulonephritis and hypertension had an apparent NESR of 26.1 mg per 24 hours. ¶ The t statistic for testing the equality of the apparent NESR means of the hypertensive and normotensive groups is 5.34 with 19 degrees of freedom. Since the 0.05 percentage point (one tailed) of the t distribution is 3.89, we have significant evidence that the mean apparent NESR of normotensive patients is greater than that of hypertensive subjects in the population from which this sample was drawn. The apparent NESR appears to be a valid test for identifying patients with primary hypertension.

The Effect of Neuraminidase on the Fate of Transfused Lymphocytes. JUDITH J. WOODRUFF AND BERTRAM M. GESNER, New York, N. Y. (introduced by Angelo Taranta*).

Small lymphocytes transfused intravenously normally concentrate in lymphoid tissues and subsequently recirculate to the lymph. In the present study it was found that rat thoracic duct lymphocytes incubated with a neuraminidase preparation (*Vibrio cholerae*) before intravenous transfusion into syngeneic or allogeneic recipients do not circulate normally. At 30 minutes after transfusion of

^{51}Cr-labeled, neuraminidase-treated lymphocytes, less radioactivity was found in recipients' lymph nodes and spleens, and more radioactivity in the livers, than in recipients of equivalent samples of untreated lymphocytes. A maximal effect was observed incubating 50×10^6 lymphocytes with 12.5 U neuraminidase for 15 minutes at 37° C. The effect of the enzyme was destroyed by heating it at 65° C for 10 minutes before use and was inhibited by addition of neutralized sialic acid to the *in vitro* reaction mixture. The enzyme had no effect on the distribution of radioactivity when transfused separately from untreated lymphocytes. Before transfusion, the treated lymphocytes were unagglutinated, showed no significant loss of radioactivity, and were "viable" as determined by studies of uptake of trypan blue and motility. Recipients of neuraminidase-treated cells followed at later intervals after transfusion showed a fall of radioactivity in the liver accompanied by a rise of counts in the lymph nodes, and a sequentially associated increase in recovery of labeled lymphocytes from the thoracic duct. These and other findings suggested that at least some of the treated cells, initially trapped in the liver, regained their ability to "home" to lymphoid tissues and recirculate to the lymph. The findings suggest that alteration of lymphocytes by neuraminidase profoundly affects their fate in the body and that the sialic acid constituents of the lymphocyte surface play an important role in determining the circulation of these cells.

Impaired Secretion of Epinephrine in Response to Hypoglycemia in Hypophysectomized Dogs. RICHARD J. WURTMAN, ALFRED CASPER, JULIUS AXELROD, AND FREDERIC BARTTER,† Cambridge, Mass., and Bethesda, Md. (introduced by Seymour S. Kety†).

The synthesis of epinephrine in the mammalian adrenal medulla depends upon the *N*-methylation of norepinephrine, a rate-limiting step catalyzed by the enzyme phenylethanolamine-*N*-methyl transferase (PNMT). The activity of PNMT declines after hypophysectomy and can be restored by very large doses of carbohydrate-active steroids; it can also be restored by small doses of ACTH, presumably because ACTH delivers high concentrations of steroids directly into the adrenal portal system. ¶ Experiments were done to determine whether the secretion of epinephrine in response to insulin hypoglycemia is also impaired after hypophysectomy. Mongrel dogs were hypophysectomized and maintained for 5 months on cortisone (12.5 mg per day). One adrenal vein was then cannulated, and blood was collected before and at 10-minute intervals after the injection of insulin (0.1 U per kg intravenously). Blood was analyzed for glucose, epinephrine, and norepinephrine. The adrenal was then removed and assayed for epinephrine, norepinephrine, and PNMT. ¶ Among control animals (pair matched for per cent decline in blood glucose), basal epinephrine secretion averaged 7.5 ± 0.4 (SEM) ng per gland per kg per minute and rose sevenfold in response to hypoglycemia; $83 \pm 3\%$ of the catecholamine in the adrenal blood was epinephrine. Among hypophysectomized animals, basal epinephrine secretion averaged 2.8 ± 1.2 ng per gland per kg per minute and rose three- to fourfold with hypoglycemia; $55 \pm 5\%$ of the catecholamine was epinephrine. Control adrenals contained 121 ± 12 U of PNMT activity, 530 ± 116 μg of epinephrine, and 174 ± 16 μg of norepinephrine; corresponding values for glands from hypophysectomized animals were 26 ± 4 U PNMT, 170 ± 39 μg epinephrine, and 246 ± 30 μg norepinephrine ($p < 0.02$ for epinephrine). ¶ These data indicate that there is an impairment in the secretion of epinephrine, as well as in its synthesis, in hypopituitarism. This impairment, which is not corrected by physiologic doses of carbohydrate-active steroids, may contribute to the insulin sensitivity of pituitary insufficiency.

Effect of Thyrotropin and Inorganic Iodide on Thyroid Gland Output. JAMES WYNN,* Durham, N. C.

The influence of continuous administration of thyrotropin and inorganic iodide on the blood levels of thyroxine and triiodothyronine has been studied. Two normal volunteers received 100 μc ^{125}I to label their thyroid glands. Five days later daily blood collections were begun. For 3 days control blood samples were collected. For the next 6 days 5 U TSH was given daily intramuscularly. During the last 3 days 10 drops of SSKI was given daily with the TSH. Serum was prepared and subjected to column chromatography. The proportion of radioactivity distributed in thyroxine and triiodothyronine was estimated. In both subjects the proportion of triiodothyronine in the blood increased for the first 3 days of TSH until it accounted for as much as 30% of the radioactivity in the blood. After iodide was started the proportion of labeled triiodothyronine in the blood decreased daily until after 3 days of iodide there was virtually no identifiable triiodothyronine in the blood. Continuous TSH stimulation causes increased triiodothyronine output by the thyroid gland. Treatment with iodide caused an abrupt cessation of triiodothyronine output. It seems likely that this effect of iodide may account for the rapid therapeutic effect of iodide in hyperthyroidism.

Pituitary-Thyroid Feedback Relationship in Rats Treated with Thyroxine and Antithyroid Drugs. TAKASHI YAMADA, Boston, Mass. (introduced by E. B. Astwood†).

Rats fed methimazole (0.05%) were injected with graded doses of thyroxine (T_4) (0.5 to 2.5 μg) daily for 2 weeks. In spite of high concentrations of PBI in the serum with doses of 1.0 and 1.5 μg of T_4, goiter was still observed. Goiter was prevented only when the PBI was increased to 1.8 times the control. The findings were not simply explained by the classic feedback hypothesis. Similar effects were found with sulfaguanidine. Thyroidectomized, T_4-maintained rats were used to compare the influence of propylthiouracil, sulfaguanidine, methimazole, and methylthiouracil upon deiodination of T_4. PBI values were increased in all drug-treated animals, suggesting that deiodination, thought to parallel effectiveness of T_4, was inhibited by all four compounds. The

data suggest that methimazole and sulfaguanidine distort pituitary thyroid relationships by reducing deiodination of T_4. Alternatively, goiter may have been prevented at normal concentrations of PBI had a mixture of T_3 and T_4 in proper proportions been given.

Evaluation of Remission Status of Acute Leukemia with *In Vitro* Uptake of Tritiated Thymidine by Peripheral Blood Leukocytes. THEODORE S. ZIMMERMAN, HERMAN A. GODWIN, AND SEYMOUR PERRY, Bethesda, Md. (introduced by Warren E. C. Wacker*).

Peripheral blood leukocytes from patients with acute leukemia in bone marrow remission (less than 5% blasts and abnormal forms) were isolated from peripheral blood by dextran sedimentation and incubated with tritiated thymidine (TdR-^{3}H). After washing the DNA-containing precipitate with cold perchloric acid, we determined its radioactivity in a liquid scintillation spectrometer. Leukocyte specific radioactivity (counts per minute per 10^7 cells) in nine patients was significantly elevated above that found in normal controls. Three of these patients developed frank bone marrow relapse within 1 month. Decrease of specific activity to normal values after additional chemotherapy was observed in two patients. One subsequently died of intercurrent disease while still in bone marrow remission. The other remains in remission 3 months later. The remaining four patients are still under observation. In addition, seven patients with remissions beyond 1 year showed normal values. Normal values were also seen in two patients in marrow relapse, but both were receiving antileukemic therapy when tested. One "normal" control with a tenfold elevation of specific activity had circulating atypical lymphocytes and a positive heterophile test of 1:448. Although false negative as well as false positive results will occasionally occur, the measurement of *in vitro* uptake of TdR-^{3}H by peripheral blood leukocytes may prove useful in evaluating the completeness of remission in acute leukemia.

Hyperabsorption of Calcium in Patients with Nephrolithiasis as Measured by a New Isotopic Technique. ELIAS ZISMAN, CHARLES Y. C. PAK, AND FREDERIC C. BARTTER,† Bethesda, Md.

Increased intestinal absorption of calcium was shown in patients with recurrent nephrolithiasis (calcium stones) without bone disease by means of a new radioisotopic technique. This technique consists of hourly measurement of forearm radioactivity for 4 hours in a large-sample scintillation counter after oral administration of 4 to 6 μc of Cl_2-^{47}Ca. Since this measurement includes chiefly accretion by bone, which represents more than 90% of absorbed radioactivity, counting efficiency is ten times that obtained from counting blood alone; it is not appreciably affected by variations in blood and urinary radioactivity. Error from variable counting geometry of the forearm, as opposed to absorption, was small, since the 1- to 4-hour uptake of ^{47}Ca by the forearm after intravenous injection was essentially the same in all groups. ¶ At 1 hour, mean forearm radioactivity expressed as per cent of oral dose was 1.00 ± 0.06 (SEM) in 16 patients with nephrolithiasis (group I) versus 0.63 ± 0.06 in 10 normal subjects (group II) ($p < 0.001$). At 4 hours, radioactivity was 1.59 ± 0.04 for group I versus 1.24 ± 0.09 for group II ($p < 0.005$). Seven of the patients with nephrolithiasis had idiopathic hypercalciuria (group IA); the others had normal urinary calcium (group IB). Whereas both group IA and group IB had significantly higher absorption of calcium than the control subjects, values in group IA (1.23 ± 0.04 at 1 hour and 1.67 ± 0.04 at 4 hours) were higher than those in group IB (0.82 ± 0.05 at 1 hour and 1.53 ± 0.05 at 4 hours) ($p < 0.001$; $p < 0.05$). With calcium loading the maximal amount of stable calcium absorbed was much higher in group I than in group II, substantiating the above findings. Customary methods for determining calcium absorption from blood radioactivity did not distinguish group I from group II. ¶ These findings suggest that *1*) this use of bone trapping, which "integrates" blood radioactivity over time, provides a simple, sensitive measure of intestinal calcium absorption; and *2*) patients with recurrent nephrolithiasis have hyperabsorption of calcium.

Abnormal Estrogen Metabolism in Cirrhosis. BARNETT ZUMOFF, JACK FISHMAN, T. F. GALLAGHER, AND LEON HELLMAN,* New York, N. Y.

An abnormality of estrogen metabolism has been found in cirrhosis after administration of intravenous tracers of estradiol-^{3}H to 6 patients and 23 healthy controls. The major abnormalities observed involved estrogen metabolites other than the 3 "classic" ones, i.e., estrone (E1), estradiol (E2), and estriol (E3). Urinary recovery of radioactivity was regularly elevated in the patients, to an average of 70% of the dose compared to 51% in normals. This is considered to reflect the component of intrahepatic cholestasis in cirrhosis, since patients with extrahepatic biliary obstruction show similarly elevated recovery. The per cent dose recovered as urinary glucosiduronates (42%) was normal in cirrhotics in contrast to impaired glucuronidation of cortisol metabolites in this disease. E1 and E2 were present in normal amounts, and E3 was slightly elevated to 21% of the extract compared to 14% in controls. There were strikingly decreased excretion of 2-hydroxyestrone (3% compared to normal 20%) and 2-methoxyestrone (2% compared to 5%) and correspondingly increased excretion of 16α-hydroxyestrone from the normal value of 4% to 12%. Thus cirrhosis, too, is characterized by the reciprocal relationship between decreased 2-hydroxylation and increased 16-hydroxylation we have described in hypothyroidism and male breast cancer. In the latter diseases, however, the elevated 16-hydroxy metabolite was estriol. The data for cirrhosis indicate that, in addition to depressed 2-hydroxy metabolite formation, there is a metabolic block between 16α-hydroxyestrone and estriol, a finding so far unique to this disease. Estradiol loading studies in one cirrhotic patient confirmed this conclusion. The finding of abnormal peripheral metabolism of estrogen in cirrhosis provides a new approach to the origin of the hyperestrogenic syndrome in this disease.