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ABSTRACTS

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ABSTRACTS

Pressor Responses of Renal Small Vessels during Vasomotor Stimulation. FRANÇOIS M. ABBOUD,* DENNIS R. BALLARD, NABIL W. MOUKHEIBIR, AND JOHN W. ECKSTEIN,* Iowa City, Iowa.

The correlation between arterial blood pressure and plasma renin has not been consistent. In the present experiments the possibility that changes in renal small artery pressure occur without similar changes in systemic arterial pressure was tested in 19 anesthetized dogs. ¶ Stimulation of renal nerves at 3, 6, and 12 cycles per second for 20 seconds caused reductions in renal blood flow averaging 71, 83, and 120 ml per minute and increases in small artery pressure averaging 7, 17, and 22 mm Hg, respectively. Aortic pressure at the level of the renal arteries did not change significantly and small vein pressure decreased. Injections of norepinephrine (2, 4, and 8 μg) into the aorta caused reductions in renal flow averaging 31, 56, and 76 ml per minute, respectively; there was a transient rise followed by a more sustained decrease in small artery pressure; aortic pressure increased slightly and small vein pressure decreased. Bilateral carotid occlusion did not change renal flow or small vein pressure; both aortic and small artery pressures increased by averages of 47 and 44 mm Hg, respectively. ¶ Acute reductions in renal blood flow by compression of the renal artery caused significant reductions in both small artery and small vein pressures. When the kidney was perfused at constant flow, the increases in perfusion pressure and small artery pressure were similar during the interventions. ¶ Perfusion of a portion of the venous return from the kidney into the hind paw indicated that a vasodilator substance is released from the kidney during the ischemia associated with nerve stimulation. A vasoconstrictor substance was released during nerve stimulation at constant renal flow. ¶ The results indicate that changes in systemic arterial pressure do not always reflect the magnitude or direction of changes in small renal artery pressure.

Identification, Quantification, and Regulation of β-MSH in Man. KAORU ABE, WENDELL E. NICHOLSON, DONALD P. ISLAND, DAVID N. ORTH, AND GRANT W. LIDDLE,* Nashville, Tenn.

A radioimmunoassay for β-melanocyte-stimulating hormone (β-MSH) was developed that would distinguish β-MSH from α-MSH, ACTH, and several synthetic analogues of MSH and ACTH. By use of extracts of human plasma and tissues, dose-response curves parallel to that for standard synthetic human β-MSH could be constructed. One picogram (pg) of β-MSH could be detected, and quantitative assays of plasma β-MSH could be performed when the concentrations were 20 pg per ml or greater. ¶ Normal human plasma contained 20 to 90 pg β-MSH per ml. Two human pituitaries contained approximately 350,000 pg β-MSH per mg of wet tissue. Plasma of 11 hyperpigmented patients (2 Addison's disease, 2 ectopic ACTH syndrome, and 7 Cushing's disease postadrenalecetomy) contained 500 to 5,000 pg β-MSH per ml. In these patients with chronic elevation of β-MSH, the degree of hyperpigmentation correlated well with the concentration of β-MSH in the plasma. In all cases elevated plasma β-MSH was accompanied by elevated plasma ACTH (rat adrenal corticosterone assay). Tumors from 11 patients with the ectopic ACTH syndrome contained 3 to 1,600 pg β-MSH per mg of wet tissue. Here, too, ACTH concentrations tended to parallel β-MSH. In all of these circumstances, bioassays for MSH were also performed using the *in vitro* frog skin method, and it was found that most of the biologic MSH activity of the plasma and tissues could be accounted for by β-MSH. ¶ In response to dexamethasone, the plasma β-MSH of 9 normal subjects was suppressed in all cases and became undetectable in 5. In response to metyrapone, plasma β-MSH of 7 normal subjects increased from 36 ± 8 pg per ml (mean ± SE) to 65 ± 9 pg per ml ($p < 0.025$). ¶ Conclusions: *1*) β-MSH has invariably been detectable in plasma or tissues containing detectable quantities of ACTH. *2*) β-MSH is the principal pigmentary hormone in man. *3*) Like ACTH, β-MSH is regulated, in part, by corticosteroids.

Selective Stimulation of Amino Acid Transport in Embryonic Bone by Thyroid Hormones. LUCILE F. ADAMSON AND SIDNEY H. INGBAR,* Boston, Mass.

Since thyroid hormones are necessary for the normal growth and development of osseous tissue, this seemed a potentially sensitive end-organ for seeking a fundamental hormonal action. Therefore, the transport and incorporation of 14 neutral amino acids from an artificial medium by the cartilaginous anlage of the pelvic bone of the 10-day chick embryo were studied, and the effects of thyroid hormones *in vitro* evaluated. Initial studies demonstrated mechanisms for both active inward transport and active efflux of free amino acids. Studies of competitive transport indicated the presence of at least two transport sites, corresponding well to the alanine (A)- and leucine (L)-preferring sites of Christensen, and amino acid accumulation conformed to typical Michaelis-Menton kinetics. In general, thyroid hormones stimulated accumulation of amino acids preferring the L-site, but not those preferring the A-site. Stimulation of free amino acid accumulation did not result from decreased amino acid incorporation, from inhibition of free

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¶ New paragraph.

amino acid efflux, or from change in extracellular or cellular water content. No lag period for stimulation of amino acid transport was evident, and the effect was not prevented by actinomycin-D or puromycin. A good correspondence between rank order of potency *in vivo* and those effects observed here was evident. For example, isopropyl-diiodothyronine and L-triiodothyronine were more active than L-thyroxine. All were consistently active at 10^{-6} mole per L and occasionally at 10^{-7} mole per L. D-Thyroxine was relatively inactive. ¶ Conclusions: An effect of thyroid hormones in embryo bone *in vitro,* corresponding well to their physiological effect *in vivo,* is described. Thyroid hormone induces an almost immediate and specific stimulation of the accumulation of amino acids transported at the leucine-preferring site. The effect is not dependent upon new protein synthesis, but may represent a direct interaction between the hormone and the specific site carrier.

Phase Equilibrium Technique and Its Application to Human Cholesterol Gallstone Formation. WILLIAM H. ADMIRAND AND DONALD M. SMALL, Boston, Mass. (introduced by Franz J. Ingelfinger†).

The major constituents of human bile are bile salt, lecithin, and cholesterol. Although cholesterol is insoluble in water, in normal bile it is made soluble by being carried in mixed micelles of bile salt and lecithin. However, during the formation of cholesterol gallstones part of the cholesterol becomes insoluble. ¶ Under the assumption that cholesterol solubilization in bile is determined by the relative concentrations of its major constituents, a model system was constructed. Mixtures representing all possible combinations of bile salt, lecithin, and cholesterol were hydrated, allowed to equilibrate, and examined to determine the presence or absence of insoluble cholesterol. Each mixture was plotted, in terms of concentration of bile salt, lecithin, and cholesterol, as a single point on triangular coordinates. The triangular phase diagram thus derived formed two zones: a micellar zone consisting of mixtures which were liquid and a second zone which consisted of mixtures that contained not only liquid but insoluble cholesterol. ¶ The triangular phase diagram was then used to study cholesterol solubilization in human bile. The composition of bile samples from 65 patients with cholesterol gallstones was compared with bile from 25 normal subjects by plotting each sample, in terms of relative concentrations of bile salt, lecithin, and cholesterol, on the triangular diagram. The plotted values from all normal biles fell within the micellar zone, whereas those from bile of patients with cholesterol gallstones fell outside the micellar zone. ¶ We conclude that the physical state of cholesterol in bile is determined by the relative concentrations of bile salt, lecithin, and cholesterol and can be accurately predicted from a triangular phase diagram. Through the application of phase equilibrium technique, complete separation of normal bile from bile of patients with cholesterol gallstones has been achieved.

Nephron Function during Hypotension: Persistence of Glomerular Filtration in Anuria. EDWARD A. ALEXANDER AND NORMAN G. LEVINSKY,* Boston, Mass.

Indirect studies have suggested that glomerular filtration (GF) may continue even when anuria occurs in hypotension. To investigate GF during hypotension directly, we injected lissamine green, a dye excreted by GF, into the aorta during graded reductions of renal blood pressure (BP) by aortic clamping. Continuing GF could be recognized microscopically by the appearance of dye and movement of filtrate determined by disappearance of dye from surface tubules. Dogs dehydrated overnight became anuric when BP was reduced to 50 to 65 mm mercury. Despite anuria, all cortical nephrons continued to function in one-half of dogs, judged by rapid entrance and complete disappearance of dye; the transit time (TT) was prolonged by 50 to 100%. In the remaining dogs, there was heterogeneity of function among nephrons; GF continued in 10 to 25% of nephrons, but no dye entered the remaining tubules. Strikingly different results were obtained in saline-loaded dogs. At BP 50 to 60, urine flow was always present; GF was reduced 25 to 85% by clearance measurements. Dye entered all tubules and TT increased only 10 to 50%. Anuria did not occur until BP reached 30 to 40. In half of the dogs, GF continued in all visible nephrons even at BP 30 to 40, although TT increased markedly. In the remaining dogs, 10 to 50% of nephrons still filled and emptied. Thus, although all saline-loaded dogs were anuric at BP 30 to 40, GF continued in at least some tubules in every dog. These observations demonstrate directly that GF may continue during anuria, indicating that the filtrate is completely reabsorbed distally. Prevention of anuria during moderate hypotension by saline infusion is due both to decreased reabsorption of filtrate and to maintenance of GF in a greater proportion of nephrons in saline-loaded than in dehydrated dogs.

The Absorption and Metabolism of Vitamin D in Chronic Renal Failure. LOUIS V. AVIOLI AND EDUARDO SLATOPOLSKY, St. Louis, Mo. (introduced by Stanford Wessler†).

The resistance to vitamin D observed in patients with chronic renal failure prompted an evaluation of vitamin D metabolism in this disorder. After an overnight fast, 10 μc of tritiated vitamin D (D_3-^3H) was administered orally to four normal adults and four patients with chronic renal disease (CRD), characterized by hyperphosphatemia, hyperphosphatasia, and inulin clearances ranging from 1.3 to 11.4 ml per minute. Total radioactivity in plasma was determined by liquid scintillation counting of combusted samples. D_3-^3H and lipid-soluble metabolites thereof were separated by thin layer and gradient elution chromatography and plasma chylomicrons fractionated by ultracentrifugation. D_3-^3H and its circulating metabolites were bioassayed according to general USP procedures. Plasma D_3-^3H levels in normal subjects rose gradually, reaching peak levels at 8 hours ($3.37 \pm 0.38\%$ injected

dose), declining exponentially thereafter with a half-time of 27 hours (range 26 to 29). Eight hours after the oral dose, chylomicron-D_3-^3H in these subjects ranged from 6.5 to 13.9% total plasma radioactivity. At 24 hours 70% (range 68 to 74) of lipid-soluble plasma radioactivity represented biologically active vitamin D. By contrast 8-hour peak D_3-^3H values in CRD subjects averaged 1.39 $\pm$ 0.40% injected dose, declining thereafter with a rapid half-time of 14 hours (range 7 to 20). Eight-hour chylomicron-D_3-^3H values were also decreased in CRD ranging from 1.8 to 3.3% total plasma radioactivity. At 24 hours only 40% (range 17 to 64) of lipid-soluble plasma radioactivity could be identified as biologically active vitamin D_3, and a twofold increase in polar biologically inactive metabolites was noted. These results demonstrate that the metabolism of vitamin D is abnormal in chronic renal failure as manifested by *1*) decreased gastrointestinal absorption of vitamin D, *2*) an increase in the vitamin D fractional turnover rate, and *3*) increased formation of biologically inactive metabolites. These abnormalities provide the explanation for the known resistance to vitamin D among subjects with prolonged renal insufficiency.

Evidence for Hypercoagulability in Heat Stroke. F. BACHMANN, St. Louis, Mo. (introduced by G. T. Perkoff*).

In July 1966, 87 patients with heat stroke were admitted to St. Louis City Hospital. Fifty-one had temperatures over 106° F; mortality was 40%. Thrombocyte counts were <200,000 in 20 of 27 patients. Eighty-one extensive coagulation profiles were performed on 21 patients. Seventeen of 21 had significantly shortened plasma recalcification times upon admission. Kaolin-activated partial thromboplastin times were normal in 16 of 20 patients, prolonged in 3 of 20, shortened in 1 of 20. Quick time, fibrinogen, Factors II, V, VII, VIII, IX, and X, and plasminogen in general were normal. Compared to normal plasma standards, 15 of 21 patients showed higher Hageman Factor (XII) activity when measured by a method sensitive to activated XII than by one measuring total XII. Serial determinations in 12 patients showed significantly decreased total XII over a 1- to 3-day period in 6, and in one XII remained low throughout the course. Six of these 7 patients died. Increased levels of circulating plasminogen activator were observed in a few cases: 2 of 19 had a positive fibrin plate test; 4 of 19 had shortened euglobulin lysis time. Six of 21, however, had increased thrombin times, and in 7 of 21 fibrin breakdown products were found in the serum, suggesting intense fibrinolysis before examination. Fibrinogen was normal in 16 of 21 and above 400 mg per 100 ml in 5 of 21. Subsequent increases of fibrinogen >25% were observed in 8 of 12 and >50% in 6 of 12. The hyperfibrinogenemia reached average levels of 570 mg per 100 ml with maximal single values between days 3 to 8. ¶ In patients with heat stroke a hypercoagulable state appears to be initiated by excessive activation of Hageman Factor, succeeded by Hageman Factor exhaustion in the majority of the severe cases. The presence of fibrin breakdown products provides evidence for a fibrinolytic state, usually of short duration. ¶ The hypercoagulable state may prepare the terrain for later thromboembolic episodes. Thus, of 5 autopsied patients dying more than 9 days after admission, all showed massive pulmonary embolism and/or myocardial infarction.

Heavy Chain: Light Chain Relationships among Erythrocyte Autoantibodies. RICHARD F. BAKEMEIER AND JOHN P. LEDDY, Rochester, N. Y. (introduced by John H. Vaughan*).

In an earlier report RBC autoantibodies from a high proportion of patients with Coombs-positive hemolytic anemia resembled myeloma proteins in having only one detectable light chain type. By analogy with myeloma proteins, antibodies of "monoclonal" origin would also be expected to be homogeneous by other criteria. In the present study, eluted γG autoantibodies have been analyzed with specific antisera for their contents of heavy chain subclasses and light chain types by radioprecipitin inhibition (RPI) as well as by hemagglutination and gel precipitation. The RPI technic is capable of detecting 1 to 2 μg γG per ml. Eluates from patients Fr. and Tr. had exhibited only type K molecules by hemagglutination and gel precipitation. RPI analysis of Fr. eluate, containing 400 μg γG per ml, showed only type K and γ_{2B} (We) molecules (type L, γ_{2A}, γ_{2C} <2%). Electrophoretic mobility was sharply restricted. By RPI, Tr. eluate (500 μg γG per ml) also contained not more than 2% type L molecules; however both γ_{2A} (Ne) (250 μg per ml) and γ_{2B} (185 μg per ml) heavy chain subclasses were clearly present. Furthermore, this eluate was electrophoretically heterogeneous. This demonstrates that an autoantibody population with light chain homogeneity is not necessarily homogeneous in other respects. Re eluate (600 μg γG per ml), exhibiting only type K molecules by hemagglutination, was shown by RPI to contain 5% type L molecules; γ_{2B} heavy chains predominated, but 4 to 6% of γ_{2A} and γ_{2C} (Vi) molecules were also detected. Eluates in which both light chain types were detected contained at least two heavy chain subclasses. γ_{2B}, the major subclass of normal serum γG, usually predominated in the latter group. One autoantibody eluate, containing both light chain and three heavy chain types, exhibited multiple specificities within the Rh system. Autoantibodies with anti-e specificity separated from this eluate showed a marked enrichment of γ_{2B} heavy chains as compared to the parent eluate. Structural homogeneity or heterogeneity of an autoantibody population may be determined by the number of antibody specificities involved. The structural data presented cast doubt on the "monoclonal" origin of the autoantibodies of most patients with Coombs-positive hemolytic anemia.

The Relationship of Pulmonary Artery Pressure to Increased Breath-holding Diffusing Capacity (D_{Lco}) during Muscular Exercise. DUKE H. BAKER AND WALTER J. DALY,* Indianapolis, Ind.

Theoretical considerations and observations when using isolated lungs suggest that D_{Lco} correlates well with pulmonary vascular pressure and is nearly independent of pulmonary blood flow (PBF) per se. This investigation was designed to study the relative importance of pulmonary artery pressure (PAP) and PBF in determining the large increase in D_{Lco} observed during muscular exercise. ¶ In 13 anesthetized dogs, a large caliber right atrial reservoir was inserted via the jugular vein. PAP, 10-second breath-holding D_{Lco}, and PBF were measured during electrically induced muscular exercise. Subsequent measurements were made during similar exercise while PAP increases were prevented by varying the atrial reservoir level. ¶ In the control exercise situation, D_{Lco} increased from 13.6 ± 6.4 at rest to 24.4 ± 9.2 ml per minute × mm Hg; PBF increased from 3.68 ± 1.78 to 7.09 ± 2.82 L per minute; PAP increased from 11 ± 4 to 23 ± 8 mm Hg; and O_2 consumption increased from 130 ± 37 to 528 ± 248 ml per minute. When pulmonary artery pressure was maintained at a resting level during exercise sufficient to produce similar increases in O_2 consumption, D_{Lco} did not rise during the exercise. Analysis of covariance showed that changes in D_{Lco} correlated with changes in PAP ($0.02 > p > 0.01$) but were independent of changes in PBF ($p > 0.50$). ¶ This study shows: *1*) D_{Lco} increases can be prevented during muscular exercise if the PAP increase is prevented. Therefore, potential chemical changes in exercise blood such as decreased pH, increased O_2-CO competition for CO binding on hemoglobin, or a chemical "x" factor affecting the pulmonary capillary bed do not increase D_{Lco} if PAP increases are prevented. *2*) Exercise D_{Lco} does not correlate with increased PBF per se but does correlate well with increased PAP. These findings are consistent with a unifying hypothesis which relates D_{Lco} in exercise to the "pressure-volume" behavior of the effective pulmonary capillary bed.

Modification of Intestinal Motility and Heart Rate by Instrumental Training. ALI BANUAZIZI AND NEAL E. MILLER, New Haven, Conn. (introduced by Howard M. Spiro*).

It has traditionally been held that visceral and glandular responses, being under the control of the autonomic nervous system, can only be modified by classical (Pavlovian) conditioning, whereas skeletal responses of the striped muscles, being controlled by the somatic nervous system, can be learned as rewarded instrumental acts. Recent evidence indicates, however, that at least some autonomic reactions, such as salivation, the electrical potential of the skin, and heart rate, may be modified by instruments (trial-and-error) learning. ¶ A major difficulty in the interpretation of these results is that an apparently learned change in an autonomic response may often be mediated by subtle skeletal responses which can, of course, be brought under instrumental control. One possible way to minimize the effects of skeletal responses appears to be to use curare, which blocks the motor end plates of nerves to skeletal muscles and paralyzes them, without blocking glandular or visceral responses. ¶ In this study two visceral responses, the spontaneous motility of the intestine and changes in the heart rate, were independently modified by instrumental reinforcement. Rats, with all skeletal muscles paralyzed by curare and maintained at a constant rate of artificial respiration, were used. The reward consisted of direct electrical stimulation of the medial forebrain bundle of the lateral hypothalamus ("pleasure centers") via chronically implanted electrodes. The frequency and amplitude of intestinal contractions increased in the group rewarded for contractions, whereas these decreased in another group of rats when the reward was administered during periods of intestinal relaxation. Similarly, animals rewarded for heart rate accelerations or decelerations showed reliable increases or decreases, respectively, in their heart rate compared to their initial base lines. Concomitant measurements of the heart rate, when intestinal motility was being reinforced, and of intestinal motility, when heart rate was being reinforced, revealed no significant correlations between the two responses during training. ¶ The results have possible implications for the neurophysiological basis of autonomically mediated behavior and the origins of psychosomatic symptoms.

The Effect of Inhibitors of Protein Synthesis on the Production of Pyrogen by Granulocytes. HARRY N. BEATY AND ROBERT G. PETERSDORF,* Seattle, Wash.

Most studies related to the production of pyrogen by granulocytes conclude that during incubation in saline, pyrogen is either synthesized *de novo* or released from an inactive precursor. Experiments were undertaken to assess the effect of chloramphenicol, actinomycin D, and puromycin on pyrogen formation by leukocytes. Adult rabbits were given a 2-day course of 600 mg per kg per day of chloramphenicol intramuscularly. Leukocytes were then harvested from inflammatory peritoneal exudates, washed in cold saline, and incubated for 20 hours at 37°. Extracts of these cells were analyzed for protein content and pyrogenicity. When compared with extracts of leukocytes from untreated animals or animals which had received equivalent doses of an antimicrobial not affecting protein synthesis, extracts of cells from chloramphenicol-treated animals exhibited a 45% reduction in pyrogenicity and a decrease in protein content. The role of protein synthesis in the production of pyrogen by granulocytes *in vitro* was then examined by adding chloramphenicol, 25 μg per ml, actinomycin D, 10 μg per ml, or ampicillin, 25 μg per ml, to leukocyte suspensions from pooled peritoneal exudates. The febrile response induced by extracts of cells incubated for 2 hours with inhibitors did not differ from the response seen with control extracts. When extracts were incubated for 20

hours, however, there was a slight reduction in the pyrogenicity of extracts of cells incubated with puromycin. These results indicate that pyrogen formation by granulocytes is dependent on protein synthesis *in vivo,* but that pyrogen production *in vitro* is not affected by inhibitors known to interfere with protein synthesis at three separate loci. This excludes significant *de novo* synthesis as the mechanism of pyrogen production *in vitro,* and therefore, supports the thesis that pyrogen is released from an inactive precursor.

Vitamin B_{12}-dependent Deoxyribonucleotide Synthesis: Regulatory Features and Mechanism of Action of Cobamide Coenzyme. WILLIAM S. BECK,* Boston, Mass.

Previously reported indirect evidence implicating vitamin B_{12} in the synthesis of deoxyribonucleotides was accounted for by the discovery in the vitamin B_{12} auxotroph *Lactobacillus leichmannii* of a repressible ribonucleotide reductase that requires dimethylbenzimidazolylcobamide coenzyme (DBCC), the active form of vitamin B_{12}, and a dithiol reductant [R-$(SH)_2$]. Pure enzyme (mol wt, 110,000) reduces the four major ribonucleoside triphosphates to deoxyribonucleoside triphosphates. Choice of substrate by the enzyme is determined by divalent cations and deoxyribonucleoside triphosphates, each an allosteric effector promoting reduction of a different ribonucleotide (i.e., dATP promotes CTP reduction; dGTP, ATP reduction; dCTP, UTP reduction; and dTTP, GTP reduction) by inducing different states of enzyme conformation or dissociation. Such a network of regulatory effects may control the balance of deoxyribonucleotide synthesis *in vivo.* ¶ Mechanism studies (with R. H. Abeles) have shown that DBCC is an acceptor-donor of hydrogen in ribonucleotide reduction and in the several known DBCC-dependent nonreductive rearrangements (i.e., methylmalonyl CoA isomerase, glutamate isomerase, and dioldehydrase). Reductase catalyzes a transfer of ^3H from H_2O-^3H [or R-$(S^3H)_2$] to DBCC and then to C-2′ of the nucleotide sugar moiety. Experiments with enzymatically and synthetically tritiated DBCC have shown that C-5′ of the DBCC deoxyadenosyl moiety is the locus of hydrogen transfer and that hydrogen transfer—intermolecular (from dithiol to C-2′) in the reductase reaction and intramolecular (from one carbon to another) in the nonreductive rearrangements—is common to DBCC-dependent reactions of both types. ¶ Interestingly, the ribonucleotide reductase of *Escherichia coli,* an organism lacking a vitamin B_{12} requirement, reduces ribonucleoside diphosphates and is cobamide-independent; yet it transfers ^3H from R-$(S^3H)_2$ to C-2′ (Larsson and Reichard). It is unclear, therefore, what agency in this system serves the hydrogen-transferring function performed by DBCC in the lactobacillus system. Which system is present in animal tissues is still unknown. A reductase of the *E. coli* type is found in Novikoff hepatoma (Moore), but it remains to be seen whether this tumor system is a prototype for vitamin B_{12}-dependent animal tissues such as bone marrow.

Micropuncture Study of the Primate Nephron. C. M. BENNETT, B. M. BRENNER, AND R. W. BERLINER,† Bethesda, Md.

Renal tubule functions studied by micropuncture differ in dog and rat. To characterize a species more closely related to man, we obtained samples of tubule fluid from the rhesus monkey. Proximal TF/P ratios for Na^+, K^+, and osmolality in six normal animals approximated unity. At 50 to 70% of proximal tubule (PT), $(TF/P)_{In} = 2.15 \pm 0.09$ SE similar to the dog; at 70 to 90% of the PT, $(TF/P)_{In} = 2.76 \pm 0.17$ SE. During hydropenia [mean $(U/P)_{osm} = 2.67$], DT fluid was always hypotonic to plasma [$(TF/P)_{osm} = 0.49 \pm 0.12$ SD and $(TF/P)_{Na^+} = 0.43 \pm 0.16$ SD] and did not change along this segment. In early DT $(TF/P)_{In} = 4.22 \pm 0.35$ SE; minimal water reabsorption occurred in this segment [late DT $(TF/P)_{In} = 5.21 \pm 0.38$ SE]. Five monkeys were given furosemide (3 mg per kg prime and per hour iv) and urinary losses ($\frac{1}{3}$ of the filtered Na^+ and water) were replaced. Electrolyte and water reabsorption along the PT did not differ from controls. The hypotonicity in the DT was nearly abolished [$(TF/P)_{osm} = 0.89 \pm 0.06$ SE and $(TF/P)_{Na^+} = 0.85 \pm 0.08$ SD], and the Na^+ remaining at this site (22% of filtered) was twice control values (10%). Water reabsorption to this point was not significantly decreased [$(TF/P)_{In} = 3.95 \pm 0.21$ SE], indicating that inhibition of Na^+ reabsorption occurred primarily in the water impermeable segment rather than in the PT. ¶ In normals $(TF/P)_{K^+}$ showed considerable scatter in 26 collections along the DT (range, 0.26 to 3.43); of these, 11 were less than unity. No net secretion or reabsorption in DT was detected and the K^+ present (22.6% of amount filtered) was the same as in urine (22.1%). Furosemide doubled the K^+ in the DT (48% of amount filtered) and in all 11 samples, $(TF/P)_{K^+} \geq 1$ (range, 0.98 to 3.43), suggesting inhibition of K^+ reabsorption at or before this site.

Hemodynamic and Metabolic Effects of Phosphate Supplements in Paget's Disease of Bone. HERBERT BENSON, RALPH S. GOLDSMITH, SIDNEY H. INGBAR,* WALTER H. ABELMANN,* AND LEWIS E. BRAVERMAN, Boston, Mass.

Three patients, aged 61 to 69, with extensive involvement of the skeleton by Paget's disease were studied before, during, and, in two cases, after cessation of oral supplements of inorganic phosphate (1 g daily). Without supplementation, all three patients displayed abnormally high cardiac outputs (6.3 to 7.7 L per minute), low total peripheral resistance (984 to 1,188 dynes-sec-cm^{-5}), and low arteriovenous oxygen differences (3.7 to 4.4 vol per 100 ml). Greatly accelerated bone turnover rates were associated with negative calcium balance in one patient and essentially equilibrium calcium balance in two. During supplementation (7 to 10 months), all three patients showed reduction in cardiac output (−20 to −26%), increased total peripheral resistance (+22 to +46%), decreased left ventricular work index (−12 to −46%), and increased arteriovenous oxygen difference (+3 to

+39%). Within 3 months after being switched to placebo, all cardiovascular parameters had returned to near control levels. The one patient in mild high output failure showed a significant reduction in central venous pressure and cardiac size during supplementation, with reversal on placebo. In addition, during supplementation, all patients displayed moderate to complete relief of pain, decreased urinary calcium excretion, and positive calcium balance. ¶ These data suggest that oral phosphate supplements favorably affect the abnormal cardiovascular dynamics of Paget's disease and promote calcium retention in this disorder. These effects persisted during prolonged administration (7 to 10 months in this study) and regressed during substitution by placebo. Hence, these effects can probably be ascribed to phosphate supplementation.

Genetic Polymorphisms of Human Serum β-Lipoprotein. KÅRE BERG AND ALEXANDER G. BEARN,* New York, N. Y.

Genetically determined antigenic variations have been demonstrated in normal human serum β-lipoprotein. The Ag and Lp systems are independent, as are the Ld(a) and Lt(a) antigens. The genetic variants of the β-lipoprotein have been investigated further, with antisera from immunized animals and transfused patients. All animal antisera demonstrated anti-Lp(a) specificity, whereas multiple specificities have been found by human-antihuman sera. More than 20 human sera containing precipitating antibodies against β-lipoprotein antigens have been studied. Eleven different specificities have been revealed by these antisera. Association studies disclosed that several specificities were related and clustered into four groups, corresponding to the Ag, Lp, Ld, and Lt systems. Some of the antisera contained antibodies with more than one specificity. In several instances the association was caused by one antibody being present in two antisera. ¶ The anti-Ld serum F. J. was originally considered monospecific, but recent studies have revealed that it contains two antibodies, one shared with the anti-Ag(a) serum, the other unique. The unique antigen, designated Ld(a), was associated with an antigen revealed by another antiserum. No serum lacking both antigens was observed in a panel of 58 normal sera. The frequencies of the different phenotypes indicated that the two antigens were products of allelic genes. Thus, the product of an allele of the gene Ld^a has been demonstrated and has been termed Ld^b. ¶ Investigation of the genetics of human serum β-lipoprotein is useful in mapping the human genome, and because of the possible relation of the β-lipoprotein to structural cell lipoproteins, to problems of transplantation immunity.

Anomalous Osmosis and Gastric Cation Exchange. JESSE M. BERKOWITZ, IRVING F. MILLER, AND HARRY P. GREGOR, New York, N. Y. (introduced by Irving L. Schwartz†).

The purpose of this study was to evaluate anomalous osmosis of water, secondary to the diffusional exchange of hydrogen for sodium across a cation exchange membrane, as the basis of the hypotonicity of basal gastric fluid. In prior studies, in stomachs of dog and man, the introduction of isosmotic HCl solutions resulted in the exchange of hydrogen for sodium accompanied by the flow of water from mucosa to lumenal fluid and a drop in tonicity. Calculations of net solute and water fluxes in these experiments suggested the flow of hypotonic fluid into the lumen, i.e., anomalous osmosis. To test this we placed a cation exchange membrane (18.5 cm in diameter) in a plastic chamber. On one side of the membrane, isosmotic NaCl was circulated and on the other, isosmotic HCl. A flow of free water (5 ml H_2O per 5 minutes) from the Na to the H side was observed. The flow decayed as the Na and H gradients declined. This would fit the theory that anomalous osmosis is related to the potential produced by the differential mobilities of the mobile ions. Other solute systems with smaller differential mobilities between the mobile ions gave less water flow. In contrast, glycerol, 2.0 moles per L, on one side of the membrane and distilled water on the other side gave a flow of 1.5 ml H_2O per 5 minutes. These experiments suggest that the potential produced by the flow of mobile ions is more potent than an osmotic gradient in effecting net water movement across ion exchange membranes. Furthermore, the mucoid substances lining the gastric mucosa may be the cation exchange materials for the transport processes described.

Bone as a Buffer: The Role of Inorganic Phosphate as a Hydrogen Ion Acceptor. DANIEL BERNSTEIN, AMNON WACHMAN, AND CHARLES GURI, Boston, Mass. (introduced by George Thorn†).

Previous studies by Goldsmith and Ingbar showed that oral and intravenous phosphate were effective in reducing plasma and urinary calcium levels in hypercalcemic cases. Studies in our laboratory concerning the effect of oral phosphate in patients with idiopathic hypercalcuria and renal calculi showed a marked reduction in urinary calcium excretion and formation of renal calculi. In order to delineate the role of phosphate, we have studied the physiologic function of bone salts (calcium and phosphate) in the regulation of $[H_3O]^+$ excretion. Five normal human subjects (three male, two female), 19 to 23 years old, were fed a soybean protein diet and studied under balance conditions for 26 to 30 days. After baseline studies, a constant NH_4Cl load was administered, and the urinary calcium, phosphate, magnesium, and hydroxyproline excretion increased significantly over base line. At the peak of the NH_4Cl load, intravenous phosphate was administered (1 to 1.5 g P each day over 4- to 5-day periods) to three of the subjects, and the urinary excretion of calcium, magnesium, and hydroxyproline fell significantly, even though the same acid load was imposed, indicating that bone resorption decreased. The buffering of $[H_3O]^+$ ion by phosphate was accompanied by a rise in urine pH, titratable acidity, and serum pH. We believe that the administration of exogenous phosphate spares the necessity of utilizing endogenous stores of bone phos-

phate for the buffering of $[H_3O]^+$. Implied in this new concept is that parathyroid hormone secretion, while responding to changing levels of ionized serum calcium, may also be responsive to changes in acid-base metabolism by virtue of its regulatory function on phosphate transfer in bone and kidney.

Augmentation of Hepatic Accumulation of Thyroxine (T_4) by Phenobarbital and Chlordane. G. Bernstein, J. Hasen, S. A. Artz, and J. H. Oppenheimer,* New York, N. Y.

Previous studies in man and experimental animals have demonstrated a rapid exchange of T_4 between plasma protein and tissue pools. In the rat, the liver accumulates about 25% of a dose of tracer T_4-^{125}I within 35 minutes after intravenous injection. In order to elucidate the factors responsible for hepatic uptake, several drugs known to stimulate detoxifying enzymes in the liver were injected intraperitoneally daily for 3 to 5 days. Paired groups of treated and control animals (140 to 200 g) were used. Phenobarbital (100 mg per kg) and chlordane (25 mg per kg) markedly increased hepatic uptake of T_4-^{125}I. Phenobarbital induced a 51% and chlordane a 74% increase ($p < 0.01$). Appropriate corrections were made for T_4-^{125}I associated with plasma proteins in tissue. The enhanced hepatic uptake was due both to an increased liver mass and to a slight increase in the concentration of T_4-^{125}I per gram tissue. A concomitant reduction in plasma T_4-^{125}I was observed in the treated animals. Histologic examination of phenobarbital-treated animals revealed a marked increase in the cytoplasmic content of hepatocytes. Chromatographic studies indicated that more than 95% of liver and plasma radioactivity was in the form of T_4-^{125}I both in control and treated animals. The observed alterations in T_4 distribution were not due to a primary disturbance of the T_4-plasma protein interaction since no changes in T_4 binding were noted on equilibrium dialysis of serum of phenobarbital-treated animals. Moreover, a single intravenous injection of phenobarbital (1 mg) did not alter the plasma disappearance curve of T_4-^{125}I. Injection of 3,4-benzpyrine (25 mg per kg) and chlorpromazine (25 mg per kg) did not increase liver weight or hepatic uptake of tracer T_4. Since phenobarbital and chlordane are known to stimulate the formation of smooth endoplasmic reticulum (SER) and 3,4-benzpyrine does not, these findings raise the possibility that the SER may play an important role in the reversible intracellular accumulation of T_4 by hepatic cells.

The Hemoglobin of Irreversibly Sickled Erythrocytes. John F. Bertles, Eleanor Roth, and Vincent Anku, New York, N. Y. (introduced by James G. Hilton†).

The presence of deformed erythrocytes [irreversibly sickled cells (ISC)] in peripheral blood led to the original description of sickle-cell anemia (Hb SS disease). These cells retain their elongated, crescentic shape despite equilibration with O_2. Allegedly ISC are relatively old erythrocytes that have suffered prior sequestration in areas of low O_2 tension. Old erythrocytes in Hb SS disease should, through processes of selective survival, contain more Hb F than young erythrocytes. On the other hand, a low content of Hb F presumably would facilitate ISC formation during sequestration. Experiments were conducted to resolve these conflicting predictions. ¶ The proportion of ISC varied from 10 to 40% among patients studied. Optical densities of projected images of individual erythrocytes, stained for Hb F by the Kleihauer-Betke technique, were measured with a gun-type photometer: mean values for ISC were consistently less than half those for non-ISC. After ultracentrifugation of Hb SS blood, fractions taken along the packed cell columns were analyzed for proportions of alkali-resistant hemoglobin (Hb F). This value increased in parallel with increasing specific gravity, but then fell sharply as the proportion of ISC rose to over 90% in the heaviest fraction. Cell content of total hemoglobin (MCH) was constant in all fractions: thus absolute amounts of Hb F and S per cell varied reciprocally. ¶ The physical state of hemoglobin within ISC was examined by *1)* exposure of these cells to a 5,000 gauss magnetic field and *2)* electron microscopy. Whereas, as expected, Hb SS cells after sickling *in vitro 1)* became oriented with their long axes perpendicular to the magnetic field and *2)* contained bundles of parallel rod-like structures (crystals), oxygenated ISC remained randomly arranged in the magnetic field and showed no internal fine structure. ¶ We conclude that net synthesis of Hb F is least in those erythroid cells destined to become ISC and that the membranes of these morphologically curious cells are "irreversibly sickled," but the hemoglobin is not.

Interaction of Glucagon with Hepatic Adenyl Cyclase. Mark W. Bitensky, Joanna W. Clancy, and Elise Gamache, New York, N. Y. (introduced by Lewis Thomas†).

Sutherland, Rall, and co-workers have established that adenyl cyclase plays a key role in the hormonal regulation of glycogen metabolism. Because the amount of cyclic-AMP formed by adenyl cyclase is miniscule, previous cyclase assays have utilized a conjugated series of enzymes as an amplifying device. This system has proved extremely difficult to prepare and use, giving high blank values and a nonlinear response. Work in this laboratory using ATP-^{14}C as substrate and descending thin layer chromatography for product isolation has resulted in the development of a direct assay for membrane-associated adenyl cyclase *in vitro,* which is both linear and sensitive. This technique has permitted the study of the kinetic mechanism of activation of hepatic cyclase by glucagon. ¶ Glucagon stimulation is observed without reduction over a wide range of magnesium and ATP concentrations. The stimulation caused by glucagon is not attenuated even at the highest substrate concentrations employed. This suggests that glucagon stimulation

does not result from an improvement in the efficiency of substrate utilization. Incubation of whole liver homogenate before preparation of washed membrane fragments results in a reduction of base-line liver cyclase activity to virtually zero. Such enzyme preparations showed full activity after glucagon addition, suggesting that hormone stimulation of liver cyclase is a prerequisite for enzyme activity. ¶ It was further observed that the glucagon-treated enzyme remained stimulated after dialysis or washing by repeated sedimentation and resuspension. This persistence of the glucagon effect was not reversed by sugars or insulin, but was reversed by a soluble fraction from liver. ¶ The question is raised whether glucagon stimulation is accompanied by persistent glucagon binding or some glucagon-mediated change in adenyl cyclase or both. In addition, the nature of the soluble fraction from liver responsible for the reversal of the glucagon effect remains to be determined.

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

Effects of a 20-day period of bedrest were studied in five subjects, ages 19 to 21. Total body weight remained constant. Lean body mass, total body water, intracellular fluid volume, red cell mass, and plasma volume decreased. Electron microscopy of quadriceps muscle showed no significant changes. There was no effect on total lung capacity, forced vital capacity, 1-second expiratory volume, or membrane diffusing capacity for carbon monoxide. Total diffusing capacity and pulmonary capillary blood volume were slightly lower after bedrest. These changes were related to changes in blood flow. Total heart volume decreased from 860 to 770 ml. ¶ Maximal oxygen uptake fell from 3.3 to 2.4 L per minute. Cardiac output, stroke volume, and arterial pressure at rest in supine and sitting positions did not change significantly. Cardiac output during supine exercise at 600 kpm per minute decreased from 14.4 to 12.4 L per minute, and stroke volume fell from 116 to 88 ml. Heart rate increased from 129 to 154 beats per minute. There was no change in arterial pressure. Cardiac output during upright exercise at submaximal loads decreased approximately 15% and stroke volume 30%. Calculated heart rate at an oxygen uptake of 2 L per minute increased from 145 to 180. Mean arterial pressures were 10 to 20 mm Hg lower with no change in total peripheral resistance. The av-O_2 difference was higher for any given level of oxygen uptake. Cardiac output during maximal work fell from 20.0 to 14.8 L per minute and stroke volume from 104 to 74 ml. Total peripheral resistance and av-O_2 difference did not change. ¶ The fall in maximal oxygen uptake was primarily due to a reduction of stroke volume and cardiac output. The response to upright exercise after bedrest suggests impaired venous return. However, stroke volume and cardiac output were reduced also during supine exercise. A direct effect on myocardial function can therefore not be excluded.

The Haptoglobin Locus and Chromosome 13. GERALD E. BLOOM, PARK S. GERALD,* AND LEONARD E. REISMAN, Boston, Mass., and Louisville, Ky.

A previously reported child with congenital anomalies and a ring D chromosome was found to lack the paternal haptoglobin gene. It was hypothesized that the locus for the haptoglobin alpha chain was situated on one end of a D chromosome and was lost during formation of the ring. A second unrelated child with a ring D chromosome has now been studied with findings supporting this interpretation. Studies using tritiated thymidine have shown that in both the ring was derived from a no. 13 chromosome. ¶ No haptoglobin could be detected in the second patient. His father and several relatives exhibited a haptoglobin variant resembling a normal 2–1 pattern except that duplication of the haptoglobin bands was present. This abnormal haptoglobin pattern, identified as the Carlberg type, is believed to result when normal amounts of "2" alpha chain are produced in the presence of very small amounts of "1" alpha chain. The Carlberg pattern is presumably a mixture of 2–1 bands and 2–2 bands (formed from excess "2" chains). The Carlberg pattern was simulated by mixing normal sera of 2–1 and 2–2 haptoglobin types. On subtyping, the father's haptoglobin yielded only type 2 alpha chains, the type 1 alpha chains presumably being in too low a concentration to detect. ¶ These observations support the contention that this ring 13 child possesses only the paternal variant haptoglobin gene (which produces minimal amounts of type 1 haptoglobin), whereas the maternal haptoglobin gene was lost during formation of the ring chromosome. The studies in this family provide confirmatory evidence that the Carlberg pattern results from a $Hp^2/Hp^{1\,Weak}$ genotype and that the haptoglobin gene is present on one end of the no. 13 chromosome. This is the only structural locus so far identified with a specific autosome.

The Reciprocal Effects of Myocardial Wall Force and Length on Mean Rate of Left Ventricular (LV) Contraction in Man. JOSEPH BLUMENTHAL, H. W. PALEY, AND RICHARD REEVE, San Francisco, Calif. (introduced by Meyer Friedman†).

In studies of left ventricular volume (thermodilution) in this laboratory, mean rate of LV circumferential shortening (MRCS) was derived by assuming a spherical LV and by dividing the difference of end-diastolic and end-systolic circumferences by LV ejection time. Cardiac output was determined (indicator dilution) and mean LV wall force during ejection (MFE) was calculated. ¶ In 16 subjects, means of end-diastolic and end-systolic volumes fell from 187 to 130 ml and from 100 to 72 ml, respectively, in the 60° head-up tilted posture as compared to the supine position, while arterial blood pressure remained practically unchanged. Considering force-velocity relationships of cardiac muscle, it was anticipated that MRCS would increase in the smaller heart, in which, according to the LaPlace law, MFE was significantly diminished. However, MRCS remained unchanged in

the tilted posture (14.3 ± 1.2 cm per second) as compared to the supine (13.9 ± 1.1 cm per second). The relatively constant MRCS with differences of EDV suggests a reciprocal relationship between the force and length factors of the intact LV wall, for as EDV decreases causing a reduction of MFE, LV fiber length diminishes, reducing velocity of contraction according to the length-tension diagram. MRCS as a contractility index was further tested by infusing isoproterenol in five subjects (1.5 to 2.5 μg per minute). In response, MRCS increased from a mean of 13.0 ± 1.2 cm per second to 21.7 ± 0.8 cm per second in the supine posture and from 13.4 ± 1.2 cm per second to 20.9 ± 1.5 cm per second in the head-up tilted position. This study indicates that in the intact heart of man the effects of left ventricular wall force and length associated with changes in end-diastolic volume appear to cancel each other so that mean velocity of LV wall shortening during systole tends to remain constant for any given state of myocardial contractility.

Effect of a Germfree Environment on Granulocytopoiesis. D. R. Boggs, P. A. Chervenick, J. C. Marsh, H. I. Pilgrim, and G. E. Cartwright,† Salt Lake City, Utah.

This study was designed to determine if the absence of environmental microorganisms would result in reduced neutrophil production. The total number and type of marrow neutrophils, the number of marrow neutrophils in mitosis, as well as blood neutrophil concentration, were compared in germfree and conventional mice. The effects of endotoxin and of removal from germfree environment upon the rate of release of neutrophils from marrow to blood were also studied in germfree mice. ¶ The number of nucleated cells washed from the humerus was slightly less in germfree (mean ± SE = 7.77 ± 0.17 million) than in conventional mice (8.15 ± 0.27). This slight difference reflected a difference in lymphocytes since erythroblasts and neutrophils were virtually identical in the two groups (erythroblasts = 2.28 ± 0.16 and 2.27 ± 0.13; neutrophils = 3.29 ± 0.16 and 3.38 ± 0.21). Numbers of potentially mitotic neutrophils (myeloblasts, promyelocytes, and myelocytes) were similar in the two groups, being 1.02 million in germfree mice and 0.91 in conventional mice. The mitotic index for this marrow compartment was 11% in both groups. Blood neutrophil concentration was 1,710 ± 140 per mm^3 in conventional mice but slightly less in germfree mice (1,120 ± 120, $p = < 0.05$). ¶ Within 16 hours of removal from germfree environment, 30% of postmitotic marrow neutrophils (metamyelocytes, band and segmented) had been released to the blood. Administration of endotoxin as mice were removed from germfree environment increased this percentage to 76%. ¶ Although a subtle difference in blood neutrophil concentration was observed, these studies suggest that the rate of production of neutrophils in germfree and in conventional mice is similar. Furthermore, a prompt acceleration of rate of release of neutrophils from marrow was evident in response to endotoxin and infection (removal from germfree environment). Thus, in this model system, there is little to suggest that microorganisms or their products play a role in controlling neutrophil production in the normal steady state.

Effect of Actinomycin C on Adrenal, Parathyroid, and Immunoglobulin Responses in Man. William E. Braun, Joseph Tucci, Jean-Marie Idatte, and John P. Merrill,† Boston, Mass.

Although actinomycin, an inhibitor of DNA-directed RNA synthesis, usually blocks protein synthesis, in very low doses (0.01 to 0.025 μg per ml) it is capable of stimulating protein synthesis. In this study its effect on adrenal and parathyroid function and immunoglobulin levels was evaluated in four renal transplant patients on maintenance azathioprine and dexamethasone therapy. Three patients ($C_{Cr} = 75$, 70, and 25 ml per minute) received standard ACTH infusions before and after one injection of actinomycin C (Act) (5 μg per kg), and one patient ($C_{Cr} = 57$ ml per minute) received them before and after five daily doses of Act (5 μg per kg). In each patient the adrenal ACTH response as measured by plasma and urinary steroid levels increased by 1½ × to 2½ × after Act administration. In two patients given Act alone, plasma cortisol levels rose transiently (0.5 to 2.0 and 1.0 to 6.9 μg per 100 ml). On a 200 mg Ca, 500 mg P diet supplemented with $Al(OH)_3$, the three patients receiving one injection of Act increased their urine Ca excretion above control levels. One patient ($C_{Cr} = 75$ ml per minute) developed borderline hypercalcemia and hypercalcuria (5.3 → 5.6 mEq per L and 118 → 204 mg per 24 hours, respectively), and another ($C_{Cr} = 70$ ml per minute) developed frankly elevated blood and urine Ca levels (5.5 → 6.0 mEq per L and 185 → 299 mg per 24 hours, respectively). Measured by the immunoplate method, serum IgG rose transiently after Act in eight of nine studies (56% ± 33%). IgM, IgA, and β_{1C} changes were slight and variable. No significant changes in serum urea nitrogen or creatinine clearance occurred after Act. It appears from these data that Act as used for immunosuppression in kidney transplant patients may have a stimulatory effect on the systems studied. This may result from Act interference with m-RNA linking a pleiotropic regulator gene and its repressors.

Mechanism of Action of Hypoglycin, Etiologic Agent in the "Vomiting Sickness" of Jamaica. R. Bressler* and M. Entman, Durham, N. C.

"Vomiting sickness" is a toxic disease that occurs in Jamaica and has been attributed to the ingestion of the unripe fruit of the tropical plant *Blighia sapida* (ackee fruit). The illness is characterized by pernicious vomiting in man and by profound hypoglycemia in animals and man. The causative agent was found to be an amino acid, L-α-amino-β-methylenecyclopropranepropionic acid (hypoglycin), which caused hypoglycemia and impairment of fatty acid oxidation (FAO) *in vivo*, but was inactive *in vitro*. The amino acid was shown to undergo transamination and oxidative decarboxylation in the liver to

yield methylene-cyclopropaneacetic acid (MCPA), which was active both *in vitro* and *in vivo*. Van Holt has shown that MCPA causes hypoglycemia and a decrease in the oxidation of palmitate but not of octanoate. Because of the effect of MCPA on long but not short chain FAO, we undertook a study of the effect of hypoglycin on the long chain acyl CoA-carnitine acyltransferase (LCAT). This enzyme has been shown to be the rate-limiting step in long chain FAO. ¶ Intravenous administration of hypoglycin to mice resulted in marked hypoglycemia, which was preceded by a decrease in palmitate oxidation in myocardial homogenates from treated animals. These homogenates carried out normal rates of glucose and hexanoate oxidation. LCAT was markedly depressed. The addition of (−)−carnitine to myocardial homogenates from toxin-treated mice restored both the depressed palmitate oxidation and the decreased LCAT activity to normal. The administration of (−)−carnitine to hypoglycin-treated animals prevented the decreases in myocardial palmitate oxidation, LCAT activity, and the hypoglycemia. The data suggest that hypoglycin administration causes an inhibition of the rate-limiting step in long chain FAO, which can be reversed by (−)−carnitine, and that the impairment in FAO is responsible for the hypoglycemia.

Leukemic Lymphocyte Antibody Synthesis Induced by Extracts from Immune Normal Lymphocytes. JEROME I. BRODY,* Philadelphia, Pa.

The purpose of this study was to define more closely the basis for the failure of the leukemic lymphocyte to respond immunologically to newly introduced antigens by determining whether extracts, obtained from normal lymphocytes that had been previously stimulated *in vivo* with a specific immunogen, could supply immune information to the nonsensitized neoplastic cell. Normal volunteers were immunized with commercial salmonella vaccine. After a suitable waiting period, their lymphocytes were collected by differential centrifugation and grown in suspension tissue culture in the presence of autologous plasma, TC 199, and sonicated salmonella. On the fourth day culture supernatants were removed, the cellular sediments thoroughly washed, and the normal lymphocytes resuspended in buffer and fragmented by ultrasound. The disrupted lymphocytes were centrifuged, and the optically clear, lymphocyte-derived, supernatant was added to freshly made cultures containing intact leukemic lymphocytes obtained from nonimmunized donors, fragmented salmonella antigen, and a ^{14}C amino acid hydrolysate that should be incorporated into protein synthesized by growing cells. These cultures were terminated, and leukemic lymphocyte sonates, prepared as described earlier, were incubated with whole salmonella as for conventional agglutination. Radioactivity was retained by the bacteria, as assayed by scintillation spectrometry, implying that leukemic lymphocyte antibody biosynthesis had been induced by the immune normal lymphocyte extract. This result is particularly significant since, as shown in a companion study, antibody to salmonella could not be made by leukemic lymphocytes when the antigen was administered directly to the leukemic patient. The observations strongly suggest that the immunologic capability of the leukemic lymphocyte may be augmented if this cell is provided with preformed information in an appropriate fashion. Limited immune reactivity of the leukemic lymphocyte, therefore, may be related to an abnormality of the pristine mechanism responsible for initial antigen recognition but not necessarily for ultimate immunoglobulin formation.

The Effect of Drugs on the Distribution of Pulmonary Vascular Resistance. JEROME S. BRODY, EDWARD J. STEMMLER, AND ARTHUR B. DUBOIS,* Philadelphia, Pa.

The pressure drop along the pulmonary arteries, capillaries, and veins, and the resistance to blood flow in each of these segments have been measured before and during drug infusion using a method recently described by the authors. This method depends on combining three techniques: *1*) recording the decrease in perfusion pressure vs. time following the injection of a bolus of low viscosity fluid into the pulmonary circulation (J. Piiper); *2*) ether-plethysmographic determination of pulmonary artery volume (forward perfusion) and pulmonary venous volume (reverse perfusion); and *3*) dye dilution measurements of total blood volume in the isolated lobe of a dog's lung perfused with blood via a donor dog at a constant flow. ¶ Isoproterenol caused vasodilation of the entire pulmonary vascular bed during forward and reverse perfusion of the lobe. Low doses of serotonin (1.5 μg per minute per kg body weight) constricted both large and small arterial vessels during forward perfusion but did not affect the capillaries or veins. During reverse perfusion pulmonary venous constriction predominated. High doses of serotonin (5.0 μg per minute per kg body weight) constricted the pulmonary arteries and veins simultaneously. Histamine constricted the pulmonary veins during forward and reverse perfusion but did not affect the pulmonary arteries. ¶ These findings demonstrate vascular tone in both arteries and veins in the isolated lung and differential contractility in the pulmonary vascular bed. The method described allows, for the first time, measurements of pulmonary capillary pressure.

Regulation of Liver Glucokinase Activity in Man and Dog. JOSIAH BROWN AND RICHARD HORNICHTER, Los Angeles, Calif. (introduced by Joseph Ross†).

Glucokinase, an enzyme present only in liver, has properties which indicate its role is to phosphorylate the tide of glucose reaching the liver after a carbohydrate meal. Since the liver cell is freely permeable to glucose, the rate of phosphorylation controls glucose utilization and is an important determinant of oral glucose tolerance. We have demonstrated the presence of glucokinase in the liver of human beings and dogs and its dependence on diet and insulin. ¶ Glucokinase activity is assayed spectrophotometrically; confirmation is obtained by separation of hexokinase isoenzymes and glucokinase by starch gel

electrophoresis followed by staining. The intensity of staining of the bands provides an estimate of enzyme activity. In extracts of liver biopsies obtained during surgery on four well-nourished humans, glucokinase activity made up two-thirds of total glucose phosphorylating capacity but was absent or almost so in the livers of four poorly nourished humans. In dogs fed a high carbohydrate diet, glucokinase activity in liver obtained by needle biopsy made up 80% of total phosphorylating activity but virtually disappeared after 5 days of starvation. Refeeding the diet for 1 week resulted in glucokinase activity double the normal control value. Pancreatectomy was followed by virtual disappearance of glucokinase activity, and insulin administration resulted in return of glucokinase. ¶ Thus, glucokinase in the liver of humans and dogs is an inducible enzyme dependent on dietary substrate (glucose) and requiring insulin for synthesis. It represents the majority of glucose-phosphorylating capacity in the liver and may in great measure determine oral glucose tolerance. The characteristics of glucokinase regulation suggest that it is an important homeostatic regulator of glucose utilization by the liver adjusting to the intake of carbohydrate in the diet.

The Effect of Endotoxin and Immune Precipitates on PMN Motility, Aggregation, and Degranulation. R. BRYANT, Nashville, Tenn. (introduced by R. Des Prez*).

Phagocytosis of bacteria by granulocytes results in granulocyte aggregation and impairment of granulocyte migration. The present studies demonstrate that both bacterial endotoxin and immune complexes cause similar changes in granulocyte function. Heparinized human blood was incubated with endotoxin and rotated in a standard phagocytic system. As little as 1 μg per ml of *Escherichia coli* endotoxin caused granulocyte aggregation and impaired migration. Similar experiments using egg albumen (25 μg per ml) and rabbit anti-EA plasma (2.5%) demonstrated the same effects. Studies using rabbit blood incubated with endotoxin or immune complexes demonstrated marked leukocyte degranulation. Enhancement of cell particle contact by rotation increased the effects of endotoxin and immune precipitates on PMN aggregation and migration. Impairment of phagocytosis by divalent cation depletion, temperature reduction, and removal of heat-labile plasma factors greatly reduced the effect of endotoxin. High speed centrifugation of endotoxin or immune precipitates removed factors effecting leukocyte migration and aggregation. These findings suggest that the effects of endotoxin or immune precipitates on granulocytes are consequences of phagocytosis and represent a general pattern of leukocyte response to ingestion of macromolecular substances.

Demonstration of a Circulating Plasma Antagonist of LATS. GERALD BURKE, Chicago, Ill. (introduced by Eric Reiss*).

The existence of a circulating inhibitor or antagonist of the long-acting thyroid stimulator (LATS) has been suggested by the repeated demonstration in our laboratory that unconcentrated immunoglobulin G (IgG) fractions derived from LATS-negative hyperthyroid sera possess LATS activity. LATS activity was demonstrated in 23 unconcentrated IgG fractions prepared from a total of 37 LATS-negative hyperthyroid sera; the inhibitor is apparently removed in the process of IgG isolation. This concept gains support from the results of eight of twelve experiments in which significant reduction (30 to 55%, $p < 0.01$) in LATS activity has been noted after incubation of LATS-IgG with IgG-free protein fractions of plasma obtained from euthyroid controls. ¶ Although we have confirmed the observation that bioassay of concentrated IgG fractions obtained from LATS-negative hyperthyroid sera increases the frequency with which LATS activity is detected, bioassay of these whole LATS-negative sera concentrated to an equivalent degree has revealed highly significant inhibition of murine thyroidal ^{131}I release (36 to 58% decrease in 8-hour blood radioactivity in three consecutive experiments to date). ¶ These data suggest that the demonstrated antagonism of LATS by IgG-free human plasma protein fractions is mediated by direct thyroidal suppression and militate against a primary etiologic role for LATS in the pathogenesis of Graves' disease. The nature of the circulating LATS antagonist and its role in normal thyroidal economy remain to be defined.

CNS Control over Glucose Homeostasis. IAN BURR, Melbourne, Australia, and San Francisco, Calif. (introduced by Melvin M. Grumbach*).

Data from single injection studies on 23 normal subjects led to development of an intravenous glucose load-constant infusion test in which nonphysiological disturbances of glucose homeostatic mechanisms were minimized. The plasma glucose response to this test was determined in 37 normal and 24 diabetic subjects. Variation in plasma glucose was minimal in normals and marked in diabetics—assessed by the difference between maximal and minimal glucose concentrations over the final 25 minutes of a 50-minute infusion (95% confidence limits, 2.11 to 3.40; 5.24 to 6.62). This parameter successfully predicted the course of 16 pregnant women with clinical suspicion of prediabetes unresolved by oral glucose tolerance tests. ¶ Plasma glucose, cortisol, growth hormone, and insulin concentrations were estimated after glucose load infusion in eight normal subjects with and without prior pempidine administration. Pempidine increased the magnitude of glucose fluctuation from 5.6 to 22.3 mg per 100 ml (mean) and of insulin secretion as indicated by the difference between final and fasting levels (pretreatment < 1 μU per ml; post-treatment, 16 μU per ml, $p < 0.01$). Pempidine did not influence either plasma cortisol (mean fall 56%) or growth hormone response to glucose. Hormonal responses after pempidine did not appear to account for alterations in glucose concentrations. Pempidine administration to normals resulted in a plasma glucose response to glucose load infusion similar to that in pre-, late, and early onset diabetes and

an insulin response similar to that in 10 "late-onset" diabetics. These results led to the hypothesis that the CNS exerted a dampening effect on glucose fluctuation by controlling the phasic relationship between the activity of factors tending to raise and lower blood glucose levels, this effect being mediated partly by the autonomic nervous system and possibly by a direct effect of the peripheral nervous system on tissue metabolism. Disturbance of this postulated control mechanism may be involved in the onset of clinical diabetes.

Changes in Immunoglobulins in Nasal Secretion during Viral Respiratory Infection. W. T. BUTLER, T. A. WALDMANN,* R. D. ROSSEN, R. G. DOUGLAS, AND R. B. COUCH, Houston, Texas, and Bethesda, Md. (introduced by Vernon Knight†).

Immunoglobulins A and G and albumin were measured daily in nasal secretions and serum before, during, and after viral respiratory illness induced in three volunteers with rhinovirus 15 and in three others with Coxsackie A-21. ^{131}I-labeled IgG and ^{125}I-labeled albumin were given intravenously to four of the volunteers before virus inoculation, and the specific activities of each were measured in nasal secretions and serum during the same period. Albumin and IgG concentrations, together constituting about 25% of the nasal secretion protein, increased several-fold to maximal values that coincided in time with illness (3 to 4 days after virus inoculation) and then returned toward base-line values within 3 days. In contrast, 11 S IgA, constituting about 50% of the nasal secretion protein, increased more slowly, reaching a peak concentration several days after subsidence of illness and remaining high thereafter. This may represent local formation of antiviral antibody that is known to be associated with 11 S IgA in nasal secretions. Evidence for locally formed IgA is further supported by the finding that labeled 7 S IgA given intravenously appeared in nasal secretions in only small amounts, was unchanged in sedimentation behavior, and was not incorporated into 11 S nasal secretion IgA. ¶ Before, during, and after illness the specific activity of albumin was the same in nasal secretions and serum. In contrast, before illness the specific activity of IgG in nasal secretions was about two-thirds of that in serum, suggesting that normally about one-third of IgG is synthesized locally. During and after illness the specific activity of IgG in nasal secretions approximated that of serum, suggesting that virus infection caused a replacement of locally synthesized IgG with IgG from serum. These findings suggest that during viral respiratory infection IgA is formed locally whereas IgG is derived from serum.

Renin Activity in Anephric Subjects. JOHN P. CAPELLI, LAURENCE G. WESSON, JR., GONZALO E. APONTE, ELEANOR JAFFE, AND CELIA FARALDO, Philadelphia, Pa. (introduced by William A. Sodeman†).

Although extrarenal sources of renin have been suggested, experimental studies have not yielded conclusive results because of the inability to maintain laboratory animals in a chronically nephrectomized state. Anephric subjects awaiting renal homotransplantation, maintained on chronic hemodialysis, afforded the opportunity to investigate this area in man. Four female patients are the subject of this study. Plasma renin was assayed according to the enzyme kinetic technique of Lever and associates. The patients and plasma renin concentrations at various times postnephrectomy were as follows: M.B.—1 month, 0 renin units per liter plasma (U/L). D.T.—2 months, 0.4; 4 months, 0.6 U/L. B.S.—3 months, 2.3 U/L. B.R.—2 months, 2.6; 3 months, 4.3; 6 months, 7.7; 7 months, 11.6; 8 months (postmortem plasma), 7.4 U/L. Identity of chemical properties, including Michaelis-Menten constant, pH sensitivity, and renin-substrate reaction product characteristics, indicate that the extrarenal renin was indistinguishable from human renal renin. Tissues were obtained within 4 hours of death in patient B.R. Extraction and assay of adrenal gland, heart, ileum, liver, lung, ovary, submaxillary gland, stomach, thyroid, and uterine tissues demonstrated renin-like activity only in uterine tissue: 9.7 R.U. per kg per hour; (human renal renin standard: 30.9 R.U. per kg per hour). Stain of uterine tissue with thioflavine-T and the periodic acid silver-methenamine stain demonstrated aggregates of stainable material within myometrium and around arterioles. These findings, indicating the presence of an extrarenal source of renin in the uterus, not only suggest a role for renin in the pregnant state, but also that excessive uterine renin secretion may be implicated in the pathophysiologic syndrome of toxemia of pregnancy.

Role of 12α Hydroxylase Deficiency in Continuing Liver Injury. J. B. CAREY, JR.,* I. DODD WILSON, G. ONSTAD, AND F. G. ZAKI, Minneapolis, Minn.

Cholic acid (3α,7α,12α-trihydroxycholanic acid) is normally the predominant primary bile acid formed from cholesterol in man. With severe liver damage, however, a relative deficiency of 12α hydroxylase activity often develops and the principal primary bile acid formed is chenodeoxycholic acid (3α,7α-dihydroxycholanic acid), which colon bacteria rapidly convert to lithocholic acid (3α-hydroxycholanic acid), a potent hepatic toxin. Serum bile acid concentrations measured by gas-liquid chromatography in 57 patients with hepatic cirrhosis disclosed 45 with mainly chenodeoxycholic acid of which 18 had 2- to 5-fold increases in serum lithocholic acid concentrations. When fed to 36 rats, lithocholic acid produced hydropic degeneration of hepatic cells, inflammatory reaction, ductular cell proliferation, and fibrosis. The rats protected themselves, however, by converting most of the lithocholic acid fed to the less toxic 3α,6β-dihydroxycholanic acid, which precipitated with lithocholic acid in the common bile duct to form gallstones. All signs of hepatic injury disappeared when lithocholic acid was withheld but the gallstones remained. Two patients with total bile fistulas did not metabolize intravenously administered lithocholic acid-^{14}C to other compounds but excreted it largely unchanged (90%) in the bile. The human liver is thus unable to detoxify lithocholic acid

as readily as the rat liver. ¶ Removal of bile salt anions from the enterohepatic circulation in four jaundiced patients with Laennec's cirrhosis by cholestyramine resin (anion exchange) and in two similar patients with neomycin (anion exchange + antibacterial) for 10 days resulted in a lowering of serum lithocholic acid and chenodeoxycholic acid concentrations to normal. This was associated with a decline in serum bilirubin, alkaline phosphatase, and SGOT values. No such changes occurred in four untreated but otherwise similar control patients. Thus 12α hydroxylase deficiency leading to increased serum lithocholic acid concentrations may perpetuate liver injury in man, and treatment with cholestyramine, neomycin, or both, warrants consideration.

Neonatal Regulation of Erythropoiesis. ALBERTO CARMENA, DONALD HOWARD, AND FREDERICK STOHLMAN, JR.,* Boston, Mass.

We previously observed that erythropoiesis in the newborn rat was unaffected by bilateral nephrectomy. Accordingly the production of erythropoietin (EP) and its effectiveness in the newborn animal were advanced as well as the effect of nephrectomy on EP production. Animals were exposed to 0.4 atmosphere for 16 hours and the plasma was assayed in polycythemic mice. The concentrations of EP per milliliter of plasma were as follows: 1 day old, 0.76 U; 5 days old, 2.5 U; 14 days old, 4.8 U; 10 weeks old, 5.4 U. Within 2 hours of nephrectomy 5-day-old and adult rats were exposed to 0.4 atmosphere for 16 hours. Nephrectomy reduced the plasma concentration of EP in the 5-day-old animals for 2.5 to 1.17 U per ml, but in similarly treated adult rats EP could no longer be demonstrated. ¶ Eleven units of EP per 100 g of body weight was given to hypertransfused rats on the ninth and tenth days of life; the reticulocyte count increased from 1.5×10^5 to 3.4×10^5 per mm^3. In contrast 3.5 U per 100 g in hypertransfused adults on each of 2 successive days increased the reticulocyte count from 0.11×10^5 to 1.7×10^5. ¶ The data suggest that the capacity to produce EP is limited at birth but increases as the animal matures. During the early neonatal period there is significant extrarenal production of EP that diminishes with age. The degree of hypoplasia resulting from polycythemia in the neonatal animal is significantly less than in adults. Exogenous EP appears to be somewhat less effective in neonatal than adult animals. These observations are consonant with the suggestion that neonatal erythropoiesis in the rat is governed in significant part by factors other than EP.

Stimulation of Bone Formation by Inorganic Phosphate and Inhibition of Bone Resorption by Thyrocalcitonin. EVELYN CARROLL AND MAURICE PECHET,† Boston, Mass.

The mechanism of the hypocalcemic action of porcine thyrocalcitonin and of inorganic phosphate was studied in a special unanesthetized rat preparation designed for a metabolic study in a single animal. Normal and thyroparathyroidectomized rats were used. Urine was collected at 30-minute intervals over a 4-day period, and specimens were analyzed for Ca, P, Mg, hydroxyproline, and radioactivity. Thyrocalcitonin infusions induced a sharp decrease in serum Ca and P and a marked decrease in Ca, P, Mg, and hydroxyproline excretions. Parathyroid hormone infusions induced an increase in serum Ca and a marked increase in Ca, P, Mg, and hydroxyproline excretions. Thyrocalcitonin infused concomitantly with parathyroid hormone negated the effects of parathyroid hormone. Inorganic phosphate infusions induced a marked decrease in Ca excretion but did not negate the effects of parathyroid hormone when infused concomitantly with parathyroid hormone. Animals whose bones had been labeled with ^{90}Sr at least 90 days before infusion studies responded to parathyroid hormone infusions with increased excretion of ^{90}Sr. Thyrocalcitonin infused concomitantly with parathyroid hormone negated the parathyroid-hormone effect of mobilizing ^{90}Sr from bone, but inorganic phosphate infused with parathyroid hormone did not. Thus, the mechanism of the hypocalcemic effect of thyrocalcitonin is quite distinct from that of inorganic phosphate. Thyrocalcitonin inhibits bone resorption and the action of parathyroid hormone on bone, whereas inorganic phosphate stimulates bone formation.

Antibody Production and Transfer in Congenital Murine Reovirus Infection. MARY M. CARRUTHERS AND A. MARTIN LERNER,* Detroit, Mich.

A previous study has shown that when 20,000 hemagglutinating units of reovirus, type 2 (strain 988) was inoculated intraperitoneally into pregnant ICR Swiss albino mice at 72 hours after fertilization, viremia and hemagglutinating inhibiting antibody (HIA) were demonstrated in apparently well progeny during the first 3 weeks of life. At autopsy viremic mice were histologically normal. Three, 7, and 9 months later these mice appeared well and had no HIA. At 7 months these sera were tested for type-specific neutralizing antibodies, and these also were absent. Mothers continued to show high HIA, and the congenitally infected progeny when reinoculated at 3 months were capable of manufacturing specific antibody. ¶ The nature and source of antibody in the baby mice that were transiently immunologically tolerant were determined. Adult mice produced high titered HIA to live and formalin-killed virus. Mice whose mothers were inoculated with live or inactivated virus at 72 hours after the onset of gestation, but were immediately nursed by foster mothers without reovirus antibodies, had no HIA reovirus. In marked contrast, similar mice who were not separated from their mothers had no antibodies in the first 24 hours of life, developed gradually increasing HIA levels over the next several days, and by 14 days had levels approximating those of their mothers. These levels then declined over the next several days. Reduction with mercaptoethanol of sera from progeny with HIA showed the reovirus antibodies to be predominantly, but not exclusively, 19 S gamma globulins. These studies indicate that reovirus antibodies in immunologically tolerant congenitally infected

mice are passively transferred through the milk and that murine fetal membranes do not transfer 19 S or 7 S reovirus HIA antibody. Also, the epithelium of the gastrointestinal tract of the suckling mouse can absorb these antibodies for only 14 days.

***In Vitro* Activation of Glutathione Reductase.** PAUL E. CARSON,* BARBARA SCHARRER, BARBARA MORGAN, AND FRANCO AJMAR, Chicago, Ill.

We previously reported the apparent activation of glutathione reductase (GSSG-R) in hemolysates of normal blood after incubation with red cell stromata; activation did not occur in hemolysates of G6PD-deficient blood (in which GSSG-R activity is already consistently increased). For additional analysis of this phenomenon, stromata-free hemolysates of heparinized blood were prepared by centrifugation and Millipore filtration; essentially hemoglobin-free stromata were prepared by modification of Dodge's method. Stromata in all preparations were monitored with a particle counter and plotter. Results obtained using these preparations are: *1*) Incubation of normal or G6PD-deficient, stromata-free hemolysates does not cause increased GSSG-R activity. *2*) Incubation of normal hemolysates with hemoglobin-free stromata from either normal or G6PD-deficient blood does cause increased GSSG-R activity (30 to 40%). *3*) Incubation of G6PD-deficient hemolysates with stromata from either normal or G6PD-deficient blood does not cause increase in GSSG-R activity. At 45° C activation is complete in 2 hours; at 37°, between 8 and 15 hours. Similar results were obtained with hemolysates either of Negro (A-) or Caucasian (B-) G6PD-deficient blood. ¶ The GSSG-R activity of the hemoglobin-free stromata appears to be insufficient to account for the activation in normal hemolysates, but proof that this phenomenon is not a result of elution of enzyme from stromata has not yet been obtained. Preliminary results suggest that similar activation of GSSG-R occurs in hemoglobin-free fractions when incubated with stromata. Whether this phenomenon is due either to elution from stromata or to activation of the enzyme, it fails to occur in hemolysates from G6PD-deficient blood even though stromata from G6PD-deficient blood can exert the effect on hemolysates from normal blood. This suggests that the increased GSSG-R secondary to G6PD deficiency may involve an intrinsic change in this enzyme.

Erythroid Cell Development: Decay Rates of Synthetic Capacity for Different Proteins. ALBERT DE LA CHAPELLE, ANTONIO FANTONI, RICHARD A. RIFKIND,* AND PAUL A. MARKS,* New York, N. Y.

Cellular differentiation is characterized by changes in pattern of proteins synthesized. Erythroid cell (EC) development is particularly suited for studying specific protein synthetic capacities. During fetal development in C57Bl/6J mice (gestation = 21 days), EC form in yolk sac (YS) during days 8 to 12, then in liver (L), during days 12 to 16. YS-EC synthesize embryonic hemoglobins; L-EC synthesize adult type. Between 11 and 13 days the average rate of hemoglobin synthesis (separated by electrophoresis) per YS-EC remains constant, while the rate of nonheme protein synthesis declines from 2-fold that of hemoglobin at 11 days, to nil at 13 days. Between 13 and 15 days, circulating YS-EC lose the capacity to form hemoglobin, associated with loss of cytoplasmic ribosomes (electron microscopy), while the nucleus is retained. Incubation of 11-day YS-EC with actinomycin to inhibit RNA synthesis reduces nonheme protein synthesis 55%, while hemoglobin synthesis is sustained. ¶ Developing L-EC lose the nucleus, while cytoplasmic ribosomes are retained. As erythroblasts develop to reticulocytes, hemoglobin synthesis increases 2- to 4-fold per cell. By the reticulocyte stage, hemoglobin comprises about 95% of the protein synthesized. Incubation of L-EC (erythroblasts and reticulocytes) with actinomycin causes little or no inhibition of hemoglobin synthesis. YS-EC and L-EC incubated with actinomycin develop changes in the nucleolar fine structure associated with the inhibition of RNA synthesis, but no detectable loss of cytoplasmic ribosomes, sites of protein synthesis. ¶ Thus, in developing EC the rates of decay in synthetic capacity for specific proteins differ. Hemoglobin synthesis proceeds on relatively stable templates, which are established in YS-EC line before day 11 and in L-EC line, before day 13. As YS-EC and L-EC develop, synthetic capacity for nonheme proteins is lost more rapidly than that for hemoglobin.

Atypical Bacterial Forms in "Abacteremic" Endocarditis. PATRICIA CHARACHE, Baltimore, Md. (introduced by Philip S. Norman*).

Because organisms are not identified in up to 20% of patients with apparent bacterial endocarditis, blood from patients with possible endocarditis has been processed for fragile, atypical bacterial forms. Atypical forms have altered morphology and altered cultural characteristics consistent with defective bacterial cell wall. ¶ Atypical forms have been identified in eight patients with diagnosed bacterial endocarditis over a 30-month period. In each case, routine processing gave negative results, multiple cultures were positive with the same organism (controls negative), and clinical course was consistent with the bacteriology. All patients had previous cardiac surgery or rheumatic valvulitis. Endocarditis occurred more than 2 years after surgery in four of five cases. ¶ Comparison of patients with atypical bacterial endocarditis with patients having endocarditis owing to classical bacterial forms showed no significant differences in bacterial species isolated (*Staphylococcus: aureus, albus; Streptococcus: viridans, fecalis, microaerophilic*), number or per cent positive cultures, incidence of underlying heart disease, or patient response to appropriate therapy. The atypical group differed in incidence of previous cardiac surgery, duration of illness before diagnosis (average >2 months), and incidence of associated skin rash (four cases), arthritis, or arthralgia (four cases). ¶ In three cases therapy was not begun before demonstration of septicemia. Treatment was unsuccessful in three other potients until therapy was altered

as a result of identification of atypical bacteria. Other diagnoses considered included pneumonia, two cases; rheumatic fever, three cases; and rheumatoid arthritis or Still's disease, four cases. Latex fixation was positive in four cases, but fell promptly in each with antibiotics. ¶ The identification of atypical bacteria in endocarditis was found to be significant *1*) in establishing correct early diagnosis and *2*) in identifying pathogens for proper drug selection. In some patients, immunologic mechanisms may have been important in producing both atypical bacterial forms and clinical manifestations resembling serum sickness.

Effects of Sex Hormones on the Metabolism of the Arterial Intima. ARAM V. CHOBANIAN, Boston, Mass. (introduced by Robert W. Wilkins†).

Although a marked sex difference in predilection to atherosclerosis is well recognized, little information is currently available to explain this difference. The present investigation was undertaken to determine whether sex hormones influence the metabolism of the arterial wall. Intact segments of human, dog, and rat arteries were incubated with various estrogenic hormones (10^{-7} to 10^{-9} mole per ml) and testosterone (10^{-8} mole per ml), and the effects of these hormones on the incorporation of labeled precursors into lipids, RNA, and proteins were examined. Similar studies of arterial wall metabolism were carried out *in vivo* in immature rats treated with estradiol (0.5 to 10 μg) or testosterone (50 μg). The uptake of estradiol-^{3}H and testosterone-^{3}H by human and canine arteries was also examined. The results indicate: *1*) Estrogenic hormones stimulate arterial phospholipid synthesis both *in vivo* and *in vitro*. The enhancement of phospholipid synthesis occurs in atherosclerotic as well as normal intima and is inhibited by puromycin. The intimal synthesis of other lipids does not appear to be influenced by estrogens. *2*) Estrogens appear to increase RNA and protein synthesis *in vivo*. *3*) Testosterone inhibits in whole or in part the *in vitro* enhancement of phospholipid synthesis produced by estrogens. *4*) Rapid entrance of both estradiol and testosterone into the arterial intima is present. Testosterone does not appear to inhibit the rate of uptake of estradiol by the intima. These studies suggest that sex hormones can act locally on arterial wall metabolism in both man and animals. Estrogens appear to stimulate arterial synthesis of phospholipid, RNA, and protein, whereas testosterone may inhibit the phospholipid stimulatory effects of estrogens.

Demonstration of an Apical Site of Action of Vasopressin in Toad Bladder Epithelium. MORTIMER M. CIVAN AND HOWARD S. FRAZIER,* Boston, Mass.

Vasopressin stimulates transport of sodium ions across the urinary bladder of the toad. Indirect evidence has suggested that the hormone acts by selectively decreasing the resistance of the apical permeability barrier, while not significantly affecting the basal barrier or active transport mechanism of the mucosal cells. ¶ To determine more directly the site of hormonal action we introduced glass micropipettes into individual epithelial cells *in vitro*, and monitored the electrical characteristics of each of the permeability barriers before and during the administration of vasopressin. The electrical potential differences between the intracellular micropipette and reference solution and across the entire bladder were measured while pulses of depolarizing or hyperpolarizing current were applied; these observations permitted calculation of the total transbladder resistance and the fractional resistance across the apical membrane. After base-line measurements, vasopressin was added to the serosal bathing medium without moving the micropipette from its position within the cell. In each of 12 successful impalements, the transbladder resistance decreased. In 6 of these, the entire resistance change was localized to the apical membrane; in the remaining experiments, the resistance change was predominantly apical in location. ¶ These data provide the first direct evidence that the major action of vasopressin is to reduce selectively the resistance to sodium movement across the apical permeability barrier of the transporting cells of toad bladder. The results further suggest that the vasopressin-sensitive pathway for sodium entry communicates with the cytoplasm of the epithelial cells.

Identification of Distal Nephron Sites of Action for Certain Diuretics. JAMES R. CLAPP* AND ROSCOE R. ROBINSON,* Durham, N. C.

Although it is generally accepted that diuretic drugs inhibit sodium reabsorption by the renal tubule, their exact sites of action within the nephron remain controversial. In these studies, renal micropuncture techniques were used in the dog to examine the effects of chlorothiazide, chlormerodrin, acetazolamide, and furosemide, and furosemide and chlorothiazide in combination, on sodium reabsorption by the ascending limb of Henle's loop and the distal convoluted tubule. Osmolalities were measured in 144 samples of distal fluid; a diuretic-induced increase in the osmolality of the normally hypotonic early distal fluid (15 to 20% length) was used as presumptive evidence of decreased sodium transport by the ascending limb. Furosemide (30 to 200 mg per kg), chlormerodrin (2 mg Hg per kg), and acetazolamide (15 mg per kg) were administered during antidiuresis; chlorothiazide (5 mg per kg) was given during water diuresis. The normal hypotonicity of distal fluid (average osmolal TF/P ratio: 0.41 ± 0.13) was not altered by acetazolamide (osmolal TF/P ratio: 0.41 ± 0.08). In contrast, the administration of chlormerodrin or furosemide was followed by a significant elevation of the distal osmolal TF/P ratio to 0.61 ± 0.10 and 0.83 ± 0.08, respectively. The distal TF/P ratio rose even further to 0.93 ± 0.01 when chlorothiazide and furosemide were administered in combination. Similar qualitative effects were noted when chlorothiazide was given alone during water diuresis: the distal TF/P ratio rose from 0.23 ± 0.07 to 0.38 ± 0.04. In all experiments, diuretic-induced alterations of distal fluid osmolality were maximally apparent in the earliest portions of the distal tubule; further change along the length of the distal tubule was not apparent.

These data demonstrate that the sites of action of chlorothiazide, chlormerodrin, and furosemide (but not acetazolamide) must include the ascending limb of Henle's loop. The effects of chlorothiazide and furosemide are additive within this nephron segment, but their combined administration does not inhibit sodium reabsorption by the distal nephron completely.

Effect of Phenobarbital on Endogenous Carbon Monoxide Production in Normal Man. R. F. COBURN, Philadelphia, Pa. (introduced by R. Austrian†).

It has been shown previously in our laboratory that there is a fraction (20%) of the total CO production in normal man that cannot be accounted for by catabolism of hemoglobin in circulating erythrocytes. It seems that this fraction, termed "excess CO," might originate in part as a product of catabolism of the hepatic microsomal heme enzymes, cytochrome P_{450} or cytochrome b_5 or both. It has been shown by others that administration of phenobarbital to rats and dogs results in up to a 10-fold increase in these enzymes in liver; therefore, if "excess CO" were originating from the heme moiety of these compounds the rate of CO production ($\dot{V}_{CO}$) might increase after "induction" with this drug. Phenobarbital was given orally (60 mg per day) to five normal subjects for 7 days. $\dot{V}_{CO}$ was determined using the rebreathing method developed in our laboratory. Control $\dot{V}_{CO}$ averaged 16.7 ± (SE) 1.8 μmoles per hour. After 7 days of phenobarbital, $\dot{V}_{CO}$ ranged from 25 to 56 μmoles per hour and averaged 37.5 ± (SE) 3.1 μmoles per hour in the five subjects. $\dot{V}_{CO}$ decreased toward the control value with a $t_{\frac{1}{2}}$ of approximately 2 days after termination of the drug. Reticulocyte counts and blood hemoglobin concentrations remained normal. These data are consistent with the concept that "excess CO" may arise, at least in part, during catabolism of hepatic microsomal heme enzymes. If it is assumed that the rate of hemoglobin catabolism remained constant, it is calculated that "excess CO" increased 2- to 10-fold after 7 days on phenobarbital and made up 40 to 70% of the total $\dot{V}_{CO}$. "Excess CO" has been shown to parallel "early labeled bilirubin" after glycine-2-^{14}C administration in normal man. This correlation together with the above observations suggests that hepatic microsomal heme enzymes may be a precursor of bilirubin as well. The measurement of $\dot{V}_{CO}$ may be valuable in studies of induction of hepatic microsomal enzymes with various agents.

Diethylstilbestrol Influence on the Reassembly of Lactate Dehydrogenase (LDH) Isozymes after Salt Depolymerization. L. COHEN, J. DJORDJEVICH, AND J. SECKLER, Chicago, Ill. (introduced by William R. Barclay†).

Despite the similarity in serum total LDH in males and females, we were previously able to demonstrate a sex difference in the distribution of serum LDH isozymes separated electrophoretically. Menstruating or pregnant females have relatively and absolutely more LDH_1, and less LDH_2 and LDH_3, than do men or postmenopausal women. Also, all men receiving an estrogenic substance (diethylstilbestrol) for prostatic cancer therapy develop the female pattern. It was hypothesized that the sex hormones might be influencing the tetrameric assembly of the serum and tissue LDH isozymes. A study to test this assembly hypothesis was therefore undertaken. ¶ Mixtures of liver and heart extracts, containing LDH isozymes, were frozen in aqueous 1 M NaCl. Under these conditions, LDH isozymes depolymerize into two different monomers and on thawing reassemble approximately at random in a distribution quite different from the control. When the freezing and thawing are allowed to take place in the presence of diethylstilbestrol concentrations (10^{-4} to 10^{-6} M) that do not alter LDH activity, the formation of LDH_1 and LDH_2 is visibly favored at the expense of LDH_3. These effects are not seen in the absence of NaCl, or after adding 10^{-4} to 10^{-6} M concentrations of estradiol, estrone, testosterone, or progesterone in ethanol, or ethanol alone (10^{-1} to 10^{-2} M). ¶ These observations suggest that only compounds of a very specific molecular configuration can alter the random *in vitro* tetrameric reassembly of the two different LDH monomers. The findings that sex-related differences in the serum LDH isozymes distribution exist, and that a synthetic estrogenic substance taken orally can influence the serum LDH isozyme distribution, or when added *in vitro* that in the tissues, support the hypothesis that estrogenic substances play a role in the molecular assembly of LDH tetramers.

Disparity between Biological and Immunological Characteristics of Apparent IF-B_{12} in Human Ileum during B_{12} Absorption *In Vivo*. BERNARD A. COOPER,* Montreal, Canada.

During absorption of B_{12}-^{57}Co, a specimen of ileum was obtained from two patients with carcinoma of the colon. When an extract of ileum was fed to a totally gastrectomized subject, 57 and 67% of the ^{57}Co activity was excreted in the urine during two Schilling tests. None (<0.00%) of an identical quantity of B_{12}-^{58}Co fed with the extract was excreted in the urine. Schilling test with normal gastric juice and B_{12}-^{57}Co concentration identical with the total B_{12} in the intestinal extract resulted in excretion into the urine of 53% of the B_{12}-^{57}Co, indicating that most of the B_{12}-^{57}Co in the intestinal extract functioned *in vivo* as if bound to intrinsic factor. The ^{57}Co activity also functioned *in vitro* (guinea pig intestinal homogenate assay) as if it were vitamin B_{12} bound to intrinsic factor. ¶ Despite its biological activity, only 30% of the ^{57}Co activity in the extract combined with antibody to human IF during filtration through Sephadex, and even this dissociated during precipitation of gamma globulins with ethanol. B_{12}-^{58}Co bound to human IF reacted with anti-IF even when incubated with intestinal extract. ¶ Most of the ^{57}Co activity in the intestinal extract filtered through Sephadex with mobility identical with that of human IF. Some, however, filtered as if its molecular weight were 80 to 100,000. Although TC-2 labeled with B_{12}-^{57}Co *in vitro* filters through Sephadex

with an apparent molecular weight of 40,000, the ^{57}Co activity in human portal venous blood during B_{12} absorption was restricted to a fraction with apparent molecular weight of 80 to 100,000. Its mobility on DEAE cellulose, however, was identical with that of TC-2. ¶ It is suggested that during absorption of B_{12} *in vivo*, a biologically intact IF-B_{12} complex enters the wall of the ileum. The IF then is degraded, degradation first altering the portion responsible for antigenicity. The B_{12} then probably enters the portal vein as a dimer of TC-2.

Modifications of Control Mechanisms during Artificial Circulation. T. COOPER,* D. V. PRIOLA, R. L. FULTON, AND W. R. BLAKELY, Albuquerque, N. M.

Systems of total and partial circulatory support are designed to insure adequate flows of oxygenated blood throughout the body. However, regulation of arterial blood flow at tolerable mean arterial pressures (MAP) does not insure normal distribution of blood flow. We have consistently observed striking changes in circulatory reflexes and drug responsiveness during extracorporeal circulation. Reflexes and pharmacological reactivity to *l*-norepinephrine (NE) were studied in 20 dogs during cardiopulmonary bypass at 34 to 38° C, pH 7.3 to 7.6, with net arterial perfusion rates of 75 to 110 ml per kg per minute and in MAP of 60 to 200 mm Hg. ¶ During total cardiopulmonary bypass, bilateral cervical vagotomy and mechanical exclusion of the coronary bed were unassociated with consistent changes in central hemodynamics, and bilateral occlusion of the carotid arteries did not result in the usual cardioaccelerator and pressor responses, indicating significant modification of baroreceptor control during perfusion. In contrast, carotid occlusion in the same animals, at identical flow rates, but on a right ventricular bypass, was accompanied by a response pattern similar to that observed in the intact animal. ¶ Increases in total peripheral resistance (TPR) induced by NE infusion (0.5 to 10.0 μg per kg per minute) were *1*) inversely related to control TPR, *2*) significantly attenuated (50%) after vagotomy, and *3*) not consistently modified during exclusion of the coronary bed. Venous constriction during NE infusion was *1*) diminished or reversed by vagotomy but *2*) significantly (3- to 10-fold) augmented after exclusion of the coronary bed. ¶ These studies suggest that artificial circulation at so-called physiological parameters is associated with modified and perhaps inappropriate activity of circulatory control mechanisms and emphasize the importance of indirect (neural) factors as determinants of the response of the perfused vascular system to drugs.

Effect of Sodium Phenobarbital on the Metabolism of Bilirubin-^{3}H and ^{14}C in an Infant with Congenital Nonhemolytic Jaundice and Kernicterus. JOHN F. CRIGLER, JR., AND NORMAN I. GOLD, Boston, Mass. (introduced by Charles A. Janeway†).

We have previously reported the ability of sodium phenobarbital (15 mg, twice daily, by mouth) to lower serum bilirubin concentration in an infant with congenital nonhemolytic jaundice and marked kernicterus. To study kinetic aspects of bilirubin metabolism in the same infant, bilirubin-^{3}H was administered during two control periods (serum bilirubin, 20 and 23 mg per 100 ml, respectively), and bilirubin-^{3}H and ^{14}C (to confirm the ^{3}H-label results) were administered simultaneously during phenobarbital treatment (serum bilirubin, 6.7 mg per 100 ml). Bilirubin specific activities were determined from frequent serum samples obtained on the day of administration of the labeled bilirubin and 1 or 2 times daily for 14 days. Stools and urines were collected continuously for 18 days, and total radioactivity was determined. The following metabolic parameters were obtained for the first control, second control, and phenobarbital studies, respectively: total bilirubin pool (mg)—229, 210, 82; serum bilirubin pool (mg)—86, 99, 30; bilirubin half-life (hours)—111, 84, 38; bilirubin turnover (mg per 24 hours)—34, 42, 36; radioactivity in feces (% administered)—48, 70, 86 (the low recovery in the first control remains unexplained); radioactivity in urine (% administered dose)—3, 6, 6. Values calculated from ^{14}C were similar to those calculated from ^{3}H. The decrease in serum bilirubin concentration is not attributed to a transfer of bilirubin from the serum into extravascular pools, since the values for serum pool/total body pool were similar. These data indicate that phenobarbital decreased serum bilirubin concentration by enhancing its rate of excretion from the body pool.

The Uptake of Native Insulin by Isolated Fat Cells. OSCAR B. CROFFORD, Nashville, Tenn. (introduced by C. R. Park†).

The significance of previous attempts to measure the uptake or binding of insulin by various tissues has been difficult to assess owing to the use of superphysiological concentrations of insulin, the doubtful validity of radioactive insulin as a tracer of biologically active insulin, and uncertainty concerning the amount of insulin trapped within the interstitial spaces. In the present investigation, these difficulties have been overcome by measuring the binding or uptake or both of native insulin by a suspension of fat cells from rat epididymal tissue incubated in low concentrations of the hormone. Insulin uptake was measured as a change in insulin concentration of the incubation medium estimated both by radioimmunoassay and by the stimulation of glucose-^{14}C conversion to $^{14}CO_2$ in fat cells. ¶ Cells incubated in concentrations of insulin varying from 4 to 1,000 μU per ml showed an immediate (<1 minute) uptake of hormone that was proportional to its concentration in the incubation medium. Cells exposed to insulin, then removed from the incubation medium but not washed, and exposed to insulin again within 5 minutes showed an undiminished uptake of insulin from the fresh medium. When insulin treated cells were washed, no immunologically or biologically reactive insulin could be recovered in the medium. However, washing or exposure to insulin antibody abolished the hormone effect on glucose metabolism. It is concluded that insulin is rapidly bound, rapidly metabolized, and must

be continually replenished to maintain its effect on glucose utilization.

Determination of Total Body Oxygen Stores in Normal Man and Seal. CARROLL E. CROSS, BERNARD S. PACKER, MICHAEL ALTMAN, J. B. L. GEE, H. VICTOR MURDAUGH,* AND EUGENE D. ROBIN,† Pittsburgh, Pa.

Total body O_2 stores (TBO_2) consist of O_2 in lung air (LO_2) and nonlung O_2 in blood and tissues (NLO_2). Although TBO_2 is of interest as a measure of oxygen reserve, no method is available for direct experimental determination. In these studies TBO_2 was determined by the dilution of stable isotope $^{18}O_2$. ¶ In a rebreathing circuit it can be shown that: $V_1(F_1{}^{18}O_2/F_1{}^{16}O_2) = (V_t + TBO_2)(F_t{}^{18}O_2/F_t{}^{16}O_2) + \int_0{}^{t}V^{18}O_2\,dt$. ($V_1$ and V_t refer to initial and final volumes of oxygen in the external circuit; $F_1{}^{18}O_2/F_1{}^{16}O_2$ and $F_t{}^{18}O_2/F_t{}^{16}O_2$ refer to initial and final specific activities of $^{18}O_2$; $V^{18}O_2$ refers to $^{18}O_2$ consumption; t is time.) Since $V_t = V_1 - t(V^{16}O_2)$ and since the simplifying assumption that $\int_0{}^{t}V^{18}O_2\,dt = t(F_t{}^{18}O_2/F_t{}^{16}O_2)(V^{16}O_2)$ can be made, the relationship becomes: $TBO_2 = V_1[(F_1{}^{18}O_2/F_1{}^{16}O_2)/(F_t{}^{18}O_2/F_t{}^{16}O_2) - 1]$. ¶ V_1 was determined by direct measurement and specific activity by mass spectroscopy. LO_2 was determined by measuring neon dilution. NLO_2 equals $TBO_2 - LO_2$. Measurement of $F^{18}O_2/F^{16}O_2$ ratios in expired air, arterial blood, and venous blood showed that equilibrium was achieved within 5 minutes. Duplicate measurements agreed within 1%. The variation of TBO_2 in a series of eight studies performed over a 12-month period in a single individual was 5%. ¶ Measurements of TBO_2 and its subdivisions were performed in six normal men, and for comparison in three female harbor seals (selected for the known high red cell mass in this species). The results (mean ± SD) were as follows: TBO_2 (ml per kg, BTPS), 6 men (20 studies), 16 ± 2, 3 seals (12 studies), 43 ± 6; NLO_2 (ml per kg, BTPS), 6 men (20 studies), 12 ± 3, 3 seals (12 studies), 28 ± 3. The relatively large NLO_2 in the seal is consistent with previous observations of increased red cell mass. ¶ This technique may supply insight into such problems as the nature of adaptations to clinical and biological O_2-depletion states.

The Influence of the Plasma Sodium on Proximal Tubule Sodium Reabsorption. BERNARD B. DAVIS, FRANKLYN G. KNOX, FRED S. WRIGHT, AND STUART S. HOWARDS, Bethesda, Md. (introduced by Maurice B. Burg*).

Expansion of the ECF by intravenous saline causes a depression of fractional sodium reabsorption by proximal tubule. Recollection micropuncture was used to study the influence of plasma sodium (P_{Na}) on this depression. Tubule fluid to plasma inulin concentration ratio was used to estimate fractional sodium reabsorption. To produce hyponatremia, we gave dogs low sodium diet and vasopressin for 7 days. The first 2 days 25 mg of ethacrynic acid was given. Water was given by gastric tube daily to maintain initial weight. Isotonic saline given seven normal dogs (mean P_{Na} 144) to expand ECF 5% of body weight depressed fractional sodium reabsorption 48.1% ± SE 3.6. Without changing P_{Na} the same ECF expansion of seven hyponatremics (mean P_{Na} 126) resulted in less depression of fractional sodium reabsorption, 19.4% ± SE 7.5 ($p < 0.005$). Without changing plasma osmolality this expansion induced a water diuresis in all seven dogs. Six additional hyponatremics (mean P_{Na} 130) were expanded a like amount with simultaneous restoration of the P_{Na} to normal (mean 146). Depression of fractional sodium reabsorption, 48.5% ± SE 5.6, was similar to that of normals, but significantly greater than in the first group of hyponatremics ($p < 0.02$). In this restored group there was a mean increase in P_{osm} of 29.8, yet water diuresis resulted in four of six dogs. The difference in depression of fractional sodium reabsorption between normal and hyponatremics was eliminated by restoration of P_{Na} to normal. For uncertain reasons ECF expansion can induce a water diuresis in hyponatremic dogs. It is concluded that hyponatremia inhibits depression of fractional sodium reabsorption in proximal tubule in response to ECF expansion.

Passive Anaphylaxis to Endotoxin. CHARLES DAVIS, WILLIAM TATE III, HERNDON DOUGLAS, AND ABRAHAM I. BRAUDE,† Pittsburgh, Pa.

Because antibiotics are ineffective in endotoxin shock, serum therapy has been investigated as an alternative. In our previous studies rabbit antiserum against smooth and rough bacteria, as well as rabbit antiserum against heterologous organisms, prevented death in mice given lethal doses of endotoxin. This suggested that protection was due to antibody against an antigen common to various endotoxins and was independent of O antibody. ¶ In the present study similar protection was attempted with antisera prepared in mice and rats. Instead of protecting, such sera sensitized mice so that otherwise sublethal doses of intravenous homologous endotoxin produced deaths from anaphylaxis in 60% of animals within 1 hour. The small doses of endotoxin never produced anaphylactic deaths in mice given immune rat serum prepared against heterologous endotoxins. Mice receiving these sera died rapidly from anaphylaxis only after challenge with doses of heterologous endotoxin large enough to produce the characteristic delayed death of nonsensitized mice. ¶ Further analysis disclosed that anaphylaxis to endotoxin was *1*) provoked only by the 7 S component of immune serum fractionated by column chromatography, *2*) not elicited by antiserum against a rough *Escherichia coli* O:113 mutant whose endotoxin was shown by gas chromatography to be free of galactose and therefore O side chains, *3*) unaffected by elimination of protein from the endotoxin, *4*) not demonstrated with the light, nontoxic polysaccharide separated from endotoxin by density gradient ultracentrifugation, and *5*) accompanied by heavy immediate pulmonary localization of ^{51}Cr-labeled *E. coli* O:113 endotoxin. ¶ These findings suggest that anaphylaxis results from a reaction between 7 S O antibody and lipopolysaccharide. The differential effects

of protective rabbit antibody and anaphylactic rat antibody are thus attributed to reactions with different antigenic sites on the lipopolysaccharide molecule.

A New, Accurate, and Simple Technique for the Assay of Antistreptolysin O Antibodies. FRANK H. DELAND AND HENRY WAGNER, JR.,* Baltimore, Md.

The present method for the routine determination of antistreptolysin O titer in serum has several disadvantages: The procedure requires multiple pipettings for a single determination (approximately 65, if controls are included); all control sera must be standardized in relation to an international standard by indirect methods; and variation in susceptibility of RBC to lysis can result in errors of as much as 30%. The latter can be minimized by colorimetric assay of hemolysis, but this is cumbersome and inaccurate. We have devised a new procedure that is simpler and much more accurate. The technique is as follows: The unknown serum sample is incubated with streptolysin O until the reaction between the antibody and streptolysin is complete. A control tube contains buffer in place of serum. A standard quantity (1 ml of a 4% suspension) of ^{51}Cr-labeled RBC is then added, and the amount of free radioactivity is measured after 15 minutes. The antistreptolysin O titer is the ratio of the amount of erythrocyte-bound activity in the unknown to the amount of free radioactivity in the control. The method eliminates variation in erythrocyte susceptibility to lysis. Titration errors are minimized by using an internal standard for both specimen and control. Since the number of RBC lysed is linearly related to the amount of antistreptolysin present, it is possible to obtain an absolute rather than a relative measurement of streptolysin activity. The error of the new method varies from 3% at the lowest titer level to 4 to 5% at the level of 3,000 Todd units. The advantages of this procedure are its accuracy and simplicity and the fact than an exact level of antibody can be determined with a single specimen tube, without the necessity of making measurements of 12 serial dilutions. The time and effort required are less than 25% of the present method.

Early Identification of Bacterial Growth in Blood Cultures. FRANK H. DELAND AND HENRY N. WAGNER, JR.,* Baltimore, Md. (introduced by William H. Zinkham*).

The initial detection of positive blood cultures depends upon subjective evaluation of the culture media 24 hours after inoculation. We have developed a simple and objective means of detecting bacterial growth in a blood culture within 12 hours. The principles of the test are: *1)* Eighty per cent of the organisms found in bacteremia produce hemolytic toxins; *2)* hemolysis can be detected easily, objectively, and with great sensitivity by means of ^{51}Cr-labeled RBC. The technique is as follows: Two-tenths of a ml of ^{51}Cr-labeled RBC is added to the blood cultures at the time of inoculation. Growth is identified in 10 to 14 hours by measuring the free radioactive chromium in 1 ml of the supernatant of the culture media. To evaluate the procedure blood culture media were inoculated with streptococci, staphylococci, pneumococci, coliform bacilli, and diphtheroids together with ^{51}Cr-labeled RBC. Hemolysis increased logarithmically as a function of time and was not greatly influenced by the size of the original inoculum. Bacterial inoculations of 10^1 to 10^7 organisms per ml produced little hemolysis for 8 to 9 hours. Thereafter hemolysis increased rapidly in an exponential manner. Within 24 hours the amount of hemolysis was about the same over a wide range of inocula. Thirty-five positive cultures were artificially produced by inoculations of various organisms, and the new procedure was compared with present techniques. All positive cultures were correctly detected from an unknown group of 50 specimens. No false positives were found in this group or in 100 consecutive negative routine blood cultures. The method is presently undergoing clinical trials.

Mucopolysaccharide, Hydroxyproline, and Mucoprotein Excretion by Severely Burned Patients. D. JOSEPH DEMIS* AND BRUCE M. MCALLISTER, St. Louis, Mo.

Thirty-five severely burned patients were studied. The extent of the burn varied from about 18% to 98%. The patients ranged in age from 3 to 88 years (6 were children and 23 were males). Total mucopolysaccharide (MPS) excretion was quantitated on a daily basis using the carbazole method and expressed as uronic acid. Severely burned individuals had a significantly elevated MPS excretion with values as high as 70 mg a day (14 times the normal average excretion). After a burn there was an initial lag period of 1 to 4 days before MPS excretion became elevated and reached a peak at about 1 to 2 weeks. In surviving patients there was a correlation between the size of the burn (expressed as per cent of skin involved) and the amount of MPS appearing in the urine. Nonsurvivors tended to have lower daily MPS excretions than did survivors with comparable burns. In a few patients there appeared to be some correlation between urine volume and MPS excretion. However, studies of normal subjects showed urine volume and MPS excretion to be independent. The urinary MPS was characterized from 4 severely burned patients, and large amounts of chondroitin-4-sulfate and a trace of keratosulfate were isolated, in addition to small quantities of heparitin sulfate and dermatan sulfate. ¶ These patients were also found to excrete large amounts of hydroxyproline (usually 10 to 20 times base-line levels); some patients excreted in excess of 1,000 to 1,200 mg daily. An increased excretion of mucoprotein of the same magnitude was also detected with values as high as 800 to 900 mg daily. There was a good correlation between the increased excretion of MPS and elevated mucoprotein and hydroxyproline excretions. ¶ The data suggest that the increased quantities of MPS appearing in the urine were not derived from the original burned skin, but rather from granulation tissue that appears with healing. The increased quantities of hydroxyproline and mucoprotein excreted by burn patients also appear to originate in the granulation tissue.

The Role of Bile Salt in Controlling the Rate of Intestinal Cholesterogenesis. JOHN M. DIETSCHY, Dallas, Texas (introduced by Jay P. Sanford*).

We have previously shown that biliary diversion markedly enhances intestinal cholesterogenesis. In the present investigation the inhibitory constituent of bile was identified, and its anatomic and biochemical sites of action were demonstrated. ¶ *1)* An inverse relationship exists down the small intestine between total bile salt concentration ([TBS]) in the lumen and the rate of incorporation of acetate-2-^{14}C into cholesterol by the mucosa; thus maximal [TBS] occurs in the mid-intestine (18 ± 3 mmoles per L) where mucosal cholesterogenesis is minimal (20 ± 5 mμmoles per g tissue per 2 hours). *2)* Biliary diversion or feeding of the bile salt sequestrant cholestyramine enhances cholesterogenesis (to 348 ± 59 and 269 ± 52 mμmoles per g per 2 hours, respectively) without affecting the rates of acetate-^{14}C incorporation into CO_2 or fatty acids. *3)* Infusion of whole bile into the bowel of animals with biliary diversion suppresses cholesterogenesis, whereas whole bile depleted of bile salt with cholestyramine does not suppress synthesis; however, the suppressive effect can be restored by adding pure taurocholate back to the treated bile. Finally, infusion of taurocholate alone specifically suppresses cholesterogenesis but has no effect on other parameters of cell metabolism. *4)* Anatomically, suppression of cholesterol synthesis by bile salt occurs in cells of the intestinal crypts but not those of villi. *5)* Since the incorporation of ^{14}C-mevalonate into cholesterol is not inhibited by bile salt, the biochemical site of suppression is before mevalonate synthesis, presumably at the conversion of hydroxymethylglutarate to mevalonic acid. ¶ The gastrointestinal tract is known to be a major source for circulating serum cholesterol. This demonstration that alterations in luminal bile salt concentrations exert a specific rate-controlling effect upon intestinal cholesterogenesis provides the first known mechanism whereby physiologic control of sterol synthesis in this organ may be mediated.

Release of Leukocyte Pyrogen by Etiocholanolone. MORRIS DILLARD AND PHYLLIS BODEL, New Haven, Conn. (introduced by P. K. Bondy†).

Etiocholanolone, a naturally occurring steroid in man, produces fever when injected intramuscularly. Unusual features of this reaction include the long latent period before fever is induced and the apparent inability of these agents to produce fever in animals other than man. The pyrogenicity of related steroids depends on particular structural features. Although inflammation and granulocytosis accompany experimental steroid fever, the pathways by which steroids induce fever have remained obscure. ¶ With the recent finding that human leukocyte pyrogen causes fever in rabbits, experiments were set up to determine if etiocholanolone could activate human blood leukocytes to produce pyrogen *in vitro*. These studies have demonstrated that an endogenous pyrogen is released when etiocholanolone or related steroids are incubated with human blood leukocytes in a serum-buffer medium. The characteristics of this reaction closely resemble those of experimental steroid fever. ¶ First, release of pyrogen varies directly with the concentration of steroid added. Second, 4 to 8 hours of contact between steroid and leukocyte is required for activation of the cell. Third, rabbit leukocytes are not activated by etiocholanolone. Fourth, the varying ability of steroids of both the C-19 and C-21 series to activate leukocytes *in vitro* correlates closely with their reported pyrogenic activity when injected. For example, androsterone is nonpyrogenic in the *in vitro* system, as it is *in vivo*. Fifth, cortisone, an agent which suppresses steroid fever, reduces pyrogen production by steroid-activated leukocytes *in vitro*. ¶ Studies of the mechanism of this reaction indicate that actinomycin D prevents activation of the cells and that puromycin also blocks pyrogen production. Thus, alterations in RNA and protein synthesis appear to be involved. ¶ Abnormalities of steroid metabolism occur in certain clinical fevers. Our studies, in addition to providing an explanation for the occurrence of experimental steroid fever, suggest that naturally occurring steroids may play a similar role in producing some clinical fevers.

Myosin-synthesizing Polyribosomes from Embryonic Chick Muscle. ROBERT M. DOWBEN,* STUART M. HEYWOOD, AND ALEXANDER RICH, Cambridge, Mass.

Muscle is a system uniquely suited for studying the biosynthesis of relatively insoluble structural proteins, their organization into an architecturally complex pattern, the effects of various hormones, and the alterations in disease. With these goals in view, a polyribosomal system from chick embryonic muscle has been prepared which incorporates ^{14}C-labeled amino acids into myosin *in vitro*. Leg muscles from 14-day chick embryos were homogenized in high ionic strength buffer, $\mu = 0.27$. Active preparations could not be obtained with isotonic salt or sucrose buffers because myosin forms nonspecific coprecipitates with polyribosomes of all classes at low ionic strengths. The supernatant fraction obtained after centrifugation at $11{,}000 \times g$ was layered on a 15 to 40% linear sucrose gradient and centrifuged at 25,000 rpm for 2 hours in a Spinco 25.1 rotor. A 260 mμ absorbance profile of the gradient showed a peak corresponding to polyribosomes of 50 to 60 U. Such polyribosomes might be expected to synthesize a protein of molecular weight 170,000 to 200,000; myosin (mol wt, 580,000) is thought to consist of three identical subunits. *In vitro* amino acid incorporation was carried out using 0.5 mg heavy polyribosomes per ml buffer (0.15 M KCl, 0.01 M $MgCl_2$, 0.006 M 2-mercaptoethanol, 0.01 M Tris, pH 7.4), containing 2 μmoles ATP, 0.5 μmoles GTP, 10 μmoles phospho*enol*-pyruvate, 5 μmoles ^{14}C-labeled amino acid mixture, 50 μg pyruvate kinase, 0.2 mg chicken liver t-RNA, and 0.4 mg pH 5 enzyme fraction from muscle, and incubating at 37°. The labeled protein obtained possessed the solubility properties of myosin, gave a monodisperse single peak corresponding to authentic chicken myosin upon electrophoresis in 12 M urea-acrylamide gel, and reacted with antibody prepared by using chicken myosin as antigen.

Plasma 17-Ketosteroid Levels during Adolescence. WALTER R. EBERLEIN,* ALFRED M. BONGIOVANNI,* AND ROBERT L. ROSENFIELD, Philadelphia, Pa.

To study adrenal maturation at adolescence, we collected pooled blood from prepubertal males, ages 6 to 9 years, and from pubertal males, ages 12 to 14 years. Plasma steroid sulfates were extracted in the form of the methyl green salts, which were solvolyzed and then successively subjected to separation by means of the Girard T-reagent, digitonin precipitation, thin-layer chromatography, and gas-liquid chromatography (free steroid, acetate, trimethylsilyl ether) on the phases SE-52 and QF-1. In the 6- to 9-year-old pool, the level of androsterone sulfate (AS) was 8.6 μg per 100 ml, and of dehydroepiandrosterone sulfate, 13.5 μg per 100 ml. In the 12- to 14-year-old pool AS was 56.7 μg per 100 ml; dehydroepiandrosterone (DHAS) was 80.3 μg per 100 ml. In adult males, studied for comparison, plasma levels of AS were 40 to 53 μg per 100 ml and of DHAS, 89 to 209 μg per 100 ml. ¶ In the nonketonic fraction of both pools traces of presumed 17α,20α-dihydroxy-cholesterol were detected. Provisional identification was based upon the behavior of the unknown on thin-layer chromatography, column chromatography, and gas-liquid chromatography as the free steroid on SE-52 and QF-1, as well as the trimethylsilyl ether on the latter phase. ¶ The dramatic rise of plasma AS and DHAS during adolescence appears to occur simultaneously with beginning testicular maturation under gonadotropin stimulation. Since ACTH secretion is not known to change at the time of adolescence, the above observations suggest that adrenal maturation at this age is not directly related to ACTH stimulation but to some other stimulus. The finding of a sterol behavior like 17α,20α-dihydroxy-cholesterol before as well as during puberty may suggest that adrenal maturation at adolescence may represent the induction of a desmolase, which cleaves the sterol to dehydroepiandrosterone.

Abnormal Ventilatory and Circulatory Responses to Hypoxia in Familial Dysautonomia. NORMAN H. EDELMAN, ERNEST C. RICHARDS, AND ALFRED P. FISHMAN,† New York, N. Y.

Abnormal reflexes are characteristic of familial dysautonomia. The present study evaluated the reflex ventilatory and circulatory responses to hypoxia in this disorder. Six trained patients with familial dysautonomia (FD) were compared to six matched controls (C). Rebreathing and open circuit techniques using air and hypoxic gases were used to determine CO_2 response curves and the ventilatory response to hypoxia. Blood gases, heart rate, and systemic blood pressure were monitored. Patients differed from controls in the following ways: *1*) Hypoxia produced a striking decrease, rather than an increase, in the slope of the CO_2 response curve (FD: 0.65 to 0.13 L per minute per mm Hg; C: 1.18 to 3.39). *2*) Patients manifested bradycardia and severe hypotension when hypoxic, instead of tachycardia and slight hypertension. Ventilation varied directly with blood pressure in these studies. *3*) Hypoxia per se (Sa_{O_2}; 80 to 85%) failed to increase ventilation (FD: +1.2%; C: +66%, but abrupt relief of hypoxia with pure oxygen produced an inordinate transient fall in ventilation (FD: −76% of air ventilation; C: −27%). ¶ These results demonstrate a marked impairment of the ventilatory and circulatory responses to hypoxia. The administration of pure oxygen revealed that there had been considerable peripheral chemoreceptor activity during hypoxia that was obscured by an inordinately low central respiratory drive. The data therefore suggest that the hypotension induced by hypoxia played an important role in depressing ventilation by exaggerating cerebral hypoxia, a stimulus known to depress ventilation. The findings are of clinical importance since they may explain the high mortality during anesthesia, as well as the characteristic sequence of prolonged breath holding and syncope, in this disorder.

Oxygen Affinity of Blood in Erythrocytosis. MILES J. EDWARDS, MILES J. NOVY, AND JAMES METCALFE,* Portland, Ore.

The differential diagnosis of erythrocytosis may be difficult. A mild arterial hypoxemia may occur in polycythemia vera, and the hypoxemia of pulmonary disease may be of fluctuating severity. Construction of *in vitro* oxygen-hemoglobin equilibrium curves may aid in diagnosis. Equal volumes of fully oxygenated blood and fully deoxygenated blood (P_{CO_2} = 40 mm Hg, 37° C) are mixed anaerobically, and the resultant P_{O_2} and plasma pH are measured. The P_{O_2} at half-oxyhemoglobin saturation is corrected to a plasma pH of 7.40 and is designated P_{50}. ¶ The mean P_{50} of blood from normal subjects was 25.8 mm Hg (±0.4 mm Hg, N = 6). In blood from patients with arterial hypoxemia (arterial P_{O_2} less than 60 mm Hg) owing to either pulmonary disease or congenital heart disease, the mean P_{50} was 29.7 mm Hg (±0.9 mm Hg, N = 10). In blood from patients with polycythemia vera, with arterial P_{O_2} greater than 70 mm Hg, the mean P_{50} was 25.0 mm Hg (±1.4 mm Hg, N = 6). ¶ The observed increase of P_{50} (decrease in oxygen affinity) in patients with arterial hypoxemia allows an increased blood oxygen tension at a given oxyhemoglobin saturation and acts to facilitate oxygen release to the tissues by maintaining capillary oxygen pressure. Others have shown decreased oxygen affinity in anemia and cyanotic heart disease. Such an adjustment is not made in polycythemia vera. These findings suggest that an intraerythrocyte response to tissue hypoxia may occur by a decrease in oxygen affinity independent of plasma pH. In addition to its diagnostic value in erythrocytosis, measurement of oxygen affinity may serve as a useful index of tissue hypoxia.

Immunoglobulin Content of Intestinal Plasma Cells in Ataxia Telangiectasia. SHMUEL EIDELMAN AND STARKEY D. DAVIS, Seattle, Wash. (introduced by Cyrus E. Rubin*).

Most intestinal mucosal plasmacytes contain immunoglobulin A (IgA). Few contain immunoglobulins G or M (IgG, IgM). ¶ In previous studies of hypogammaglobu-

linemic subjects, we found that when serum IgA levels were very low, intestinal plasmacytes were virtually absent. However, in four children with ataxia telangiectasia and very low serum IgA, normal numbers of intestinal plasmacytes were found. Immunocytochemical studies were therefore done to identify the immunoglobulins contained in these cells. Fluorescein-conjugated rabbit antisera to human IgG, IgA, and IgM were used. ¶ Several rectal biopsies were studied from each of 4 ataxia telangiectasia patients and 17 immunologically normal children of similar age. IgG-containing cells were rare in both groups. In all biopsies from the normal children, the expected predominance of IgA-containing cells was found. The biopsies from the ataxia telangiectasia patients were markedly different, with few IgA-containing cells and many IgM-containing cells. ¶ To ascertain the ratio of IgA- to IgM-containing plasmacytes in the two groups, we selected three to four pairs of adjacent serial sections, 4 μ thick, from different parts of each biopsy specimen. One of each pair was incubated with a fluorescein-anti-IgA conjugate, the other with an anti-IgM conjugate. The total number of fluorescent plasmacytes in each section was counted and the IgA/IgM cell ratio computed for each section pair. Repeat counts by a second observer of 50 randomly selected coded sections showed a mean deviation of 5%. The mean ratios of IgA/IgM-containing plasma cells in the two groups of biopsies were: normals—8.1 (range, 2.9 to 33.4); ataxia telangiectasia—0.12 (range, 0.02 to 0.40). ¶ A statistical difference between the two groups was demonstrated by the Mann-Whitney nonparametric test ($p < 0.01$). ¶ These data suggest that in ataxia telangiectasia, although the number of intestinal plasmacytes is normal, their function is altered to predominant production of IgM rather than IgA.

Collagenolytic Activity in Normal and Diseased Human Skin. ARTHUR Z. EISEN, Boston, Mass. (introduced by T. B. Fitzpatrick†).

A collagenase produced by amphibian epithelium and active at physiologic pH and temperature has recently been demonstrated (Gross and Lapiere; Eisen and Gross). To date, there is little evidence for a specific enzyme responsible for removal of collagen fibers in normal human skin. Detection of a collagenolytic enzyme was undertaken in biopsy specimens of skin from normal subjects (ages, 15 to 70 years) and patients with the dystrophic, recessive form of epidermolysis bullosa (DEB). Fragments of whole skin were cultured on sterile, reconstituted ^{14}C-labeled mammalian collagen gels under physiologic conditions. Collagenolytic activity was detected by an increasing area of lysis around the explant and measured quantitatively by the release of soluble glycine-^{14}C containing peptides. Skin from the trunk and extremities solubilized an average of 21% of the collagen substrate, whereas postaural skin lysed an average of 38% of the gel. These data demonstrate the presence of a collagenolytic enzyme in normal human skin and suggest a regional difference in enzyme activity. Isolated dermis cultured separately showed no collagenolytic activity. Freezing and thawing the tissue before culture blocks the appearance of enzyme activity. Cultures of bullae produced by mild frictional trauma from patients with DEB showed collagenolytic activity approximately three times greater than that found in normal skin or in certain other bullous diseases. ¶ In addition to increasing our understanding of normal connective tissue remodeling, these studies suggest that collagenase may play a significant role in blister formation in DEB.

Stimulation of Lecithin Synthesis from Medium Lysolecithin during Phagocytosis. P. ELSBACH, New York, N. Y. (introduced by Saul J. Farber†).

The striking morphologic changes seen during phagocytosis have suggested increased formation of membranes. However, earlier studies have failed to provide convincing biochemical evidence of enhanced *de novo* synthesis of membrane constituents such as phospholipids. ¶ The present *in vitro* study concerns recently described alternative pathways of phospholipid (lecithin) synthesis as a possible source of membrane material during phagocytosis. ¶ With lysolecithin-^{32}P as substrate it was found that homogenates of rabbit granulocytes and lung macrophages contain enzymes that directly convert lysolecithin to lecithin. Intact resting cells, incubated in a medium containing lysolecithin-^{32}P, also incorporate ^{32}P radioactivity into cellular lecithin. Accumulation of lecithin-^{32}P proceeds linearly for at least 30 minutes. At equivalent substrate concentrations whole granulocytes per milligram protein incorporate considerably more lysolecithin into lecithin than do macrophages, whereas homogenates of both cell types are more active than whole cells. However, the amount of labeled lecithin associated with intact granulocytes after 30 minutes represents as much as 5% of total leukocyte lecithin. For macrophages this percentage does not exceed one. The mechanism of lecithin-^{32}P synthesis by intact cells is a direct acylation of medium lysolecithin. ¶ In the presence of polystyrene, zymosan, or starch particles, incorporation of exogenous lysolecithin into lecithin is stimulated up to three times. Virtually all newly formed lecithin remains associated with the cells, both at rest and during phagocytosis; apparently little is released or exchanged with medium lipid. This indicates net translocation of lysolecithin-^{32}P from medium to cells. ¶ Since lysolecithin is a normal constituent of plasma, direct conversion of medium lysolecithin to cellular lecithin can therefore account for appreciable addition of membrane phospholipid, especially by granulocytes. It is proposed that this mechanism of lecithin synthesis provides building blocks for increased membrane formation during phagocytosis.

The Diffusion Capacity of the Underventilated Portion of the Lung in Obstructive Disease: A Method. GEORGE EMMANUEL AND IVAN SAFONOFF, Brooklyn, N. Y. (introduced by Ludwig W. Eichna†).

The purpose of this investigation was to establish *a*) whether diffusion abnormalities exist in emphysema and

b) to what extent they contribute to hypoxia. In 12 patients with chronic obstructive lung disease the measured arterial oxygen saturation, Sa, was lower than predicted from the measured uneven ventilation and blood flow distribution and venous admixture, suggesting increased resistance to diffusion across the alveolar-capillary pathway. ¶ Results obtained from compartmental analysis of simultaneous lung and arterial blood nitrogen washout during 100% oxygen breathing are as follows: The fractional perfusion of the underventilated alveoli ($\dot{Q}c_2/\dot{Q}c_T$) averaged 0.44, their "calculated" ventilation/perfusion ratio ($\dot{V}_{A2}/\dot{Q}c_2$), 0.370, the "expected" end-capillary oxygen saturation, Sc_2 (i.e., if alveolar and end-capillary oxygen tension are in equilibrium) 88%, and the corresponding alveolar (P_{A2}) and/or end-capillary oxygen tension (Pc_2), 61 mm Hg. The respective values for the well-ventilated alveoli averaged 0.56, 2.1, 98.4, and 120. ¶ Sa predicted from these data averaged 91%, whereas the measured Sa was 87%. If alveolar-end-capillary oxygen tension gradient in the well-ventilated alveoli (P_{A1}-Pc_1) is nearly zero then a 17 mm Hg alveolar-end-capillary oxygen gradient owing to increased resistance to diffusion in the poorly ventilated alveoli (P_{A2}-Pc_2) must exist. ¶ From alveolar oxygen tension, "actual" end-capillary oxygen saturation, and mixed venous saturation, the alveolar-mean-capillary gradient (P_{A2}-Pc_2), the oxygen consumption ($\dot{V}o_{2_2}$), the diffusion capacity (D_{L2O_2}), and the $D_{L2O_2}/\dot{Q}c_2$ ratio of the underventilated alveoli were determined. These values were 22 mm Hg, 58 ml per minute, 3 mm per minute per mm Hg, and 0.0017. ¶ Conclusion: Diffusion and $\dot{V}A/\dot{Q}c$ distribution abnormalities are equally responsible for reducing Sa.

Similar Involvement of Some Chromosomal Groups in Leukemias and Birth Defects. E. ENGEL AND L. C. MCKEE, JR., Nashville, Tenn. (introduced by Elliot V. Newman†).

Cytogenetic studies of blood and bone marrow were performed in 42 patients with leukemia and various myeloproliferative disorders. The chromosomal data were derived from several thousand cells of which more than one thousand were analyzed. Eighteen cases showed consistent anomalies other than the Ph^1 mutation. The abnormalities were found in the G group (7 cases), in the D group (4 cases), in the E group (4 cases), and the C group (13 cases). Alterations in groups A, B, and F were rare. In several cases, alterations in more than one chromosome group were present in the same patient. Changes consisted of gain, loss, rearrangements, or reciprocal translocations of genetic material. In the G group, four cases showed total lack of a G member, whereas in three others a G chromosome was added in part or in all cells studied. In cases with D chromosome abnormalities, one totally lacked such a member, two others had partial long arm deletion, and one had an extra chromosome. In the cases with E_{17-18} lesions, there were two with a long arm isochromosome, one with long arm partial deletion, and one with either pericentric inversion or translocation. In two of the four cases the E alteration was the only abnormality noted. Abnormalities of G_{21}, D_{13} (or "D_1"), and E_{18} autosomes have been primarily associated with chromosomally induced birth defects. Among some of these patients and members of their family there is a higher incidence of leukemia. The concentration of chromosomal changes in the same groups or pairs of chromosomes in hemopoietic tissues of patients with leukemia or allied disorders and in patients with birth defects suggests that certain chromosomes may be more subject to damage or changes.

The Effect of the Platelet-Collagen Reaction and Blood Coagulation on Hemostasis. G. EVANS, M. A. PACKHAM, E. E. NISHIZAWA, AND J. F. MUSTARD,* Toronto, Ontario.

Arrest of bleeding from injured vessels involves three processes: adherence of platelets to collagen, adenosine diphosphate (ADP)-induced platelet aggregation, and fibrin formation. ¶ To examine the importance of the platelet-collagen reaction and fibrin formation on hemostasis, we administered phenylbutazone and acetylsalicylic acid to rabbits. These drugs inhibit platelet interaction with collagen, but do not influence blood coagulation or the action of ADP. They prolonged the time for primary arrest of bleeding (nontreated, 190 seconds; phenylbutazone treated, 501 seconds, $p < 0.001$) from transected vessels in the mesentery. Rabbits given Dicumarol in doses sufficient to prolong the prothrombin time to twice normal showed a lengthened time until primary arrest and frequent rebleeding. Light and electron microscopy showed that the principal structural defect of these hemostatic plugs was lack of fibrin. When phenylbutazone or acetylsalicylic acid was given in combination with Dicumarol, there was no arrest of bleeding during 30 minutes of observation. Histologically, the plugs had loose platelet aggregates with numerous channels. Administration of phenylbutazone to Factor IX-deficient dogs caused a further impairment of hemostasis, similar to observations from the Dicumarol-treated rabbits (primary arrest Factor IX, 222 seconds; Factor IX plus phenylbutazone, 874 seconds, $p < 0.001$). Heparin in doses of 2,500 U per kg has mainly an antithrombin effect, whereas higher doses (12,000 U per kg) also depress the platelet response to collagen. Rabbits given the lower dose showed a normal primary arrest, but an increased frequency of rebleeding, whereas those given higher doses bled throughout 30 minutes of observation. ¶ These observations show that the platelet-collagen reaction and blood coagulation are important in establishing an effective hemostatic plug. Drugs that influence the platelet-collagen reaction can impair hemostasis and when administered to animals with coagulation abnormalities produce profound aggravation of the hemostatic effect.

A Regulatory Site for the Control of Lipid Synthesis in Rat Liver. HAROLD J. FALLON, Chapel Hill, N. C. (introduced by Charles H. Burnett*).

The initial reaction in *de novo* lipid synthesis, L-α-glycerophosphate (GP)-acyl transferase (GPAT), may

regulate the endogenous synthesis of glycerides by liver. (L-αGP + 2 acyl CoA $\rightarrow$ phosphatidic acid + CoA.) GPAT activity was measured in rat liver microsomes by the GP-dependent release of coenzyme A from palmitoyl CoA. Activity was proportional to the simultaneous incorporation of GP-^{14}C into lipids. Serum albumin or dialyzed protein preparations of rat liver supernatant fractions increased GPAT activity. Albumin concentrations of 7 mg per ml increased GPAT activity 5- to 10-fold, but higher concentrations were inhibitory. Increases of substrate concentration within the physiological range for GP (2.5 to 5.0 μmoles per g) and long chain acyl CoA (15 to 60 mμmoles per g) resulted in 2- to 3-fold increases in GPAT activity. ¶ Fasting for 44 to 70 hours resulted in minimal decrease in microsomal GPAT activity, but supernatant protein fractions prepared from livers of rats fasted for 70 hours were 33% less effective than preparations from fed rats inactivating GPAT. Fasting also lowers GP and acyl CoA concentration. Thus several factors may depress lipid synthesis in fasting rats. ¶ A 2.5- to 3-fold increase in GPAT occurred in male rats fed high glucose or high corn oil diets for 7 days when compared to rats fed chow. ¶ These results suggest that several factors are important in the control of hepatic GPAT activity: *1*) tissue concentration of GP and long chain acyl CoA derivatives; *2*) possible activation of GPAT by cellular proteins; and *3*) changes in microsomal GPAT activity in response to diet. These factors probably contribute to the regulation of endogenous lipid synthesis in mammalian liver.

RNA Synthesis in Cardiac Hypertrophy. BARRY L. FANBURG AND BARRY I. POSNER, Boston, Mass. (introduced by Maurice S. Raben*).

Cardiac hypertrophy was induced in 180- to 220-g rats by constriction of the aorta at the aortic arch. After constriction, heart growth rate was increased over control leading to a 35 to 50% hypertrophy by 7 days subsequent to which the rate of growth returned to control level. RNA concentration increased 50% by 48 hours and returned to control level by 21 days. RNA content per heart doubled by 7 days and did not increase further over the next 21 days. ¶ Cardiac RNA labeled *in vivo* with ^{32}P was extracted with phenol in the presence or absence of sodium dodecyl sulfate (SDS) and fractionated by sucrose gradient. AMP obtained from extracted ATP by enzymatic hydrolysis and ion exchange chromatography was used to correct RNA labeling for changes in precursor specific activity. No increase in RNA synthesis was noted until 4 hours after constriction. Subsequently, there was a marked stimulation of all species of RNA, which reached a peak about 24 hours postconstriction. ¶ Pressure time index (PTI) obtained from left ventricular pressure recordings was constant at 40% above control in the first 8 hours postconstriction. Preliminary observations suggest a direct relation between the rate of RNA synthesis and the PTI at 4 to 8 hours postconstriction. ¶ RNA synthesis was determined during a 4-hour perfusion of the isolated heart under conditions of zero load at 6 and 48 hours after constriction. No increase above control was seen in hypertrophying hearts 6 hours postconstriction; in contrast, synthesis was increased 48 hours postconstriction. This suggests that increased rate of RNA synthesis is readily reversible with removal of the work load at early times postconstriction, but is less readily reversible as time progresses following constriction.

Evaluation of Nephron Disparity in the Dog and the Influence of Hypotension and Osmotic Loads. MELVIN H. FARMELANT, CHARLES BAKOS, AND BELTON A. BURROWS,† Boston, Mass.

Most techniques for evaluating renal function treat the kidney as a unit. Disparity of nephron function in a kidney could result from renal disease or physiological alterations. To evaluate renal disparity we have injected a bolus of Hippuran-^{131}I into one renal artery and externally monitored the radioactivity present in both renal areas, subtracting the curve of the noninjected from the injected kidney curve to eliminate the effect of recirculation. The curves of radioactivity versus time obtained in normal dogs are sigmoid when urine flow is maintained above about 1 ml per minute per kidney. Below this flow rate they are exponential in character, suggesting that pelvic mixing becomes predominant in determining the curve shape. Differentiation of the sigmoid curves yields a bell-shaped curve skewed to right in time. The median of this latter curve is the mean passage time through the nephrons and pelvis. The degree of deviation from the mean was used to measure disparity of passage times. ¶ In normal dogs increasing the urine flow rates shortens the mean passage time and results in smaller deviations from the mean. At comparable urine flow rates mannitol loads and saline loads yield similar passage times and deviations. Clamping the aorta above the renal arteries sufficient to reduce femoral artery blood pressure produces prolonged passage time and marked increase in the disparity. This occurs despite mannitol loading sufficient to produce urine flow rates comparable to the preclamped control period. The data suggest that in the hypotensive kidney tubular fluid moves more slowly and the nephron population is functioning less uniformly than in the normal kidney, possibly as a result of differing reabsorption to filtration ratios of salt and water.

Neglect of Human Decisions: A Major Source of Intellectual Morbidity in "Vital Statistics." ALVAN R. FEINSTEIN,* West Haven, Conn.

When the occurrence and mortality rates of different "diseases" change from one year or decade to another, the changes are often attributed to nature, or to medical maneuvers in prevention and treatment. These explanations do not account for two types of human decision that may greatly alter the "disease" data of "vital statistics": *1*) the iatrotropic stimuli that make diseased hosts elect to seek medical attention, thereby becoming patients; and *2*) the availability and dissemination of

changing concepts, techniques, and criteria for the diagnoses made by doctors. Since the data of "vital statistics" are based on diagnoses, rather than diseases, the frequency of a "disease" will depend on the frequencies with which affected hosts appear as patients and receive correct diagnoses. ¶ A disease that requires a specific examination for diagnosis will be undetected in "aniatric" diseased hosts who have not sought medical attention because they are asymptomatic or noncomplainant. The frequency of the disease will rise spuriously as such hosts become increasingly "iatric" via "routine checkups" and "health campaigns." ¶ The occurrence of a disease will be changed not merely as new diagnostic tests or criteria become available, but as they are generally disseminated and used. The temporal dissemination of new procedures from academic center to community is generally overlooked in "vital statistics," but can cause false rises or falls in certain diseases, together with reciprocal changes in occurrence of some other disease that can produce similar symptoms. ¶ Simple examples of these phenomena are the contemporary disappearance of dropsy, as diagnostic nomenclature changed, and the increase of lupus erythematosus, as the LE test became disseminated. Comparable phenomena may be responsible, in the United States, for the rise of emphysema and lung cancer, as chest X rays have become used routinely, and the fall in stomach cancer, as gastrointestinal X rays have been applied more often in patients with anorexia, weight loss, and an abdominal mass.

Intestinal Transport of Cholesterol. ELAINE B. FELDMAN, Brooklyn, N. Y. (introduced by David M. Kydd†).

The specific mechanisms of intestinal transport of cholesterol were investigated using ^{14}C-labeled cholesterol, 0.25 mM, in micellar solution with 5.4 mM mono-olein and 6.0 mM sodium taurodeoxycholate, in Krebs-Ringer phosphate buffer, pH 6.3. Rings of hamster small intestine accumulated 27% per 100 mg of cholesterol after 1-hour incubations at 37° C in oxygen. Tissue accumulation decreased with decreasing temperature. An Arrhenius plot was linear with a Q_{10} of 0.94. Uptake was in linear proportion to concentration (0.1 to 1.0 mM), with both linear and log-log plots indicating a process conforming to the Freundlich adsorption isotherm. Data plotted in accordance with the Langmuir adsorption equation did not yield a straight line, precluding the inference that cholesterol absorption is a simple process of Type I unimolecular adsorption. The 40 to 50% decrease in cholesterol uptake with phospholipase A or lysolecithin in the medium and the doubling of sterol accumulation in the presence of amphotericin B support the suggestion of a specific receptor mechanism at the cell membrane surface. Cholesterol may enter the membrane by exchange with membrane sterol (membranous displacement) involving reversible structural modifications of globular micelles in lamellar association. Requirement of metabolic energy was suggested by inhibitions of uptake, 19% in the absence of 10 mM glucose, 30% in an atmosphere of N_2, and 38% by 200 μg per ml Na azide. Transport of cholesterol against a gradient was observed with 2-hour perfusions *in vitro* of 10-cm segments of everted jejunum. The uptake of serosa was half that of mucosa. With perfusion *in vivo* similar quantities of cholesterol were absorbed with 13% esterification demonstrated. These data implicate two processes in intestinal sterol transport: *1*) entry into the cell membrane by a reversible physical process of exchange diffusion; *2*) rate-limiting entry into the cell by low-grade active transport.

***In Vitro* Stimulation by LATS of Thyroid Glucose Oxidation and ^{32}P Incorporation into Phospholipids.** JAMES B. FIELD,* ADRIENNE REMER, GAIL BLOOM, AND JOSEPH P. KRISS, Pittsburgh, Pa., and Palo Alto, Calif.

Long-acting thyroid stimulator (LATS) increases ^{131}I release from thyroid *in vivo* and may be of etiologic importance in thyrotoxicosis. The present *in vitro* studies were designed to determine whether LATS reproduces any other metabolic effects of TSH that might be relevant to the increased function of the gland in thyrotoxicosis. In contrast to TSH neither 0.5 U per ml LATS (4 U per mg) nor equivalent amounts of pooled human γ-globulin stimulated glucose-1-^{14}C oxidation in dog thyroid slices during a 45-minute incubation. However, when thyroid slices were preincubated with LATS, γ-globulin, or TSH for 2 hours and then glucose-1-^{14}C was added during a further 45-minute incubation, LATS, but not γ-globulin, significantly increased $^{14}CO_2$ production, but to a lesser extent than did 0.5 mU per ml TSH. When the preincubation was prolonged to 4 hours, LATS stimulation was approximately equivalent to 0.5 mU per ml TSH, whereas with 5- to 6-hour preincubations, the effect of LATS exceeded that of 0.5 mU per ml TSH. TSH-antibody did not modify LATS stimulation but abolished the TSH-mediated increase. Measurement of ^{32}P incorporation into phospholipids produced very similar results. LATS did not augment ^{32}P incorporation during short-term incubations. However, when thyroid slices were preincubated for 3.5 to 6 hours with LATS, TSH, or γ-globulin and ^{32}P was added for a further 2-hour incubation, LATS increased ^{32}P incorporation into phospholipid. The stimulation was equivalent to approximately 0.5 mU per ml TSH. Although LATS did not influence glucose-1-^{14}C oxidation in testes or kidney, stimulation was occasionally observed in spleen and liver. These studies demonstrate that *in vitro*, as *in vivo*, effects of LATS are more delayed than those of TSH and that LATS can mimic effects of TSH on other metabolic parameters that might be involved in thyrotoxicosis.

Immunoglobulins Synthesized by Human Lymphoid Cell Lines. IRA FINEGOLD AND JOHN L. FAHEY,* Bethesda, Md.

Immunoglobulin synthesis has been previously detected in several human lymphoid cell lines in continuous tissue culture. We have extended our initial observations to study the nature and prevalence of cell lines capable of

immunoglobulin production. Immunoglobulin synthesis has been detected in 19 cell lines of 27 tested from 24 patients with a variety of malignant disorders. Cell products labeled with amino acids-^{14}C were tested by radioimmunoelectrophoresis with antiserums specific for IgG, IgA, IgM, and IgD and kappa and lambda light polypeptide chains. A range of immunoglobulins synthesized *in vitro* was detected—from only one light chain type to two heavy chain and two light chain types in a single cell line. ¶ Physical and immunochemical techniques showed that many of the newly synthesized heavy and light chains are linked to form typical 7 S or 18 S immunoglobulin molecules. Immunochemical tests indicated that immunoglobulins produced by the cultured cell lines were very similar or identical to normal human immunoglobulins. Stability of immunoglobulin formation was assessed by repeat tests of 15 cell lines, and only two cell lines varied in results. The immunoglobulins produced by these cells are electrophoretically homogeneous and thus bear some resemblance to myeloma proteins. Investigation of serum from two living patients who are donors of cell lines, and of serum from patients with Burkitt's lymphoma and chronic myelogenous leukemia, failed to reveal serum immunoglobulin components comparable to the proteins identified in culture. These findings indicate that lymphoid cells producing immunoglobulin in culture are functionally different from the predominant malignant cell *in vivo*.

Specificity of Epithelial Behavior in Cultures of Skin and Oral Mucosa. B. ALLEN FLAXMAN, Bethesda, Md. (introduced by Eugene J. Van Scott*).

This work was done in part to ascertain whether seeming specific differences between cells of the epidermis and cells of the oral mucosa are inherent in each cell type and persist in tissue culture environments, or whether apparent *in vivo* differences are modulations, induced by local environments, which disappear when the cells are cultured under common *in vitro* conditions. ¶ Epithelial outgrowths from explants of both tissues were stratified and several layers in thickness. Examination with both the light microscope and electron microscope revealed that the lower layers of cells, in contact with or near the glass coverslip, had characteristics of normal epidermal basal cells. Accumulation of tonofibrils and other signs of maturation occurred in cells of the upper layers, in a gradient oriented toward the overlying medium. Formation of normal stratum corneum did not occur. Differences between outgrowths of oral mucosa and skin could not readily be identified. ¶ In cultures 1 to 3 weeks old, extensive interconnecting ductal and cisternal systems progressively developed within the outgrowth from both tissues. Examination of cross and longitudinal sections of these ductlike structures revealed the luminal contents to consist of fibrils and cell debris. The walls were several cell layers thick and were uniformly organized, with basal-type cells innermost and more mature cells found outwardly. Such ductal structures appeared similar in both types of tissues, continued to form even after removal of the primary explant along with its connective tissue, and were not qualitatively modified by variations in the culture medium. ¶ This study reveals that a significant amount of organization can occur in pure epithelial cell outgrowths from skin and oral mucosa. The system provides a means to study cell maturation gradients and other mechanisms of epithelial response, including those operative in duct formation.

Gastric Hypersecretion in Duodenal Ulcer Patients Owing to Oral Calcium Carbonate Taken One Hour after a Meal. JOHN S. FORDTRAN, Dallas, Tex. (introduced by Leonard L. Madison†).

Calcium carbonate is the most effective agent available for neutralizing gastric acidity and is commonly used to treat duodenal ulcer. However, we have observed that this drug may actually stimulate gastric acid production. Twelve patients were fed a standard meat, toast and butter meal on three or four separate test days. One hour after eating, either water (as control), 8 g of calcium carbonate, 8 g of sodium bicarbonate, or 30 ml of aluminum-magnesium hydroxide (Al-Mg) was taken orally. Two or three hours later, gastric contents were completely removed by 1-hour aspiration via Levine tube. During the following hour, gastric secretions were collected quantitatively. Acid secretory rate was 3.5 (range 0.1 to 6.1) with water, 2.9 (1.2 to 5.8) with sodium bicarbonate, and 3.0 (0.6 to 5.2) mEq per hour with Al-Mg. Every patient showed increased rates of secretion with 8 g of $CaCO_3$, mean 8.0 mEq per hour (2.4 to 11.0), and four of eight patients showed this effect with a 4-g dose. ¶ In sensitive patients, the response to even the 4-g dose may be striking, i.e., up to 90% maximal histamine response. Additional studies, mainly in one very sensitive patient who secreted <3 mEq with water but 15 to 18 mEq with 4 g $CaCO_3$, have shown that this effect *a*) does not occur in fasting patients, *b*) does not occur if the stomach is bypassed by infusing $CaCO_3$ into the duodenum in the postcibal state, *c*) cannot be reproduced by sodium bicarbonate or Al-Mg, even if given every 15 minutes for 2 hours after eating, *d*) does not occur with calcium lactate, *e*) is not due to hypercalcemia, *f*) occurs even if gastric juice is not removed from the stomach and continues to enter the duodenum, and *g*) is inhibited by oral anticholinergic agents.

Effect of Acetazolamide on Clinical and Physiological Responses to Acute Altitude Exposure. STANLEY A. FORWAND, JAMES E. HANSEN, JOHN N. FOLLANSBEE, AND MILTON LANDOWNE,† Natick, Mass.

Acetazolamide has been demonstrated by Cain to prevent the respiratory alkalosis of acute simulated altitude exposure and to increase alveolar P_{O_2}. The influence of acetazolamide on symptoms of acute mountain sickness at 12,800 feet was therefore tested in a double-blind study. Forty-three male volunteers were given either 250 mg of acetazolamide or placebo every 8 hours for 36 hours before and 48 hours after abrupt transport to Mt. Evans, Colorado. At sea level and daily for 5 days at altitude,

ventilation, alveolar P_{CO_2}, P_{O_2}, and heated hand venous blood pH and P_{CO_2} were measured. Subjects filled out symptom questionnaires and had a history and physical examination daily. ¶ At altitude, as anticipated, 21 treated subjects had lower pH, P_{CO_2}, and calculated serum bicarbonate, with greater ventilation and alveolar P_{O_2} than 22 controls. Incidence and severity of symptoms were less in the treated group. On day 2 when symptoms of acute mountain sickness were most prevalent, the following differences were present: headache ($p < 0.01$) in 22 controls (5 mild, 11 moderate, 6 severe) and 14 treated (6 mild, 7 moderate, 1 severe); gastrointestinal symptoms ($p < 0.05$) in 17 controls (10 mild, 3 moderate, 4 severe) and 10 treated (10 mild); and insomnia ($p = 0.025$) in 17 controls (6 mild, 9 moderate, 2 severe) and 8 treated subjects (4 mild, 2 moderate, 2 severe). In controls, intensity of these symptoms was best correlated with P_{CO_2} ($r = +0.56$, $+0.35$, and $+0.38$); however, waning of symptoms did not begin until HCO_3^- and pH began to fall. Ventilation increased during the first 72 hours, before pH began to decline, indicating that peripheral (H^+) receptors are not dominant regulators of ventilation during this period. In both groups, correlation of symptoms with P_{O_2} was poor. No effect of drug on blood-alveolar CO_2 gradient was noted, indicating no detectable CO_2 retention owing to erythrocyte carbonic anhydrase inhibition.

On the Relationship between Nonesterified Fatty Acids, Acetyl CoA, and Starvation Ketosis. DANIEL W. FOSTER, Dallas, Texas (introduced by Marvin D. Siperstein*).

It is generally held that ketosis in starvation and diabetes is due to mobilization of nonesterified fatty acids (NEFA) from fat stores, uptake, esterification, and oxidation of these fatty acids in the liver, expansion of the hepatic acetyl CoA pool, and a secondary increase of acetoacetate (AAA) synthesis. In the present experiments, blood NEFA and AAA concentrations were measured during fasting and after acute reversal of ketosis with insulin. After a 48-hour fast NEFA increased from 49 to 216 μEq per L while AAA rose from 1.2 to 6.6 mg per 100 ml. When ketosis was reversed by the injection of 0.05 U of insulin, both nonesterified fatty acids and ketones fell at 10 minutes, in accord with the above theory. When a large dose of insulin (0.5 U) was injected, however, a different pattern was seen. Five minutes after the injection AAA fell from 6.0 to 3.6 mg per 100 ml and NEFA decreased from 216 to 103 μEq per L. At 10 minutes AAA rebounded sharply to 6.1 mg per 100 ml secondary to hypoglycemia of 41 mg per 100 ml, but NEFA continued to fall to 53 μEq per L. Clear dissociation of the two parameters was thus obtained. Confirmation was obtained by treating fasted animals with a small dose of insulin (0.01 U). Under these circumstances ketosis was not reversed despite the fact that NEFA fell from 155 to 27 μEq per L at 5 minutes and near zero at 10 minutes. Acetyl CoA concentrations were measured in the livers of rats fasted 48 hours and 15 minutes after treatment with insulin. Blood AAA fell from 8.3 to 1.2 mg per 100 ml in this time, but acetyl CoA concentrations did not change (48 hours, 50.5 ± 3.2 mμmoles per g; 15 minutes, 47.1 ± 3.1 mμmoles per g). These experiments indicate that ketogenesis can be varied independently of plasma NEFA and acetyl CoA in the liver and strongly suggest that starvation ketosis is not causally related to increased mobilization and oxidation of NEFA or expansion of the acetyl CoA pool.

The Effects of Decreased Ventricular Filling on Left Ventricular and Coronary Hemodynamics. MARTIN J. FRANK, LEONARD J. LESNIAK, MANOUCHEHR NADIMI, AND GILBERT E. LEVINSON, Jersey City, N. J. (introduced by Harper K. Hellems†).

Decreased ventricular filling is the common denominator of such clinical conditions as cardiac tamponade, hemorrhage, and peripheral pooling of blood. The effect on ventricular performance and coronary hemodynamics is often obscured by concomitant changes in heart rate, arterial pressure, and resistances. Whether the hemodynamic collapse often associated with these conditions can be explained by inflow changes alone was investigated in nine dogs with balloon obstruction of the inferior vena cava and nine with closed chest cardiac tamponade. Left ventricular (LV) coronary flow (^{85}Kr), volumes (indicator washout), and LV and aortic pressures were measured in the steady state before and after each intervention. Fiber length ($2\pi r$) was calculated by assuming a spherical LV. Isovolumetric contractility, defined by the force-velocity relationship per unit fiber length, and the stroke work/fiber length ratio were elevated. In the caval obstruction studies, with constant heart rate (atrial pacing) and a level of balloon inflation such that mean systemic pressure did not change, the fall in stroke volume (35%) exceeded the fall in end-diastolic volume (27%). LV coronary flow and oxygen consumption decreased without important changes in coronary resistance, oxygen extraction, or excess lactate production. Isovolumetric contractility was not affected despite significant decreases in LV dp/dt, and fiber length. However, the ratio stroke work/fiber length was significantly reduced. The results during cardiac tamponade differed only in that, because of a greater decrease in stroke volume (60%), mean arterial pressure fell (35%). These results demonstrate that impaired ventricular performance results from reduced LV filling. Since force of contraction is a function of sarcomere length, it would appear that a reduced LV volume necessitates ejection on the ascending limb of the Starling curve at a fiber length too short for the prevailing contractile state.

Structural Differences between Two Subclasses of μ Chains of Human Macroglobulins. E. C. FRANKLIN* AND B. FRANGIONE, New York, N. Y.

Antigenically distinguishable subgroups were first demonstrated for the γ chains of γG globulins and have recently been shown also for the α and μ chains of γA and γM, respectively. One of sixteen antisera to pathologic

macroglobulins divided 29 isolated macroglobulins into two groups; twelve (43%) reacted with it and sixteen (57%) failed to precipitate. Two of thirteen additional macroglobulinemic sera also reacted with it. Reactivity with this antiserum was unrelated to electrophoretic mobility or light chain type of the protein. The antigenic determinants of the reactive proteins were located on the μ heavy chains. Although the μ chains and 8 S monomers failed to precipitate with the antiserum, both inhibited precipitation of reactive macroglobulins, whereas similar fractions from nonreactive proteins had no effect. ¶ Peptide maps were prepared from heavy chains and/or native proteins of eleven reactive and fourteen nonreactive proteins. The peptide maps were strikingly similar in over-all appearance with the exception of several peptides near the origin. In maps of the reaction proteins, there was either a single peptide or sometimes two peptides that were lacking in the maps from each of the nonreactive proteins. Eleven of the fourteen nonreactive proteins had no Ninhydrin-scanning material in this region, whereas the other three had two peptides with a distribution different from that of the reactive ones. ¶ These findings indicate that the two subclasses of μ chains are closely related to each other and may have evolved from each other, and suggest the existence of additional heterogeneity, and possibly, genetic polymorphism of the μ chains.

Epidermal Protein Synthesis: Subcellular Localization of the Synthetic Site. IRWIN M. FREEDBERG, Boston, Mass. (introduction by Samuel L. Gargill†).

Elucidation of the normal pathways and control mechanisms of protein synthesis in epidermis is a prerequisite to the search for defects in pathologic states. Previous studies of epidermal protein synthesis have been hindered by the failure of cell-free systems from epidermis to synthesize proteins actively, although *in vivo* and in slice systems the organ is extremely active. This inconsistency has been solved, and the epidermal fraction engaged in protein synthesis has been isolated and identified. ¶ Guinea pig skin slices that included the entire epidermis and papillary layer of dermis were incubated with a ^{14}C-labeled amino acid mixture. The epidermis was subsequently separated from the dermis, homogenized, and fractionated by differential centrifugation into "nuclear," mitochondrial, microsomal, and high speed supernatant fractions. Labeled proteins appeared in the "nuclear" fraction after a 2-minute incubation; other fractions became labeled later. During "pulse-chase" experiments, early labeled "nuclear" counts could be followed into the microsomal and high speed supernatant fractions. The "nuclei" could also be fractionated with deoxycholate into a rapidly sedimenting unlabeled pellet and a heavily labeled supernatant fraction. After 30-minute incubation the radioactivity in the supernatant fraction was 10 times higher (counts per milligram RNA) than that in the microsomes. Similar results were obtained in parallel studies with guinea pig hair follicles. ¶ Cell-free amino acid incorporation was studied in systems prepared from stretch separated epidermis and hair follicles. Microsomes and ribosomes isolated in the usual manner were relatively inactive, whereas ribosomes prepared by deoxycholate extraction of the residual "nuclear" pellet were as active (counts per milligram RNA) as liver ribosomes. Density gradient centrifugation and electron microscopy of the particles isolated with deoxycholate revealed both monomeric and polymeric ribosomes that were initially bound to membranes and filaments sedimenting at 600 *g*. Incubation with orotic acid-^{14}C proved that the deoxycholate extractable particles were not synthesizing ribonucleoprotein. ¶ It has been concluded that epidermal protein synthesis predominantly occurs on membrane- and filament-bound ribosomal particles that sediment with the "nuclear" pellet. These particles have been discarded in cell-free systems prepared in the usual manner.

Biological Clocks and Starvation. NORBERT FREINKEL,* MILTON MAGER, RICHARD SANDLER, AND LEONARD VINNICK, Chicago, Ill., and Natick, Mass.

To assess the integrative events by which endogenous fuels are recalled during early starvation, healthy young males were fasted 3 or 4 days, and bloods were analyzed for morning (7 to 8 a.m.) versus afternoon (3 to 4 p.m.) cyclicity. Plasma glucose declined 30 to 40% during the entire fast; however, on any single day, levels remained constant or rose slightly during the a.m. $\rightarrow$ p.m. interval. Plasma free fatty acids (FFA) and glycerol exhibited an integrated 200 to 300% increase, but p.m. values were 50 to 100% greater than a.m. each day. Mean levels of plasma free amino acids (AA) remained relatively constant; however, a.m. concentrations invariably exceeded p.m. (10 to 20%), and the negative correlation between FFA:AA was significant ($p < 0.01$). Diurnal excursions of plasma cortisol (fluorometric) were preserved with a.m. > p.m. and no consistent modification of the pattern by starvation. Plasma insulin displayed inverse cycling: Although mean values declined about 50% during fasts, relative a.m. $\rightarrow$ p.m. decrements were greater than p.m. $\rightarrow$ a.m., and glucose/insulin ratios were higher each p.m., suggesting that factors other than plasma glucose delimited insulinization. Urinary catecholamines tended to increase; however, increments were inconstant, and consistent cyclicity was not observed when urines were fractionated at 8-hour intervals. To evaluate the role of insulin, 3-day fasts were repeated in three subjects given 8 U insulin (4 U Lente + 4 U Ultralente) daily after the a.m. blood sampling. Such insulin lowered p.m. glucose, variably dampened p.m. increments in glycerol and FFA, and significantly attenuated the 3-day loss of nitrogen. However, a.m. $\rightarrow$ p.m. excursions of AA were not altered. ¶ Preservation of diurnal cyclicity for fuels, hormones, and their interactions during early starvation suggests that the fine regulation cannot be formulated in terms of any single, simple, negative feedback in the periphery. Instead, the adaptation to dietary deprivation must be grafted upon the same higher integrations that govern biological clocks under normal conditions.

Choleglobin Formation by Lesser Density, Reticulocyte-rich Human Erythrocytes. V. L. FROMKE, Minneapolis, Minn. (introduced by C. J. Watson†).

During the terminal event of normal reticulocyte maturation, high redox potentials and peroxides form around mitochondria, which irreversibly oxidize hemoglobin to choleglobin. Choleglobin differs from hemoglobin only in the substitution of oxygen for the alpha methene bridge of protoporphyrin. Although choleglobin formation is easily observed in oxidative systems, *in vitro,* its occurrence as a natural intermediate in hemoglobin catabolism has been in doubt. ¶ The purpose of the present study was to re-examine the question of occurrence of choleglobin in circulating erythrocytes with special reference to reticulocytes as it seemed not unlikely that their high redox activity might result in formation of choleglobin. ¶ In order to study intrareticulocyte hemoglobin degradation in normal and hemolytic states a sensitive fluorimetric modification of the method of Kench was designed to measure "native" choleglobin indirectly as total bile pigment. ¶ Without exception the lesser density, reticulocyte-rich cells were found to contain from 3- to 5-fold increase in choleglobin as compared with the greater density, reticulocyte-poor cells. The range observed with the former was 15 to 25 μg of total bile pigment per 100 mg of hemoglobin, whereas with the latter the value was invariably below 5 μg. ¶ An explanation for this evidence of hemoglobin degradation in the lesser density reticulocyte-rich fraction is best understood by the presence of metabolically active structures in the young cells that are removed as the cells mature.

Purification of Human Renin for Immunoassay Using Polyacrylamide Gel Electrophoresis. SOITSU FUKUCHI AND JEROME W. CONN,† Ann Arbor, Mich.

Because the level of renin in peripheral as well as in renal venous plasma has attained important diagnostic significance in the two major forms of surgically correctable hypertension (primary aldosteronism and renovascular hypertension), and because the role of renin in the initiation and maintenance of hypertensive disease, in general, requires further intensive study, we have emphasized the need for a relatively simple but accurate method for measuring the concentration of this enzyme in human plasma. The major limitation for the development of a sensitive immunoassay has been the unavailability of a sufficiently pure preparation of human renin. With the hope that other laboratories will join in our effort to develop such a method, we report a procedure for obtaining highly purified human renin. ¶ From 20 kg of human kidney tissue, purification of renin was accomplished by means of the following 8 steps: water extraction, fractionation at pH 2.8 with 0.8 M NaCl, fractionation with 1.0 M ammonium sulfate, precipitation of renin with 2.3 M ammonium sulfate, removal of angiotensinases with 0.1 M EDTA at pH 9.5, precipitation again with 2.3 M ammonium sulfate, DEAE-cellulose, and CM-Sephadex column chromatographies, and finally polyacrylamide gel electrophoresis. This last step provides high resolution plus the ability to separate the enzyme from large amounts of crude extract. The final product, which gives a single stained band on polyacrylamide gel electrophoresis, has a specific activity of 33.0 U (Goldblatt) of renin per milligram of protein. This represents an activity concentration of at least 10,000 times that of the watery extract. This renin preparation is believed to be suitable both as a standard and for iodination in the development of a radioimmunoassay for human plasma renin. Antibody against human renin is attainable in Dutch-belted rabbits after 8 weeks of intramuscular immunization. Such work is now in progress.

The Phosphaturic Effect of Urinary Alkalinization. MILFORD FULOP AND PAUL BRAZEAU, Bronx, N. Y. (introduced by I. Herbert Scheinberg†).

Previous studies of the effect of urinary pH on the excretion of inorganic phosphate (P) in dogs have usually entailed the infusion of P. Recent studies have shown that P infusion causes hypocalcemia that evokes increased parathormone secretion and, in turn, decreased tubular reabsorption of P. In the experiments reported here, therefore, the effect of increasing urinary pH on P excretion was studied in fasting anesthetized dogs not given P. ¶ Urinary alkalinization was produced by giving $NaHCO_3$ and/or acetazolamide while estimating clearance of P and inulin (C_P, C_{In}) and excretion of P (U_PV). In seven dogs given $NaHCO_3$ infusions, urinary pH rose, on average, from 6.56 to 7.56; the filtered load of P (F_P) decreased an average of 4%; but C_P/C_{In} increased an average of 35%, and U_PV an average of 32%. ¶ In 11 dogs given acetazolamide, urinary pH rose, on average, from 6.76 to 7.65, and F_P decreased an average of 18%, largely because of decreased GFR. Despite the fall in F_P, however, there was an average increase in C_P/C_{In} of 46%, and in U_PV of 18%. Thus, C_P/C_{In} increased, and tubular reabsorption of P decreased during urinary alkalinization, regardless of whether there was associated systemic alkalosis ($NaHCO_3$ infusion) or acidosis (acetazolamide injection). ¶ If it is assumed that renal tubular secretion of P does not occur in dogs, the explanation for the increased P excretion may be that the renal tubules do not reabsorb HPO_4^- as well as they reabsorb $H_2PO_4^-$. These findings may be relevant to the mechanism of formation of phosphate calculi.

Determination of Elastase in Mammalian Plasma and Serum. M. GEOKAS, P. WILDING, H. RINDERKNECHT, AND B. HAVERBACK,* Los Angeles, Calif.

A sensitive spectrophotometric method based on the release of dye from Congo red-stained elastin (ECR) has been recently advocated by Hall for estimation of elastase activity in plasma. An extensive study of this technique has been carried out, and it has been amply demonstrated that when electrophoretic fractions of human plasma were tested for elastase, "activity" was almost exclusively confined to the albumin fraction. The possibility that

elastase is present in this fraction was eliminated by several experiments. Boiling at 100° C for 15 minutes increased this activity, a phenomenon due to an increase of reactive basic groups following the denaturation of albumin. When increasing amounts of human serum albumin (HSA) Fraction V were incubated with constant excess of ECR, "apparent" inhibition was observed with the higher concentrations of the protein. However, a linear relationship was obtained when the same amounts of HSA were incubated with increasing excess of ECR. Addition of porcine elastase in human plasma with subsequent electrophoresis showed that the enzyme behaved as gamma globulin and its activity was unaffected by the procedure. However, when hog plasma was substituted for human, the activity of elastase was enhanced; the latter finding is attributed to two possible factors: *1*) liberation of endogenous elastase in the presence of an excess of exogenous enzyme; *2*) the enhancing effect of plasma elastomucase. It is concluded that ECR is unsuitable for assaying elastase in plasma because of the strong affinity of albumin for acidic azo dyes. The "elastase activity" in serum or plasma is not due to elastolysis but rather to the dissociating effect of albumin on the ECR-substrate. A new sensitive method of elastase assay has been developed by using an elastin substrate containing covalently bound fluorescent dimethyl aminoaphtalenesulfonic acid (DMS). No elastase activity could be detected in plasma or serum of normal subjects or of patients with acute pancreatitis. However, the presence of elastase in blood in an inactive form cannot be excluded.

Abnormal Albumin Metabolism as a Cause of "Idiopathic" Edema. JOHN R. GILL, JR., AND FREDERIC C. BARTTER,† Bethesda, Md. (introduced by James H. Baxter†).

Many patients with "idiopathic" edema manifest no other clinical abnormality. The present study demonstrates an abnormality in albumin metabolism in some of these patients in whom cardiac, hepatic, and renal abnormalities have been excluded and who have normal lymphatic vessels in the legs. Metabolic turnover studies were performed with carefully prepared albumin-^{125}I. The data were analyzed by digital computer. A compartmental model with a central, intravascular and two mamillary extravascular pools fit the data satisfactorily. ¶ Whereas albumin synthetic rate and fractional catabolic rate were normal, the total exchangeable pool (TEP) was increased (4.0 g per kg ± 0.4 SEM in the patients versus 3.2 ± 0.1 in control subjects). Pool 1 (intravascular) and pool 3 (slowly exchanging; ? skin and muscle) were normal; the increase was wholly attributable to pool 2 (rapidly exchanging; ? viscera). The relative size of pool 2, normally 12% of pool 1 and 5% of TEP, was 64% (range, 27 to 136%) of pool 1 and 16.4% of TEP in these patients. ¶ Intercompartmental fractional rate constants indicated normal exchange between pool 1 and pool 3. In independent studies, the clearance of extravascular albumin-^{131}I from the leg (? skin and muscle) was normal or slightly accelerated, in agreement with the kinetic analysis. The rate constants indicated normal egress of albumin from pool 1 into pool 2, but impaired return from pool 2 to the circulation. ¶ In conclusion, an abnormally large extravascular pool 2, probably in the viscera, has been demonstrated in some patients with idiopathic edema and could, through an increase in oncotic pressure, lead to the extravascular accumulation of salt and water. Indeed, the degree of edema in these patients, as determined from negative sodium balance with aldactone, corresponded to the increase in the rapidly exchanging extravascular pool.

Mixed γG-γM Cold Agglutinin. LEONARD S. GOLDBERG AND EUGENE V. BARNETT, Los Angeles, Calif. (introduced by Carl M. Pearson†).

Cold agglutinin antibodies have been typically characterized as γM immunoglobulins. Although only kappa light chains have been detected in the idiopathic variety of cold agglutinins, both kappa and lambda light chains have been demonstrated in these peculiar macroglobulins when they are associated with certain infectious diseases. Despite this apparent heterogeneity, no γG cold agglutinin has been described. This paper describes a mixed γG-γM cold agglutinin that occurred in a patient with hemolytic anemia associated with infectious mononucleosis. When the patient's serum was absorbed with antihuman γG or antihuman γM, a marked reduction in the cold agglutinin activity resulted. A similar response occurred after the addition of 2-mercaptoethanol. Both γG (12.0 μg per ml) and γM (16.0 μg per ml) were detected in the eluate by quantitative complement fixation utilizing specific anti-heavy chain antisera. Less than 0.41 μg per ml γA was present in the eluate. A control eluate obtained from the serum of a patient with known idiopathic cold agglutinin disease contained 24.0 μg per ml γM, 1.0 μg per ml γG, and less than 0.41 μg per ml γA. Neither the γG nor γM chromatographically separated fraction of the eluate demonstrated cold agglutinin activity alone. When these fractions were mixed cold agglutinin activity was re-established. The addition of normal γG to the chromatographically separated γM fraction or the addition of normal γM to the γG fraction did not result in detectable cold agglutinin activity. The agglutinating activity of the patient's serum against O Rh-positive red blood cells sensitized with an incomplete γG antibody (anti-CD serum Ripley) was 500 times greater at 4° C than 37° C. Evidence is presented which suggests that the γG component of the cold agglutinin was directed against red cell antigenic determinants and that the γM component represented antibody, active at 4° C, against the γG coating the red cells, i.e., cold anti-antibody.

Separate Effects of Plasma Sodium Concentration (P_{Na}) and Glomerular Filtration Rate (GFR) on Proximal Tubular Sodium Reabsorption (T_{Na}) in the Dog. MARTIN GOLDBERG,* STEVEN S. GOLDSTEIN, STANLEY GODSHALL, AND JULES B. PUSCHETT, Philadelphia, Pa.

Micropuncture studies have indicated a correlation between proximal T_{Na} and GFR at any constant level of extracellular fluid (ECF) volume. Whether this relation-

ship reflects the influence of the volume of filtrate or of filtered load of sodium ($F_{Na} = P_{Na} \times GFR$) is unclear. To study the effects of P_{Na} and GFR separately, experiments were done on eight hydropenic anesthetized dogs that received infusions of inulin, PAH, and vasopressin, and also adrenal mineralocorticoid (DOCA). Constant infusions of ethacrynic acid and chlorothiazide were given to block distal sodium transport so that excreted urine represented proximal tubular fluid. Losses of electrolytes and water were replaced. To vary P_{Na} and GFR locally without ECF expansion, a femoral-renal arterial shunt was constructed to perfuse one kidney entirely by femoral arterial blood. P_{Na} and GFR were varied either by infusing 30% saline into the shunt or by partial constriction of the shunt. Eighty-five steady-state levels of GFR and/or P_{Na} were obtained. GFR ranged from 5 to 36 ml per minute, P_{Na} varied from 145 to 197 mEq per L, and T_{Na} varied from 0.50 to 4.50 mEq per minute. ¶ The results showed *1*) T_{Na} was linearly related to GFR regardless of the P_{Na} ($r = 0.968$, slope $= 0.11$, $p < 0.001$). *2*) When GFR was constant, absolute T_{Na} remained constant despite increases of P_{Na} to 197 mEq per L. Fractional reabsorption of sodium (T_{Na}/F_{Na}) therefore decreased as P_{Na} rose and Δ Na excretion $\cong \Delta F_{Na}$. *3*) At constant P_{Na}, T_{Na}/F_{Na} was unchanged (at approximately 0.75) over the entire range of GFR. These data clearly show that proximal T_{Na} in the dog is unaffected by increments of P_{Na} or of F_{Na} per se. They support the hypothesis that T_{Na} is a direct function of the volume of fluid entering the nephron.

Regulation of Lipolysis in the Presence of Hyperglycemia; an Explanation for Ketosis-resistant Diabetes. CHARLES J. GOODNER, MARTIN J. CONWAY, AND PING CHI CHU, Seattle, Wash. (introduced by J. Thomas Dowling*).

Plasma from 70 overnight fasting diabetics was analyzed for FFA and glucose. Plasma glucose ranged from 90 to 460 mg per 100 ml. The mean FFA for the entire group was 0.58 ± 0.18 (SD) μEq per ml. A matched group of 42 nondiabetics had mean fasting FFA of 0.59 ± 0.20 (SD). Neither the whole diabetic group nor any subgroup based on range of plasma glucose displayed a significantly positive correlation between the concentration of FFA and glucose. The relative normality of fasting FFA in diabetics suggested that regulation of lipolysis was successfully taking place regardless of the fasting plasma glucose. ¶ To test this hypothesis, we infused adult-onset diabetics with varying degrees of fasting hyperglycemia with small doses of glucose to challenge substrate regulating systems. The dose necessary for insulin secretion was as small as or smaller than (100 mg per minute for 30 minutes) in diabetics compared with normals. In hyperglycemic diabetics, FFA, insulin, and glucose returned promptly to previous levels after displacement by a glucose infusion, showing that all three were being regulated to the preinfusion levels. These data indicate that regulation of lipolysis occurs with insulin levels insufficient to maintain euglycemia. In addition, although the insulin secreting mechanism was normally responsive to small increments in glucose, endogenous hyperglycemia had not stimulated corrective insulin secretion, suggesting that release of additional insulin had been suppressed to preserve fasting lipolysis. Of the two insulin-sensitive substrates, only FFA were maintained in the normal range, implying that regulation of lipolysis assumes primacy over glucose regulation in diabetics and involves controlling sites sensitive to the mixture of glucose and FFA or to FFA alone. Ketosis-resistant diabetes may therefore reflect the successful operation of this system for independent regulation of lipolysis in the presence of hyperglycemia.

The Role of Lymphatic Absorption from the Peritoneal Cavity during Peritoneal Dialysis. ARTHUR GORDON AND LAURENCE LEWIN, Los Angeles, Calif. (introduced by Morton H. Maxwell *).

Clinical experience with peritoneal dialysis has led to the conclusion that the peritoneal cavity is lined by a semipermeable membrane. However, it is also known that significant quantities of serum protein enter the dialysate during peritoneal dialysis. When radioiodinated human serum albumin (RIHSA) was added to dialyzing solution, 1 to 5% of the isotope administered was absorbed from the peritoneal cavity over a 2- to 4-hour period. This study was undertaken to evaluate the role of the abdominal lymphatics in the transperitoneal movement of serum albumin. ¶ Mongrel dogs were anesthetized and the left thoracic duct was cannulated via a thoracotomy incision. The right lymphatic duct remained intact. Peritoneal dialysis was then performed utilizing commercially prepared dialysate to which RIHSA was added. Within 15 minutes, isotope was recovered from the drainage of the left lymphatic duct and the concentration of RIHSA in the lymph increased progressively over a 4-hour period. Simultaneous blood samples were obtained and the plasma isotope concentration was determined. The total amount of RIHSA present in the plasma volume was quantitatively comparable to that obtained from drainage of the left lymphatic duct and could presumably have entered the circulation via the right lymphatic duct. ¶ These data indicate that the lymphatics are an important and possibly the sole pathway for the transperitoneal movement of serum albumin. It is therefore likely that the lymphatics of the peritoneal cavity are not invested with a semipermeable membrane and any studies relative to peritoneal permeability must take this into consideration.

Effect of Erythrocyte Age on the Susceptibility of Its Membrane to Enzymic Disruption. EDWIN E. GORDON,* EPPO MULDER, AND L. L. M. VAN DEENEN, Utrecht, Netherlands, and New York, N. Y.

Erythrocytes resist lysis when exposed to a variety of enzymes that hydrolyze proteins and phospholipids. However, rapid hemolysis by these agents can be induced by addition of sublytic concentrations of sodium deoxycholate (DOC) to suspensions of erythrocytes in saline containing boiled *Crotalus adamanteus* snake venom (rich in

phopholipase A), trypsin, chymotrypsin, or bacterial proteinase. The following preparations, however, did not induce hemolysis in the presence of DOC: *Naja naja* snake venom (phospholipase A activity), crystalline pancreatic phospholipase A, phospholipase C from *B. cereus,* and pancreatic lipase. Fifty μl of a 50% suspension of washed rabbit erythrocytes was incubated in 5 ml of 0.9% saline with shaking at 25° in the presence of DOC-*Crotalus adamanteus* venom (venom) or DOC-trypsin. After 10 minutes, the cells were sedimented by centrifugation and the hemoglobin concentration of the supernatant was determined. With constant DOC concentration (50 μg per ml suspension), a plot of the per cent hemolysis versus the concentration of venom or trypsin resulted in a sigmoid curve with 50% hemolysis occurring at 150 μg venom per ml and 70 μg trypsin per ml. These experiments led to the consideration that the cells were being selectively lysed. To test this hypothesis, we labeled erythrocytes of three rabbits by intravenous injection of 15 μc of ^{59}Fe; at five intervals spanning the period 5 days to 62 days after administration of the label, small amounts of blood were removed from each rabbit, and the erythrocytes were separated and washed. Lysis curves obtained with varying concentrations of either venom or trypsin in the presence of DOC indicated that the recently labeled cells were more resistant to hemolysis. These results suggest that low concentrations of DOC expose sites in the membrane to attack by venom and by a variety of proteolytic enzymes. The observations indicate that the membrane is altered during aging of the erythrocyte, reflected by an increased susceptibility to DOC-venom and DOC-trypsin. Such a change in the character of the membrane may be an important determinant of erythrocyte longevity *in vivo.*

The Entry of Glucose into Liver Cells. CARL A. GORESKY,* Montreal, Quebec.

The entry of glucose into liver cells has been examined by the multiple indicator dilution technique. Labeled red cells (which remain in the vascular space), sodium (which remains extracellular), water (which enters and leaves red cells and liver cells freely), and glucose were combined in a mixture with the same hematocrit as that of venous blood. The mixture was injected into the portal vein of an anesthetized dog and effluent hepatic venous blood sampled serially, its label content being expressed in terms of fractional recovery per milliliter of blood. ¶ Labeled red cells reached an early, high peak; water, a low and late peak; and sodium was intermediate. At normal plasma glucose levels, labeled D-glucose emerged slightly before the labeled water, and the fractional recovery-time curve rose to a somewhat lower and earlier peak and then dropped less quickly. When the plasma glucose level was increased, an early and distinct peak appeared in the labeled D-glucose curve and this was followed by a prolonged tail. As the plasma glucose levels were increased further, the two components, the early peak and the prolonged tail, became increasingly separated and distinct. The early peak increased in size and approached the labeled sodium curve. It was related to this curve in a manner that indicated relatively impeded entry into and exit from the liver cells. The curve for the labeled nonphysiological isomer, L-glucose, was identical with that of a substance confined to the extracellular space. ¶ These findings appear to be consistent with the presence of a saturable transport system for D-glucose in the membranes of the cells of an intact liver.

Lipids of Normal and Leukemic Human Leukocytes. EUGENE L. GOTTFRIED, New York, N. Y. (introduced by Ernst R. Jaffé*).

The lipid composition of leukocytes depends upon the morphological cell type as well as the source of the cells. Significant differences in lipid content and distribution were found between normal lymphocytes and polymorphonuclear leukocytes, polymorphonuclear leukocytes derived from peritoneal exudate, and abnormal leukocytes from patients with acute and chronic leukemia. Normal lymphocytes and polymorphonuclear leukocytes were isolated from human blood by the glass-bead column technique. Chloroform-methanol extracts of the isolated leukocytes were analyzed for total lipid weight, lipid phosphorus, cholesterol, and plasmalogens. Individual phospholipids and neutral lipids were separated by thin-layer chromatography. In general, the major phospholipids were (in descending order of concentration) lecithin, ethanolamine phosphatide, sphingomyelin, phosphatidyl serine, and phosphatidyl inositol. The neutral lipid fractions contained free cholesterol and varying amounts of triglyceride, but relatively little esterified cholesterol. Normal lymphocytes, in comparison with normal polymorphonuclear leukocytes, contained about half as much total lipid per cell. The molar ratio of cholesterol to phospholipid was similar, but lymphocytes contained relatively more lecithin and less ethanolamine phosphatide. Mature normal leukocytes, when compared with leukemic cells of the same morphological type, had a higher total lipid content, with more cholesterol per cell and a higher molar ratio of cholesterol to lipid phosphorus. Within a given morphological series, there was little difference in total lipid phosphorus per cell, but the normal mature cells contained relatively more sphingomyelin and less lecithin than the more primitive abnormal cells. The variation in lipid composition found among different leukocyte types underscores the limited value of generalizations derived from studies of mixed leukocyte populations or abnormal cells. The findings described may reflect differences in the relative content of various intracellular organelles, each with its own characteristic composition, as well as possible differences in quantity and composition of the plasma membrane.

Studies on a Peptide-Oligonucleotide Complex with Immunogenic Properties from Antigen-exposed Macrophages. A. ARTHUR GOTTLIEB, Cambridge, Mass. (introduced by Eugene C. Eppinger†).

The precise role of the macrophage in the formation of antibody is unclear. It has been shown by other investigators that the RNA extracted from macrophages that have been exposed to T2 bacteriophage specifically

stimulates the production of neutralizing antibody against phage antigen. Moreover, the immunologically active RNA has been shown to contain antigen. ¶ If RNA from antigen-exposed macrophages is subjected to banding in a cesium sulphate density gradient, a band of density 1.588 is seen in addition to the expected RNA band of higher density. This "minor" band disappears if the RNA is treated with self-digested pronase (free of detectable nuclease) before banding. Base composition analyses of the "minor" band give a guanine-cytosine content of 56% and demonstrate that this band contains all four ribonucleotides. Gel filtration studies on this molecule point to a molecular weight in the range of 3,600 to 5,000. On the basis of these determinations, the "minor" band appears to be a peptide-oligonucleotide complex. This complex is sensitive to T1 (taka-diastase) RNAse and relatively resistant to the action of pancreatic RNAse-A. The heavy RNA band is destroyed by both enzymes. The behavior of the complex to these enzymes is the same as that noted when the immunologically active RNA of antigen-exposed macrophages was similarly treated. More recent studies have demonstrated immunological activity in the minor band. ¶ It is suggested that this peptide-oligonucleotide complex may play a role in the processing of particulate antigens into a form that is then transmitted to the definitive antibody-producing cell.

The Control of Myocardial Oxygen Consumption: Relative Importance of Contractility and Tension Development. THOMAS P. GRAHAM, JR., JAMES W. COVELL, EDMUND H. SONNENBLICK,* JOHN ROSS, JR.,* AND EUGENE BRAUNWALD,* Bethesda, Md.

Although both the inotropic state of the myocardium and myocardial wall tension are known to affect myocardial oxygen utilization (MVO_2), the relative importance of these two influences has not been defined. Furthermore, the effect on MVO_2 of increasing contractility without altering the extent of contractile element shortening is unknown. Accordingly, MVO_2 was measured in the isovolumically contracting left ventricles (LV) of seven dogs over a range of peak developed tensions (PDT) produced by varying LV volume. Norepinephrine was then infused (1.9 to 7.6 μg per minute) and MVO_2 redetermined over a range of PDT comparable to those examined before norepinephrine. Since, at any level of PDT, series elastic extension was essentially constant, contractile element shortening and work were similar under the two conditions. Contractile element velocity at zero load (V_{max}) provided a measure of the level of contractile state. When, at a constant V_{max}, PDT was augmented by an average of 117% (42.3 to 91.9 g per cm^2), MVO_2 increased by 32% (39.3 to 51.7 μl per beat per 100 g LV). When, at a constant PDT, V_{max} was increased during norepinephrine infusion by an average of 29% (42.8 to 55.2 cm per second), MVO_2 increased by 41% (42.0 to 59.0 μl per beat per 100 g LV). The relative effect of changes in PDT and V_{max} in altering MVO_2 could then be expressed as: ΔMVO_2 (μl per beat per 100 g LV) = 0.27 ΔPDT (g per cm^2) + 1.32 ΔV_{max} (cm per second). It is concluded that the oxygen cost of alterations in contractile state (V_{max}) is substantial, even when contractile element shortening is constant, and the effect of changing V_{max} is of an order of magnitude similar to that associated with alterations in myocardial wall tension.

The Coordination of Heme and Globin Synthesis. A. I. GRAYZEL, JOSEPH E. FUHR, AND IRVING M. LONDON,† New York, N. Y.

Heme plays a major role in the coordination of the syntheses of heme and of globin: it controls its own synthesis by feedback inhibition and it stimulates the synthesis of globin. ¶ Participation of globin in these regulatory systems has been examined in studies of rabbit reticulocytes incubated with or without the inhibitor of protein synthesis, cycloheximide. The synthesis of heme was measured with glycine-2-^{14}C or delta-aminolevulinic acid-4-^{14}C (ALA) as the precursor; the synthesis of globin was studied with L-leucine-U-^{14}C. Cycloheximide (2×10^{-5} M) produced complete inhibition of protein synthesis within 1 minute after the start of incubation; some inhibition of heme synthesis from glycine was observed within 10 minutes and increased progressively with longer incubation. After 4 hours, the synthesis of heme from glycine-2-^{14}C was inhibited by approximately 50%, whereas synthesis from ALA-4-^{14}C was inhibited by only 10%. ¶ This effect of cycloheximide on heme synthesis may possibly result from inhibition of the synthesis of one or more proteins involved in the formation of ALA. It is more likely, however, that the inhibition of protein synthesis results in a deficiency of globin required as acceptor for newly synthesized heme; accordingly, the concentration of heme rises and results in the inhibition of heme synthesis at one or more sites before the formation of ALA. This regulatory system is concerned with the normal coordination of synthesis of heme and globin and is relevant to disorders such as the thalassemias and the structural hemoglobinopathies.

Changes in Thyroid Secretion Produced by Inhibition of Tyrosine Deiodinase. MONTE A. GREER* AND YVONNE GRIMM, Portland, Ore.

Secretion from ^{131}I-prelabeled rat thyroids perfused *in situ* with nonradioactive blood is quite similar to secretion *in vivo*. Approximately 30% of the secreted radioactivity is I^-, the rest triiodothyronine (T_3) and thyroxine (T_4). Propylthiouracil and methimazole perfused at 10^{-3} mole per L do not produce the expected marked rise in I^- secretion, although 10^{-3} M perchlorate does. We therefore questioned the validity of the concept that iodotyrosines released by hydrolysis of thyroglobulin during the process of secretion are deiodinated intrathyroidally, most of the liberated I^- being again organically bound without leaving the thyroid. We studied this problem with perfusion of dinitrotyrosine (DNT), discovered by Green to be a potent inhibitor of tyrosine deiodinase. DNT, 10^{-3} M, in the perfusate increased secretion of labeled monoiodotyrosine (MIT) and diiodotyrosine (DIT) from nondetectable levels to 50 to 75% of the total secreted radio-

activity in the thyroid effluent. There was an associated slight decrease in the absolute secretion of I^-, but no consistent change in the absolute secretion of T_3 and T_4. Stable MIT, DIT, or menadione (an inhibitor of tyrosine deiodinase *in vitro*) infused at 10^{-3} mole per L did not reproduce the effects of DNT. It is concluded that DNT produces a pattern of thyroid secretion similar to that described in patients with tyrosine deiodinase deficiency and that deiodination of iodotyrosines is an important normal concomitant of thyroid secretion. The inability of PTU and methimazole to increase secretion of I^- remains unexplained, but may be due to retention of I^- within the thyroid during the period of infusion.

Turnover of Mitochondrial and Nuclear DNA in Rat Liver. NICHOLAS J. GROSS AND MURRAY RABINOWITZ,* Chicago, Ill.

It has been shown that mitochondria contain DNA that is distinct from nuclear DNA. In mammals mitochondrial DNA is circular, of low molecular weight (10^7 daltons), and renatures readily after denaturation. In an attempt to understand the function of mitochondrial DNA, a comparison has been made of the rates of synthesis and breakdown of mitochondrial and nuclear DNA in rats. ¶ Groups of four rats were injected with thymidine-^{3}H and sacrificed at intervals. It was shown that radioactive thymidine was suitable for pulse labeling of DNA by the fact that the specific activity of purified thymidine decayed rapidly with a half-life of 15 minutes. Mitochondria and nuclei were isolated from the livers, and the DNA was extracted with phenol and purified. The purity of the mitochondrial and nuclear DNA was verified by analytical ultracentrifugation after denaturation and renaturation. ¶ One day after injection of thymidine-^{3}H the specific activity of mitochondrial DNA was over 20 times that of nuclear DNA. By measuring specific activity of whole cell DNA it could be calculated that mitochondrial DNA is between 2 and 3% of the total DNA. Measurements of specific activities of the DNA at later times showed an exponential decay with a half-life of 7 to 8 days for mitochondrial DNA as compared with no appreciable decay of nuclear DNA after 4 weeks. The turnover of mitochondrial DNA is somewhat faster than the turnover reported for other mitochondrial components. ¶ The lability of mitochondrial DNA is in sharp contrast to the marked stability of nuclear DNA. Whether the rapid turnover represents synthesis of new molecules of DNA or repair of existing molecules has yet to be established.

Latent Pulmonary Circulatory Abnormalities in Men with a Past History of Pulmonary Edema at High Altitude. ROBERT F. GROVER, L. HOWARD HARTLEY, HARRY MILLER, AND HERBERT N. HULTGREN,† Denver, Colo., and Palo Alto, Calif.

Some apparently healthy individuals develop acute pulmonary edema soon after ascending to high altitude. Previous investigations have failed to demonstrate any underlying hemodynamic abnormality in these individuals after they have returned to sea level and recovered. The present study discloses for the first time unusual reactivity of the pulmonary vascular bed in these persons. We studied four men who had developed pulmonary edema on several occasions while mountain climbing. None had any detectable cardiac or pulmonary disease. The initial cardiac catheterization was performed at sea level. Each man was then transported by plane and car to 3,100 m (Leadville, Colo.) within 8 hours. Next morning, he climbed vigorously to 3,700 to 4,000 m and returned to Leadville for catheterization that afternoon. Results were compared with our data from eight sea level residents studied after 10 days at 3,100 m ("normal"). ¶ At sea level these four subjects had normal hemodynamics and normal arterial blood gases. Rapid ascent to high altitude combined with strenuous exertion did not induce pulmonary edema by chest X ray, auscultation, or symptoms in any subject. Tachycardia was present both at rest and during exercise, and three had a high cardiac output with exercise. Hyperventilation was present in all four, which lowered arterial CO_2 tensions to 26 to 33 mm Hg at rest and 22 to 31 mm Hg with exercise. Respiratory alkalosis resulted: pH 7.47 to 7.54 at rest and 7.44 to 7.49 during exercise. The alveolar-arterial O_2 tension difference was abnormally wide: rest, 8 to 20 mm Hg ("normal" 4); exercise, 23 to 38 mm Hg ("normal" 15). This resulted in low arterial O_2 tensions: rest, 44 to 58 mm Hg ("normal" 56); exercise, 36 to 44 mm Hg ("normal" 53). Pulmonary arterial wedge pressures were normal at 10 to 11 mm Hg, but mean pulmonary arterial pressures were markedly elevated to 37 to 47 mm Hg ("normal" 15) at rest, rising with exercise to 47 to 64 mm Hg ("normal" 25). These latent abnormalities in the pulmonary circulation that became manifest at high altitude may be the precursors of frank pulmonary edema.

Serum and Urine Immunoglobulin Light Chain Concentrations and the Clinical Course after Human Renal Homotransplantation. PAUL F. GULYASSY, WALLACE V. EPSTEIN,* MARGARET TAN, AND ANGUS RAE, San Francisco, Calif.

The degradation of Bence Jones proteins and presumptively of normal immunoglobulin light (L) chains is predominantly by a catabolic process closely correlated with renal function. The concentration of free normal L-chain has been measured in the sera and urines of 13 patients in advanced renal insufficiency before and after bilateral nephrectomy and after renal homotransplantation from living donors. The sera of all 13 patients revealed L-chain concentrations 10 to 75 times normal, and serum levels usually increased after bilateral nephrectomy. Renal transplantation (16 grafts to 13 patients) resulted in a prompt diuresis of L-chain protein. ¶ In the period 7 to 25 days post-transplantation, seven individuals had detectable L-chain protein in serum or excreted more than 100 mg of this protein per day. During the period up to 372 days post-transplantation, all seven either lost the kidney graft or were having repeated rejection episodes; these results obtained whether or not GFR and proteinuria were satisfactory during the 7- to 25-day

post-transplantation period. In 8 of the 9 patients who had normal or minimal elevation of serum and urine L-chain concentration in the 7- to 25-day post-transplantation period, the subsequent course has been favorable whether or not GFR or proteinuria was abnormal during the 7- to 25-day post-transplantation period. Rising serum or urine L-chain concentrations have been observed at the time of or before changes in GFR, proteinuria, or urinary lysozyme during graft rejection episode. Measurement of the level of serum and urine L-chain concentrations would appear to add a significant new dimension to existing measures of renal function.

Salt-losing Experimental Pyelonephritis in the Nonuremic Dog. FRANK D. GUTMANN AND RICHARD E. RIESELBACH, Madison, Wis. (introduced by Edwin C. Albright†).

Previous studies in split-bladder dogs with unilateral pyelonephritis (stage 2) have indicated that fractional excretion of sodium (FE_{Na}) is not inordinately greater in the diseased kidney (DK) than in the contralateral control kidney (CK). However, after removal of CK (stage 3), uremia evolves accompanied by a 50% increase in GFR and a marked salt-losing state. This suggests that increased GFR per nephron and osmotic load are major factors in the uremic salt-losing state. The present studies were designed to investigate additional mechanisms. ¶ Ten dogs were studied 10 weeks after induction of severe unilateral pyelonephritis (¾ infarction with thermal injury to remaining viable tissue followed by intravenous *E. coli*) when mean DK/CK osmolality (U_{max}) was 500/1,700. After hypotonic expansion (50 ml per kg 0.45 normal saline intravenously plus 40 ml per kg water orally), these results were observed (expressed as mean DK/CK): C_{Cr} (ml per minute), 5.8/56; FE_{Na} (%), 4.7/0.8; U_{Na} (mEq per L), 29/8; C_{H_2O}/GFR (%), 11/6.8. Under similar conditions in stage 3, mean C_{Cr} was 8.0, FE_{Na}, 13; U_{Na}, 44; C_{H_2O}/GFR, 10; and BUN, 116. DK/CK differences in FE_{Na} and U_{Na} were minimal in stage 2 dogs restudied during hydropenia. ¶ The data suggest that the salt-losing state in these severely diseased kidneys, when studied in the nonuremic, volume-expanded state, is primarily of proximal tubular origin. Any humoral influence on sodium transport associated with volume expansion should affect both kidneys equally. Thus, it is suggested that experimental pyelonephritis per se may be instrumental in limiting the capacity of DK proximal tubules to alter their volume appropriately in response to acute volume expansion or changes in GFR. Decreased water reabsorption from the descending limb of Henle's loop in DK may account for small differences in DK/CK FE_{Na} observed during hydropenia.

Insulin-like Inhibition of Lipolysis and Stimulation of Lipogenesis by Prostaglandin E_1 (PGE_1). HERBERT A. HAESSLER AND JOHN D. CRAWFORD,† Boston, Mass.

PGE_1 is known to inhibit the release of glycerol and free fatty acids (FFA) from adipose tissue. This action appeared to be dependent upon the metabolic availability of glucose since PGE_1 had no effect on spontaneous or epinephrine-stimulated lipolysis when epididymal fat from fasted rats was incubated in glucose-free medium. PGE_1 effects, like those of insulin, became apparent with either feeding of the animals or addition of glucose to the medium and were greater when tissue from fed animals was incubated with glucose. Both PGE_1 and insulin curtailed FFA outflow by reducing lipolysis and by increasing relative re-esterification. The most pronounced effect of PGE_1 was on stimulated tissue from fed rats incubated in glucose containing medium with insulin. In these circumstances reduction in lipolysis was in excess of that seen with insulin or PGE_1 alone or predicted as an additive effect. ¶ Because these findings suggested PGE_1 might itself have an insulin-like action, studies of its lipogenic effects were undertaken. By using tissue from fed rats in media containing glucose, PGE_1 stimulated synthesis of neutral fat from acetate-^{14}C 31% with a simultaneous 7% reduction in $^{14}CO_2$. Similarly, insulin stimulated lipid synthesis 114%, reducing $^{14}CO_2$ 30%, but PGE_1 and insulin together had no additive effect. If glucose was the labeled substrate, PGE_1 had no influence on total fat synthesis, but the partition of label between glycerol and fatty acids (FA) was shifted, the latter being increased 18%. Insulin stimulated both total fat synthesis and the proportion of glucose carbon appearing in FA. With insulin present, addition of PGE_1 gave no further effect. ¶ It was concluded that PGE_1 inhibits FA release both by its known action on lipolysis and also by promoting re-esterification and synthesis of new fat. In these actions PGE_1 is qualitatively similar to insulin.

Altered Antigenic Determinant in Rheumatoid Hyaluronateprotein. D. HAMERMAN,* J. SANDSON,* AND R. JANIS, New York, N. Y.

The major noncollagenous components of connective tissue are compounds of mucopolysaccharides and protein. The polysaccharide part is joined to the protein moiety by neutral sugars. The possibility that these proteinpolysaccharides are altered in diseases of connective tissue has long been considered, but evidence for this has not been obtained. Differences between hyaluronateprotein (HP) and normal (NHP) and rheumatoid arthritis (RHP) synovial fluids were previously reported: RHP contains more protein (10%) than NHP (2%) and forms a gel at pH 4.5. However, these findings are not specific, for they are observed with HP isolated from synovial fluids in rheumatic fever, infection, or gout. Now a new finding, and one apparently specific for RHP, has been observed. Immunologic cross-reactions between HP from synovial fluids and a proteinpolysaccharide fraction (PP) from cartilage were studied. The HP was digested with streptococcal hyaluronidase to remove the hyaluronate moiety, and the glycopeptide part was isolated from Sephadex G-200. By using agar diffusion with rabbit antiserum to cartilage PP, a precipitin line was observed with the glycopeptide obtained from HP isolated from normal and a number of pathological fluids. This line fused completely with one formed by cartilage PP. However, the glycopeptide isolated from RHP formed no precipitin line or only a faint one. Since the

lining cells of the synovial membrane appear to be the source of HP of synovial fluid, indirect immunofluorescent studies were performed with antiserum to cartilage PP and a fluorescein-labeled goat antirabbit globulin. Bright fluorescence of the normal membrane lining cells was observed; fluorescence was diminished in the rheumatoid lining cells. Thus, unlike RHP, NHP and HP isolated from other types of pathological effusions all contain an antigenic determinant in common with cartilage PP. This determinant is associated with the glycopeptide part and not the mucopolysaccharide moiety of HP. If the determinant is found to be consistently modified in RHP, this finding may be relevant to the pathogenesis of rheumatoid arthritis.

The Effect of Adrenergic Agents on the Response of the Toad Urinary Bladder to Vasopressin. J. S. HANDLER,* R. BENSINGER, AND J. ORLOFF,* Bethesda, Md.

The toad's urinary bladder responds to vasopressin *in vitro* with a marked increase in permeability to water (P H_2O). The response to 1 mU per ml of vasopressin is inhibited by 10^{-5} M and 10^{-6} M epinephrine ($-78 \pm 11\%$ and $-45 \pm 11\%$, mean $\pm$ SE, respectively), and by norepinephrine. The inhibitory effect of epinephrine is blocked by 10^{-4} M phenoxybenzamine, an α-adrenergic blocker. Phenoxybenzamine alone causes slight enhancement of the response to vasopressin ($+17\% \pm 7\%$). The combination of phenoxybenzamine and 10^{-3} M isoproterenol, a β-adrenergic agent, causes a greater increase in the response to vasopressin ($+47 \pm 15\%$). The adrenergic agents and blockers alone have no effect on P H_2O. Vasopressin acts on the toad urinary bladder by stimulating the production of adenosine 3',5'-monophosphate (cyclic 3',5'-AMP). Theophylline, which inhibits the degradation of cyclic 3',5'-AMP, was the only other agent known to enhance the P H_2O response to vasopressin. The P H_2O response to cyclic 3',5'-AMP is not altered by 10^{-5} M epinephrine, whereas the response to 10 mM theophylline is inhibited ($-89 \pm 14\%$). The effects of hormones other than epinephrine that also are known to act on their specific receptor tissues via cyclic 3',5'-AMP were examined. One-tenth and 1.0 U per ml of ACTH, 5 and 50 μg per ml of glucagon, and 10 mM serotonin have no effect on the response to vasopressin. The data are interpreted as demonstrating an inhibitory effect of α-adrenergic agents on the production of cyclic 3',5'-AMP in the toad bladder. The mechanism of the enhancement of the response to vasopressin by α-adrenergic blockade and β-adrenergic stimulation remains to be clarified. These data may be indicative of the role of catecholamines in the regulation of the action of vasopressin.

Immunologic Effects of Ionizing Radiations and Radiomimetic Alkylating Agents. WILLIAM J. HARRINGTON,* EDWARD H. HOFFMANN, MARIO VUKSANOVIC, AND SHARON POCHRAN, Miami, Fla.

We have described new antigens in blood cells after exposure to ionizing radiations and radiomimetic alkylating agents. Indirect evidence has also been presented for a substantial contribution of host responses to the new antigens in the therapeutic effects of these measures. Additional observations are relevant. ¶ Antibodies have been demonstrated in patients' sera specific for post-treatment leukemic leukocytes. The new antigens appeared within 2 days in the cells of two patients with monocytic leukemia treated with nitrogen mustard, but required 12 days to be demonstrable in the cells of patients with chronic myelocytic leukemia. Complement-fixing antibodies were detected only after the appearance of the antigens. ¶ Transfer of sensitivity to ionizing radiations has been accomplished with both lymphoid cells and plasma from irradiated animals. Isogenic (Holtzman) rats were used; total body irradiation (400 R) was delivered from a ^{60}Co source. ¶ Protection can be provided by handicapping immune responses with chlorambucil, its congener para-bis-2-chloroethylamino, *n,n*-dimethyl-phenethylamine, azathioprine plus azaserine, or induction of immunologic tolerance by neonatal intravenous injection of bone marrow cells from irradiated adult donors. Both isogenic rats and mice (C57-B1) were studied. ¶ Present evidence favors the concept that the new antigens reflect mutations rather than modification of preformed cell constituents and that distal effects *in vivo* may represent transfer of the new antigenic characteristics to remote cell populations by either transformation or hybridization.

Excretion of Collagen-like Fragments in the Urine of Patients with Paget's Disease of Bone. EDWARD D. HARRIS, JR., ALBERTO J. MUÑOZ, AND STEPHEN M. KRANE,* Boston, Mass.

Urinary hydroxyproline (hypro) peptides probably originate in collagen, although most of those previously isolated contain few amino acid residues, insufficient to relate their composition to the whole collagen molecule. To define more clearly the origin of urinary hypro, we isolated hypropolypeptides of mol wt approximately 8,000 from urine of patients with Paget's disease who have accelerated bone turnover and increased total urinary hypro. These polypeptides were partially purified by dialysis, gel filtration, and ion exchange chromatography. Several hypro-containing fractions were eluted from DEAE-cellulose columns. Corresponding peaks from different patients showed similar amino acid compositions (e.g., glycine 265 to 313, hypro 128 to 144, proline 106 to 152, alanine 73 to 93, glutamic acid 50 to 70, aspartic acid 59 to 77, and hydroxylysine 7 to 11 residues per 1,000) and low absorbancy at 280 mμ consistent with low tyrosine and tryptophan content—all characteristic of collagens. These polypeptides showed negative optical rotation, which further increased on cooling (consistent with coil to helix transition), and were degraded by clostridial collagenase. To determine the source of the hypro-polypeptides, we administered single doses of 187 and 107 μc of proline-^{14}C orally to two Pagetic subjects. Specific activity of hypro-^{14}C in urine was determined in undialyzed samples, polypeptide fractions (retained after

dialysis), and small peptide fractions (dialyzable). Specific activity of hypro-^{14}C reached peaks in all fractions 3 to 5 hours after proline-^{14}C administration. The peak specific activities in the material retained (70 and 44 dpm per μmole) were higher than in undialyzed urine (19 and 17 dpm per μmole) or in dialyzates (13 and 15 dpm per μmole). Specific activity in all fractions fell rapidly over 24 to 48 hours. These studies demonstrate that collagen-like fragments in urine of patients with Paget's disease originate either from collagen newly synthesized and rapidly degraded or from fragments rapidly synthesized but not incorporated into collagen molecules.

The Effect of Vagal Influences on the Pre-ejection Period in Man. WILLARD S. HARRIS, ARNOLD M. WEISSLER,* RICHARD H. BROOKS, AND JAMES V. WARREN,† Columbus, Ohio.

The pre-ejection period (PEP), which extends from the onset of ventricular depolarization to the beginning of left ventricular ejection, can be determined easily and atraumatically from externally derived measurements. It is shortened by adrenergic β-receptor activity, unaltered by changes in heart rate produced by atropine or right atrial pacing, and prolonged by α-adrenergic and nonadrenergic vasoconstriction. The possibility that its prolongation during pharmacologic vasoconstriction may result from reflexly induced vagal impulses to the heart was investigated by dose-response studies in 35 normal subjects. The PEP was prolonged 21 ± 3 msec (SEM) by norepinephrine administered at 15 μg per minute after β-receptor blockade by 10 mg intravenous propranolol and 25 ± 4 msec by angiotensin infused at 2.5 μg per minute. Intravenous atropine (2 mg) consistently abolished these vasoconstriction-induced prolongations of the PEP although it augmented the pressor effects of norepinephrine and angiotensin. Atropine did not alter control levels of PEP in 20 normal subjects, nor did it inhibit the prolongation of PEP due to other causes, such as head-up tilt or the closing of a systemic arteriovenous fistula. By blocking vasoconstriction-induced prolongation, atropine markedly potentiated the β-receptor-mediated shortening of PEP produced by norepinephrine without propranolol. In four patients with autonomic insufficiency, who lacked vagal efferents to the heart, β-receptor-mediated shortening of the PEP by norepinephrine was markedly exaggerated, whereas vasoconstriction-induced prolongation by norepinephrine after propranolol was absent. These results are consistent with the hypothesis that, in intact man, acute pharmacologic vasoconstriction prolongs the PEP through reflex stimulation of vagal efferents to the heart.

Continuous Monitoring of Hepatic Blood Flow in the Dog by Use of a Doppler Flowmeter System. SAMI A. HASHIM AND ROBERT G. CAMPBELL, New York, N. Y. (introduced by Theodore B. Van Itallie†).

Development of the Doppler ultrasonic flowmeter has made possible a stable system for measuring the velocity of particle-containing fluids flowing through a vessel. With appropriate calibration, this system was adapted for estimation of total hepatic blood flow in the dog. At laparotomy, individual transducers were secured around portal vein and hepatic artery at the liver hilus. The signals generated from blood flowing through each vessel past the transducers were fed into separate excitor and demodulator systems that translated them into Doppler frequencies, which, in turn, were converted into d-c signals suitable for multichannel transcription. With this technique, studies of hepatic blood flow were made in anesthetized dogs. Respiration and arterial and right atrial pressures were separately monitored. Flow data were obtained during periods of artificial and spontaneous respiration. Preliminary results showed that the mean ($\pm$SD) portal venous blood flow in seven dogs was 18.8 $\pm$ 5.7 ml per kg per minute, whereas simultaneously measured mean hepatic arterial flow was 2.4 ± 0.8 ml per kg per minute. While the animals were under controlled respiration, the pattern of portal venous blood flow varied reciprocally with breathing, the decreasing phase occurring during inspiration. The pattern of hepatic arterial flow was related to aortic pressure. Certain stimuli induced profound changes in hepatic blood flow. For example, administration of hypertonic glucose via femoral vein was followed by a prompt and striking increase in mean portal venous flow sustained for 30 minutes; at the same time, hepatic arterial flow showed a sharp transient decrease followed by a gradual rise toward control levels. This technique also permits continuous monitoring of total hepatic flow in the unanesthetized animal.

Renal Hemodynamic Studies in Sickle Cell Anemia. FRED E. HATCH, SILVIA H. AZAR, THOMAS E. AINSWORTH, AND JOHN M. NARDO, Memphis, Tenn. (introduced by James W. Culbertson†).

Renal hemodynamic studies in children and young adults with sickle cell anemia have shown the presence of a supranormal glomerular filtration rate (GFR) and effective renal blood flow (ERBF), implying an increased flow of blood through the kidneys. Noncortical (medullary) blood flow has been postulated to be decreased as a result of intravascular sickling or prolonged anoxia or both. The purposes of this study are *1*) to investigate noncortical blood flow, and *2*) to determine the possible role of anoxia in altering medullary function. ¶ Renal hemodynamic studies were performed in ten sickle cell patients (SS Hgb) with no evidence of renal disease. GFR and total renal plasma flow (RPF) were increased above normal in every patient studied. Cortical plasma flow (C_{PAH}) was increased by a mean of 170 ml per minute in males and 221 ml per minute in females. Noncortical plasma flow ($RPF - C_{PAH}$) was increased by a mean of 64 ml per minute in males and 57 ml per minute in females. The renal extraction of PAH (E_{PAH}) ranged from 0.80 to 0.92 with a mean value of 0.86 ± 0.04. In three patients E_{PAH} was decreased despite no elevation in

RBF. Both an absolute and a relative increase in noncortical blood flow are suggested, which may play a role in the urinary concentrating defect seen in these patients. ¶ In ten patients bladder urinary oxygen tension (Po_2) was measured polarographically and varied from 17 to 35 mm Hg (mean 25). Calculated values for medullary Po_2 averaged 40 mm Hg, which is above the reduced Po_2 level (30 mm Hg) required to increase the degree of sickling. These data suggest that widespread ischemic changes and stasis of blood do not occur in the kidneys of young patients with sickle cell anemia under normal conditions.

Catalase Function in Glucose 6-Phosphate Dehydrogenase Deficiency. ARTHUR HAUT AND EUGENE H. TAYLOR, Little Rock, Ark. (introduced by John A. Pierce*).

Catalase and glutathione peroxidase protect the erythrocyte from hemolysis attributed to hydrogen peroxide generated either *in vivo* or *in vitro*. Glutathione peroxidase, which is dependent upon an intact hexose monophosphate (HMP) shunt, fails when erythrocytes with glucose 6-phosphate dehydrogenase (G-6-PD) deficiency are stressed by oxidative drugs. Hypocatalasia, if present, could contribute to the events resulting in oxidative hemolysis. ¶ We have found a marked reduction in blood catalase activity in Negro men with G-6-PD deficiency when compared with similar but normal subjects, but only after whole blood from both groups was incubated with sodium ascorbate and sodium cyanide; little difference in catalase activity of the two groups was found when unmodified blood was studied. Some investigators have reported catalase activity of unmodified G-6-PD-deficient red cells to be 60 to 80% of normal, but this was not found by others. ¶ For 26 normal subjects, $K_{cat.}$ (based on the method of Takahara and associates) was 3.65 ± 0.44 (mean $\pm$ 1 SD); for 23 G-6-PD-deficient subjects, we found the $K_{cat.}$ was 3.42 ± 0.40 ($p = 0.025$). After 2 hours of incubation at 37° C with sodium ascorbate, sodium cyanide, and glucose, the catalase activity of normal blood was still 94% of the initial value, but the catalase of G-6-PD-deficient blood was only 43% and met-sulfhemoglobin had formed. Even after 4 hours the catalase of normal blood was 70% of the initial value. Cyanide, a known catalase inhibitor, used alone in the same concentration, did not inhibit catalase of either group. Loss of catalase activity was accompanied by, but did not parallel, the fall in red cell reduced glutathione (GSH), and it was not recovered after the addition of GSH. ¶ We conclude that the catalase of G-6-PD-deficient red cells stressed by oxidative drugs is especially susceptible to inhibition by cyanide, although catalase activity in unstressed red cells is virtually the same as in normal erythrocytes. This abnormality may be a clue to the adverse effect of oxidative drugs on the red cell *in vivo*.

Mobilization and Storage of Fat in Congenital and Late-onset Forms of "Total" Lipodystrophy. RICHARD J. HAVEL,* LAWRENCE V. BASSO, AND JOHN P. KANE, San Francisco, Calif.

Rates of mobilization of free fatty acids (FFA) into the blood were 50 g per day in a 19-year-old male (HR) with total lipodystrophy and 80 g per day in a 40-year-old female (CK) who developed lipodystrophy in her twenties. FFA and glycerol were released in normal proportion (5.7:1) from forearm tissues of HR, although very few adipocytes were detected within his dermis or muscle. Norepinephrine and exercise increased plasma levels of FFA and glycerol in CK but not in HR. In both, 0.25 to 1.0 U per kg insulin and large amounts of nicotinic acid did not appreciably reduce plasma levels of FFA and glycerol. Composition of FFA was grossly abnormal in both: 16:0 = 39 to 49% and 18:1 = 12 to 18% of total FFA; 69 to 79% of FFA was saturated; fatty acid composition of plasma lipid esters was normal. CK required 400 U insulin daily to control hyperglycemia; withdrawal of insulin was accompanied by striking chylomicronemia (plasma triglycerides 6,000 to 7,000 mg per 100 ml) and increased levels of FFA and glycerol; under these conditions, injection of protamine rapidly reduced FFA levels by 0.32 to 0.55 μmole per ml. Fasting triglycerides were normal in HR, but clearance of ingested triglycerides from the blood was delayed and accompanied by substantial increases in FFA and glycerol levels. Lipoprotein lipase activity in postheparin plasma was moderately decreased in both subjects. We suggest that in "total" lipodystrophy either an intrinsic abnormality of the hormone-sensitive lipase system or a powerful fat-mobilizing stimulus prevents storage of triglycerides in actively functioning adipose tissue. This could account for release of newly synthesized, saturated fatty acids from adipocytes into the blood and the failure of adipocytes to incorporate triglyceride fatty acids from the blood.

Ileal Bile Salt Transport: Mutual Inhibition in an *In Vivo* Preparation. K. W. HEATON, S. T. HEATON, AND LEON LACK, Durham, N. C. (introduced by Malcolm P. Tyor*).

Previous *in vitro* studies (using everted gut sacs) demonstrated mutual inhibition of transport between pairs of bile salts consistent with the operation of a common ileal transport system. Utilizing an *in vivo* system, we have now demonstrated similar competition between pairs of bile salts for absorption from guinea pig ileum. In lightly anesthetized animals, an ileal segment approximately 20 cm long was perfused at physiological temperature, osmolality, and pH, and at a sufficient rate to avoid significant variations in intraluminal concentration. The perfusing solution contained alternately *1*) bile salt A (substrate) alone and *2*) bile salt A plus bile salt B (inhibitor). Absorption was measured by the determination of these suitably labeled bile salts in the bile collected from the common bile duct. The results showed

that absorption can be inhibited up to 75% followed by recovery to near base-line levels when the second bile salt is excluded. The liver was ruled out as the site of this inhibition by control experiments using direct liver perfusion via a mesenteric vein. Dihydroxy bile salts were more potent inhibitors than the trihydroxy compounds, and a tri-keto bile salt was the least effective. This order of potency was the same as that observed in *in vitro* experiments. The concentrations of bile salts used did not depress glucose absorption, nor was there demonstrable damage to the intestinal mucosa as judged by dissecting and light microscopy. The data agree with the concept of a common ileal transport system for bile salts and suggest that this system constitutes the major mode of bile salt absorption in the intact animal, with passive processes playing a minor role.

Incidence of Metabolic Abnormalities in Angiographically Demonstrated Coronary Heart Disease. ROBERT A. HEINLE, DONALD FREDRICKSON,* ROBERT I. LEVY, MICHAEL V. HERMAN, AND RICHARD GORLIN,* Boston, Mass., and Bethesda, Md.

An inherent defect in most *in vivo* correlative studies of coronary heart disease is the lack of morphologic evidence of coronary atherosclerosis. Intravenous glucose tolerance (GT), serum triglyceride, total and beta cholesterol, and lipoprotein electrophoretic pattern (LPEP) were determined in 123 patients (93 men, 30 women) undergoing diagnostic selective coronary arteriography. Eighty-eight were found to have coronary artery disease (CAD) and 35 normal arteries (N). Fifty-one per cent of the CAD group had abnormal LPEP, whereas only 9% of N group had abnormal patterns. Sixty-six per cent of CAD had abnormal GT compared with 25% of N. Only 15% of CAD had neither abnormal GT nor LPEP; all were men, 10 of whom were over age 51. Only 2 of the 5 major LPEP were seen: 30% type II (elevated beta lipoprotein and plasma cholesterol) and 21% type IV (elevated pre-beta lipoprotein, elevated endogenous triglycerides). This indicated that both elevated cholesterol and elevated triglyceride were associated with CAD. The mean age of CAD patients with abnormal LPEP was 8 years less than that of patients with either no abnormality or abnormal GT only. This suggested that abnormal GT was associated with increased prevalence but not accelerated age of clinical appearance of CAD, whereas abnormal LPEP is associated with both. The incidence of GT abnormality was similar in both abnormal LPEP groups (type II 58%; type IV 66%). No clear-cut distinction among the different metabolic groups could be made as to morphology or location of atherosclerosis or extent of collateral development. Significantly, 20 of 35 N subjects had typical anginal syndrome and 6 had abnormal ECG. Had they been included as CAD on the basis of clinical criteria alone, there would have been a marked reduction in apparent prevalence of metabolic abnormalities. Not only is the incidence of specific abnormalities high in CAD, but they are multiple in nature (carbohydrate, triglyceride, cholesterol). These abnormalities are related to age and sex. If these data are evaluated in the absence of morphologic evidence for atherosclerosis, one can infer falsely low estimates of prevalence of metabolic abnormalities in CAD.

Relationship between Vitamin A Metabolism and Decreased Olfactory Detection Sensitivity in Patients with Abetalipoproteinemia and Other Types of Malabsorption. ROBERT I. HENKIN AND LEONARD LASTER, Bethesda, Md. (introduced by Donald L. Fry*).

Three patients with abetalipoproteinemia were repeatedly depleted of vitamin A by withdrawal of supplementary doses of the vitamin. When serum vitamin A fell to 10 μg per 100 ml or less, each patient exhibited increased median detection thresholds for the vapors of pyridine in water, thiophene in mineral oil, and nitrobenzene in mineral oil. Their thresholds were 1, 3, and 1 mmoles per L, respectively; normal thresholds are 10^{-5}, 10^{-5}, and 10^{-4} mmole per L, respectively. Despite decreased olfactory detection sensitivity, each patient could recognize vapors of the test substances in undiluted form. Oral administration of vitamin A palmitate, in soy bean oil or in detergent, produced a three- to five-fold rise in serum vitamin A concentration within 6 hours, and in each patient olfactory detection sensitivity was restored to normal within 24 hours. These results suggest a relationship between vitamin A metabolism and olfactory detection sensitivity. ¶ When they were in relapse, three patients with malabsorption syndrome (nontropical sprue, intestinal scleroderma, or Whipple's disease) exhibited increased median detection thresholds similar to those of the abetalipoproteinemic patients. Serum carotene concentration was abnormally low in each patient, and serum vitamin A concentration was abnormally low in two. Appropriate therapy (gluten-free diet or antibiotics) produced a gradual remission of the malabsorption in each case. Serum carotene concentrations returned to normal in each patient, and serum vitamin A concentration returned to normal in one of the two in whom it was initially low. In each patient there was a restoration of olfactory detection sensitivity to normal within 8 weeks. These observations support the hypothesis that vitamin A plays an important, perhaps an essential, role in control of olfactory detection sensitivity.

Increase of Dihydrofolate Reductase Activity in Cultured Human Leukocytes Exposed to Methotrexate. B. L. HILLCOAT, V. C. SWETT, AND J. R. BERTINO,* New Haven, Conn.

Previous studies of leukocytes from patients treated with methotrexate (MTX) showed that the MTX-sensitive enzyme, dihydrofolate reductase (DHFR), increased after drug treatment. Further investigation allowed the suggestion that most or all of this increase in enzyme activity was associated with MTX tightly bound to the

enzyme. ¶ To study this phenomenon in more detail, we tested cells from a permanent line of human leukocytes (RPMI-4265) derived from a patient with chronic myelocytic leukemia. Cells were grown either in the presence of or in the absence of MTX, and DHFR activity was measured at pH 7.0 and 8.5. As untreated cells entered log phase growth, DHFR activity increased threefold after two cell divisions (70 hours) and then declined to the activity of resting cells. Actinomycin D prevented this increase. When cells were exposed to MTX at a concentration that did not inhibit cell division (4×10^{-8} M), the DHFR activity increased tenfold over that of the resting cells and persisted when the cells entered resting phase. Actinomycin did not prevent this increase. When these cells were diluted and grown in the absence of MTX, the high DHFR activity decreased to control values. At this time, this cell population showed the same sensitivity to MTX growth inhibition as did untreated cells. The level of increase of DHFR depended on the time at which MTX was added to the culture: the later the addition, the less the increase. When cells were incubated with 5-fluorodeoxyuridine, the increase in DHFR noted with MTX was not observed. ¶ These data indicate that DHFR is an enzyme of early log phase growth whose synthesis at this time depends on messenger RNA formation. MTX causes an increased amount of total enzyme in the cell, but not of free enzyme, probably by binding tightly to the enzyme and preventing its normal degradation.

Studies on Intrinsic Factor-mediated Vitamin B_{12} Uptake: Evidence That Intrinsic Factor Is Not Absorbed. JOHN D. HINES AND ARTHUR ROSENBERG, Cleveland, Ohio (introduced by John W. Harris†).

Intrinsic factor-mediated vitamin B_{12} uptake by guinea pig small intestine was studied by employing the technique of Strauss and Wilson to contrast the behavior of proximal and distal loops during two sequential incubation periods. National Formulary hog intrinsic factor (IF) when employed was 100% B_{12} saturated and without excess B_{12}. Sequential incubations were performed as follows: *1)* IF + 4.6 ng B_{12}-^{60}Co per loop × ½ hour, and *2)* 4.6 ng B_{12}-^{57}Co per loop no IF × ½ hour. After each incubation the loops were thoroughly rinsed. When IF-B_{12} "blocking" or "precipitating" antibodies were added during the second incubation, the source was PA serum containing a high titer of one or the other antibody but negligible amounts of B_{12}. No significant exchanges of B_{12}-^{60}Co with B_{12}-^{57}Co occurred in the second incubation. ¶ Sequential incubations of the two B_{12} isotopes resulted in B_{12}-^{60}Co uptakes of 115 pg per proximal, 230 pg per distal, and B_{12}-^{57}Co uptakes of 90 pg per proximal, 230 pg per distal loop. Total B_{12}-^{60}Co plus B_{12}-^{57}Co uptake after sequential incubations exceeded the B_{12} uptake after an uninterrupted 1-hour incubation by 14% for proximal and 37% for distal loops. The addition of IF-B_{12} "blocking antibody" or Na-EDTA to the second incubation step abolished B_{12}-^{57}Co uptake by the distal loops without affecting uptake by the proximal loops. The addition of IF-B_{12} "precipitating antibody" to the second incubation step produced two effects: *1)* distal loop B_{12}-^{60}Co was increased by 51%, and *2)* distal loop B_{12}-^{57}Co was decreased by 60%. ¶ These data indicate that *1)* B_{12} uptake by proximal loops during the second incubation step was not IF mediated, and *2)* IF is not absorbed during IF-mediated B_{12} uptake by distal guinea pig small intestine, but remains available for binding additional vitamin B_{12} and for interaction with IF "blocking" and "precipitating" antibodies.

Stimulation of Nonimmunized Human Lymphocytes by Antigen-Antibody Complexes. KURT HIRSCHHORN,* NAVAH BLOCH-SHTACHER, AND JONATHAN W. UHR,* New York, N. Y.

The mechanism by which nonspecific agents such as phytohemagglutinin initiate lymphocyte responses in cell cultures is not understood. The stimulation of cells from sensitized donors by the specific sensitizing antigens presumably results from an immunologic recognition process at the membrane. The nonspecific stimulation by phytohemagglutinin, observed even in cells of agammaglobulinemics and newborn infants, can be mimicked by agents that are membrane damaging, such as streptolysin S and staphylococcal α-toxin. ¶ We have studied the action of antigen-antibody complexes on cultured normal human peripheral blood lymphocytes that showed no response to the antigens alone. These were flagellar antigens derived from *Salmonella paratyphi B* and bovine serum albumin (BSA) with their respective antibodies prepared in rabbits. The addition of such aggregates to the cultures in the presence of complement resulted in a significant increase in the uptake of thymidine-^{14}C into new DNA, reaching a peak at 5 days of culture. The response to flagellar-antiflagellar complex ranged from 2 to 32 times the control in 8 experiments (median, 7-fold increase), and that to BSA-anti-BSA complex ranged from 8 to 146 times the control in 7 experiments (median, 66-fold increase). The most likely of several explanations for this increase is a nonspecific attachment to and injury of the lymphocyte membrane by the immune complex. It is probable that a similar action at the membrane is responsible for the lymphocyte reaction to all nonspecific stimulants, and possibly even specific antigens to which the cell donor is sensitized. The nonspecific action of immune complexes may play a role *in vivo,* resulting in localized lymphoid proliferation at the site of antigen-antibody interaction.

Determinants of the Rate and Site of Bile Acid Absorption in Man. I. G. HISLOP, A. F. HOFMANN, AND L. J. SCHOENFIELD, Rochester, Minn. (introduced by C. F. Code†).

Bile acids are normally conjugated with glycine or taurine, but may be hydrolyzed by bacterial action to form free bile acids under conditions of small intestinal stasis.

By intestinal perfusions with a triple lumen tube, we have shown that the ionic group of the bile acids is a major determinant of their site of absorption in man. ¶ Micellar solutions containing ^{14}C-conjugated bile acids and free 2,4-bile acids-^{3}H (prepared by alumina catalyzed tritium exchange) were perfused through a 50-cm jejunal segment, and the absorption of bile acids relative to PEG was determined (9 perfusions, 39 collection periods). Free bile acids were efficiently absorbed (60.0 ± 5.1%, SE), glycine conjugates were absorbed less rapidly (24.5 ± 5.6%), and taurine conjugates were absorbed to a negligible extent (3.3 ± 0.8%). These differing rates of absorption for the three bile acid classes correlate with the fraction of each calculated to be present in un-ionized form at the pH of small intestinal content—a finding consistent with the postulate of Dietschy and colleagues that free bile acids are absorbed by nonionic diffusion. ¶ The number and position of nuclear hydroxylic substituents for free bile acids and glycine conjugates also influenced the rate of bile acid absorption. Cholic acid (32.8 ± 3.0%), deoxycholic acid (66.2 ± 5.0%), and chenodeoxycholic acid (85.7 ± 3.2%) were absorbed with increasing rapidity; similarly, glycine dihydroxy conjugates were absorbed at a greater rate than glycocholic acid. The absorbed free bile acids were rapidly conjugated (predominantly with glycine as determined by thin layer chromatography) and returned to the small intestinal lumen. ¶ *In vitro* solubility experiments indicated that free bile acids alone could not form micellar solutions in the small intestinal lumen, but could form mixed micelles with conjugated bile acids. However, these experimental findings suggest that any free bile acids formed by bacterial hydrolysis in the jejunal lumen would be rapidly absorbed. Liberated unconjugated free bile acids would thus undergo a jejuno-hepatic circulation in contrast to the normal ileo-hepatic circulation of conjugated bile acids.

Myocardial and Hepatic Damage Associated with Acute Lipid Mobilization. JOHN C. HOAK, WILLIAM E. CONNOR,* AND EMORY D. WARNER, Iowa City, Iowa.

The effects of acute lipid mobilization were studied in geese given glucagon (0.5 mg per kg intravenously) and in rabbits given corticotropin (ACTH) (50 U per kg subcutaneously). Blood samples were taken before, during, and at the conclusion of the experiment for determination of plasma free fatty acid concentrations (FFA), glutamic oxaloacetic transaminase (SGOT), and lactic dehydrogenase (LDH) activities. ¶ In both species tachypnea and weakness developed in association with high plasma FFA. One of seven geese and one of six rabbits died during the experiment. Mean plasma FFA were as follows: geese—before glucagon, 740 μEq per L ± SE 47; 15 minutes after, 2,995 ± 280; 24 hours after, 646 ± 40; rabbits—before ACTH, 403 μEq per L ± 13; 2 hours after, 1,890 ± 120; 5 hours after, 1,245 ± 132. Mean SGOT activity was as follows: geese—before glucagon, 20 ± 2 U; 24 hours after, 102 ± 7; rabbits—before ACTH, 32 ± 8 U; 5 hours after, 92 ± 4 U. Mean LDH activity was as follows: geese—before glucagon, 303 ± 65 U; 24 hours after, 835 ± 49; rabbits—before ACTH, 670 ± 95 U; 5 hours after, 1,295 ±226. No toxic signs, significant changes in plasma FFA, enzyme activity, or pathologic changes occurred in control animals. ¶ Surviving geese were killed 24 hours after receiving glucagon, and the rabbits were killed 5 hours after ACTH. Necropsies were performed immediately, and specimens were taken for light and electron microscopy. At necropsy, most animals had fatty livers, and hemorrhagic myocardial lesions were found in some that had exhibited electrocardiographic abnormalities. Ultrastructural changes occurred in the myocardium with destruction and distortion of mitochondria and osmiophilic inclusions. ¶ These results suggest that high plasma FFA concentrations and rapid lipid mobilization may be deleterious to cellular function and warrant further consideration in the pathogenesis of myocardial and hepatic disorders.

Selenomethionine as a Trace Amino Acid. JAMES F. HOLLAND,* SANDRA PETERS, ELLEN K. PINE, BRADLEY BRYANT, AND MONTE BLAU, Buffalo, N. Y.

The administration of selenomethionine-^{75}Se leads to labeling of proteins of several species that are commonly assumed to be representative of methionine metabolism. Paired labeling of rat serum proteins with Met-^{35}S and SeMet-^{75}Se showed divergent incorporation patterns. SeMet markedly decreased the specific activity of SeMet-^{75}Se-labeled proteins but had no effect on the Met-^{35}S specific activity. Met profoundly diluted incorporation of Met-^{35}S, but caused increase of SeMet-^{75}Se incorporation. Identical specific activities for Lys-^{3}H in these proteins suggest that alterations in rate of protein synthesis did not occur. Thus Met and SeMet occupy dissimilar metabolic pools. ¶ When dialyzed at 100,000 *g* supernatant labeled mouse liver proteins were eluted from a diethylaminoethyl-cellulose column, two protein peaks were found with high specific activity for ^{75}Se without proportionate ^{35}S incorporation, and one peak was found with high ^{35}S activity without significant ^{75}Se content. After anaerobic hydrolysis of one of the ^{75}Se-labeled peaks, chromatography was accomplished. The eluted ^{75}Se activity from an amino acid analyzer column had the mobility of authentic SeMet. ¶ When radioactive SeMet and Met were incubated together with the pH 5 enzyme and additional tRNA from rat liver, reciprocal dilution of isotopic amino acids bound to the tRNA was found. Thus the disparate behavior of Met and SeMet seen in experiments concerned with protein synthesis in whole animals is probably not attributable to differences in formation of aminoacyl RNA. The presence of Se in proteins, the metabolic pathways independent from methionine, and the binding experiments suggest that selenomethionine is a physiologic trace amino acid that shares the same tRNA with methionine in rats. Available data from man are consonant with this interpretation.

Intrarenal Blood Flow Distribution in Hypertensive Man. NORMAN K. HOLLENBERG, MURRAY EPSTEIN, R. I. BASCH, AND JOHN P. MERRILL,† Boston, Mass. (introduced by Kendall Emerson, Jr.†).

The intrarenal distribution of blood flow in man has been determined at the time of renal arteriography by measuring the disappearance of ^{133}Xe from the kidney after injection into the renal artery. In 25 normal subjects who required arteriography in their evaluation as kidney transplant donors, the normal rate of flow in the most rapid component (compartment I, probably representing cortical perfusion) was 346 ± 20(SEM) ml per 100 g per minute; the percentage of total renal blood flow to this compartment was $74.0 \pm 1.7\%$. In 5 of these subjects it was shown that the arteriogram had no effect on compartment I. In 11 arteriographically normal kidneys in 9 patients with hypertension all had both normal flow rates (398 ± 26 ml per 100 g per minute) and percentage of total flow ($72.5 \pm 2.5\%$) to compartment I. In another 6 hypertensive patients with abnormal renal arteriograms, all had a significant abnormality of compartment I. In 2, the flow rate was reduced (216 and 255 ml per 100 g per minute). The other 4, however, had a normal flow rate in compartment I with a significant reduction of the percentage of total renal blood flow to this compartment (20 to 58%). It is apparent that it is not sufficient to analyze only flow rates in the kidney in assessing hypertensives. A maldistribution of blood flow within the kidney has been demonstrated to be the only significant hemodynamic abnormality in some cases of hypertension documented to be of renal origin on the basis of arteriography, split function studies, and renal venous renin determinations.

Experimental Hepatitis in Marmosets. A. W. HOLMES, RICHARD B. CAPPS,† AND FRIEDRICH DEINHARDT, Chicago, Ill.

We have attempted to infect marmosets (*Saguinus* species) with acute phase serums or plasmas from patients with viral hepatitis. Two serums caused no disease, a third serum gave equivocal results, and a fourth serum and a plasma (the latter having caused hepatitis in human volunteers) produced a disease in marmosets that was biochemically and histologically similar to viral hepatitis in man. The disease could be transferred serially from marmoset to marmoset by inoculation of serums obtained from animals during the acute stage of their disease. Hepatitis was induced in 80 to 100% of all inoculated marmosets with incubation periods of 3 weeks for the series started with human serum and 4 to 5 weeks for the human plasma series. ¶ A plasma pool that had not caused hepatitis in human volunteers was also inoculated into marmosets, and sera from these animals were drawn and transferred into new animals simultaneously with the positive series. No liver disease developed in any of these control animals. ¶ Incubation of infectious marmoset serum with convalescent serum of the patient from whom the original infectious material was obtained, or with commercial pooled human γ-globulin, reduced the infectivity of the serum strikingly. Heating of infectious marmoset serum (60° C 12 hours) in combination with ionizing radiation (2×10^6 R) completely destroyed its infectivity. ¶ These observations suggest that marmosets may prove to be the long sought regularly susceptible experimental animal for human viral hepatitis research.

The *In Vivo* Dynamics of Virilization. RICHARD HORTON, Los Angeles, Calif. (introduced by W. H. Valentine†).

The plasma concentration, clearance, and interconversion rates of testosterone and androstenedione have been studied in hirsute or virilized patients with congenital adrenal hyperplasia (CAH) and women with adult onset adrenogenital syndrome or polycystic ovaries. These measurements in blood are necessary for any meaningful study, since the sex steroids are interconvertible in the body and there are no unique urinary metabolites. In the normal adult female two-thirds of plasma testosterone is derived from peripheral conversion of secreted androstenedione, a weak androgen. Androstenedione (A) and testosterone (T) were measured with double isotope derivative assay using thiosemicarbazide-^{35}S and steroid-^{3}H indicator. The metabolic clearance rate (MCR), a measure of the plasma cleared per day of hormone, and the conversion ratio of A to T, a measure of the effective peripheral conversion *in vivo,* were determined by constant infusion of steroid-^{3}H and chromatographic purification with ^{14}C indicator from plasma. Normal female values are 140 ± 10 (SE) mμg A and 40 ± 5 (SE) mμg per 100 ml T. The mean plasma values in patients were 650 mμg A and 140 mμg per 100 ml T in CAH, 610 mμg A and 250 mμg T in adult females with the adrenogenital syndrome, and 350 mμg A and 195 mμg per 100 ml T in patients with polycystic ovaries. The MCR^A and conversion rates in all groups were not significantly different from normal adult female values. The mean per cent of T derived from A can be calculated from these data. In CAH 76% of plasma T is from conversion of A, but in the adrenogenital syndrome or polycystic ovaries only one-third of plasma T is from peripheral conversion. Cortisol or analogue in physiological suppressive dose reduces both plasma A and T to near normal values in all patients. These studies indicate that in at least three definable disorders of androgenicity: *1)* Testosterone and androstenedione concentration and blood production rate are markedly increased. *2)* The relative role of androstenedione and testosterone varies in these clinical states; A is the major source of T in CAH, but accounts for only one-third of elevated T in adult adrenogenital or polycystic ovary syndrome. Direct secretion of T is a major factor. *3)* The effect of glucocorticoid implicates the adrenal cortex as a source of androgen in all three disorders.

Cardiovascular Effects of Anaphylaxis. PAUL C. HOUK AND STEWART WOLF,† Oklahoma City, Okla.

Cardiac arrythmias and injury patterns in the EKG were induced in sensitized rabbits by injections of human

or horse serum or in nonsensitized rabbits by intravenous histamine. Twenty-three animals equipped with venous and arterial cannulas for pressure recording and sample collection were exposed to one or the other procedure. In both instances the initial manifestations were those of the oxygen-conserving (diving) reflex with bradycardia (e.g., change in rate from 242 to 170), increased arterial pressure (e.g., 100/70 to 115/85), lowered arterial pH (e.g., 7.47 to 7.10), and increased arterial lactic acid (e.g., 8 to 24 mEq per L) and potassium (e.g., 4.0 to 4.8 mEq per L). Additional effects included showers of ventricular premature contractions, atrial fibrillation, partial and complete heart block, marked elevation or depression of S-T segments, and finally death in ventricular fibrillation or cardiac arrest. When threshold amounts of histamine were introduced into the carotid artery, the cardiac and blood pressure effects occurred within 1 second, whereas they were delayed for 7 to 9 seconds when the histamine was introduced into the femoral vein. It thus appeared that the site of action of histamine was the central nervous system rather than the heart. Moreover, the entire sequence of events, including the arrythmias and EKG changes, could be blocked either by pentobarbital anesthesia or atropine. Indeed such animals were able to survive ten times the normally fatal quantity of injected histamine. It is concluded that the cardiovascular and metabolic effects of anaphylaxis or intravenous histamine injections in rabbits represent a patterned autonomic reaction that includes potentially fatal vagal influences on the heart.

Hepatic Lysosomes in Pompe's Disease: Disappearance during Glucosidase Administration. GEORGE HUG AND WILLIAM K. SCHUBERT, Cincinnati, Ohio (introduced by A. Ashley Weech †).

Type II glycogenosis is characterized by increased tissue glycogen and deficient lysosomal acid α-1,4-glucosidase (acid glucosidase). In a 3-month-old Negro girl with the clinical features of the disease, the diagnosis was confirmed by needle biopsy of brain, liver, and muscle. Glycogen concentration was 0.18% in brain (normal up to 0.08%), 7.8% in liver (normal 2.5% to 6.5%), and 7.8% in muscle (normal up to 1.5%). Acid glucosidase activity was deficient. Electronphotomicrographs showed abnormal lysosomes stuffed with glycogen in the liver and brain but relatively few lysosomes in the muscle despite the presence of large amounts of glycogen. Notable was the abundance of abnormal lysosomes in all hepatic sections. An extract of the fungus *Aspergillus niger* was prepared with a protein concentration of 25 mg per ml and a specific activity for acid glucosidase of 22 μmoles of glucose liberated from glycogen per mg of protein per minute. On analytical ultracentrifugation the extract showed three peaks, with the major peak (S_{20} = 3.8) comprising more than 90% of the total. The extract was infused intravenously in a single dose of 7.0 ml daily. The half-life of the acid glucosidase activity in the patient's serum was 110 minutes. After 18 days of treatment, 24 hours later, muscle specimens demonstrated an increase in glycogen content from 7.8 to 9.1%, continued absence of acid glucosidase, and no change in the ultrastructure. Liver specimens showed a decrease in glycogen content from 7.8% to 2.9% (i.e., to within normal range), a residual acid glucosidase of 1.62 μmoles per g per minute (three to four times normal) and complete disappearance of the abnormal lysosomes. Detectable immunologic effects have not occurred after 40 days of treatment. Slight clinical and electrocardiographic improvement has been noted rather than the progressive deterioration characteristic of the disease.

Plasma Angiotensinase Activity in Shock. HAROLD D. ITSKOVITZ, LEONARD MILLER, WILLIAM URAL, AND JOHN ZAPP, Philadelphia, Pa. (introduced by Hugh Montgomery†).

Increased levels of plasma angiotensinase activity were measured in 25 dogs with experimental shock secondary to hemorrhage or to injections of endotoxin. The increases in plasma angiotensinase were greater in expiring than in surviving dogs. They occurred more quickly after endotoxin than after hemorrhage. In hemorrhagic shock elevations of plasma angiotensinase were recorded only after 2 to 3 hours of hypotension at a time when the dogs were entering an irreversible phase of shock. ¶ Data derived from enzymatic kinetic and inhibitor studies indicated that the elevated plasma angiotensinase activity after shock was the result of a new enzyme that had entered the circulation. This conclusion was supported further by thin layer chromatographic studies that revealed differences in the reaction products after incubation of angiotensin with plasma obtained before and after shock. ¶ Studies to elucidate the mechanism by which plasma angiotensinase increased after shock suggested a relationship to tissue ischemia (especially hemorrhagic enteritis) with the subsequent release of proteolytic enzymes from injured cells into the bloodstream. Pretreatment of six dogs with the vasodilator, diazoxide (8 mg per kg), protected the animals from developing hemorrhagic enteritis after fatal doses of endotoxin and prevented the usual increases of plasma angiotensinase. ¶ The present results raise the interesting possibility that an increased destruction of regulatory polypeptides such as angiotensin (and perhaps others as well) may be an important contributory factor to the severe physiologic imbalances and refractoriness to therapy that characterize certain forms of shock.

Blockade of Membrane and Globin Thiols in the Pathogenesis of Congenital Heinz Body Hemolytic Anemia (CHBHA) with Dipyrroluria. HARRY S. JACOB,* MICHAEL C. BRAIN, AND JOHN V. DACIE, Boston, Mass., and London, England.

Mechanisms of hemoglobin precipitation, pigmenturia, and hemolytic anemia, which characterize CHBHA, were investigated in families with hemoglobin Köln$^{\beta\text{-}93\text{-methionine}}$. Reflecting globin's helical structure, the reactivity of β-93 sulfhydryls and β-92 heme-linked histidines was altered by the neighboring mutation in Köln. Glutathione bound both β-93 cysteines as mixed disulfides, rendering globin

sulfhydryl activity immeasurable, and erythrocytic "free" glutathione subnormal, despite increased total GSH as titrated with *N*-ethylmaleimide. $G^{35}SH$ binding to Köln was seven times greater than to A. Köln detached its hemes excessively at 37° C, completely transferring ferri-hemes-^{59}Fe to unlabeled hemoglobin F in 90 minutes (A transferred under half). Blockading β-93 sulfhydryls of hemoglobin A with paramercuribenzoate reproduced both the excessive heme loss and unique heat precipitability of Köln. Cyanide or carbon monoxide prevents heme detachment. As a result excessive Heinz body formation in incubated CHBHA erythrocytes and hemoglobin precipitation at 50° C were blocked. ¶ Heinz bodies appeared attached to erythrocyte ghosts and were released specifically by mercaptoethanol. Bound radioactivity of normal ghosts, incubated with Köln-^{59}Fe, tripled when Heinz bodies were generated at 50° C; mercaptoethanol released 70% of the radioactivity. Ghosts with sulfhydryls blockaded (paramercuribenzoate) preceding Köln-^{59}Fe exposure were virtually unlabeled. CHBHA erythrocytes, especially if laden with Heinz bodies from splenectomized patients, or through heating, were hypersusceptible to membrane sulfhydryl inhibitors, exhibiting inordinate cation leakage, osmotic fragility, and autohemolysis. These findings indicate that both cellular and membrane thiols bind β-93 sulfhydryls of mutant CHBHA hemoglobins as mixed disulfides. Concomitantly heme avidity to β-92 lessens, suggesting that degradation of the resulting excessively freed hemes may produce pigmented dipyrroluria. Heinz bodies, reflecting the heightened precipitability of heme-deficient globin, attach to, thereby depleting, membrane sulfhydryl groups. This, shown previously, causes membrane hyperpermeability (as also noted during disulfide linkage of membrane thiols with insulin and ADH), splenic entrapment, and osmotic destruction of erythrocytes.

The Metabolism of 26-Hydroxycholesterol in the Hamster. NORMAN B. JAVITT AND SIDNEY EMERMAN, New York, N. Y. (introduced by David S. Baldwin*).

The two primary bile acids of hamster and man, cholic and chenodeoxycholic acids, have been identified as metabolites of 26-hydroxycholesterol. These findings indicate that 26-hydroxycholesterol may be a normal intermediate in the conversion of cholesterol to bile acid. ¶ The preparation of tritiated 26-hydroxycholesterol was achieved by modifications of the Clemmensen reduction procedure for kryptogenin that made feasible the use of tritiated water for hydrogenation at C-16 and C-22, positions not altered by metabolism to bile acid. The final crystalline product had a specific activity of 33 μc per mg and was homogeneous by thin layer chromatography and reverse isotope dilution. ¶ The compound, dissolved in a mixture of Tween 20 and saline, was given by intravenous infusion (3 to 25 mμmoles per minute) to bile fistula hamsters. Excretion rates of 10 mμmoles per minute were obtained, and 60% of the isotope was recovered within 3 hours after the infusion. ¶ Thin layer chromatographic analysis of bile after alkaline hydrolysis revealed radioactivity consistent with the presence of mono-, di-, and trihydroxy bile acids. Elution of appropriate zones and crystallization to constant specific activity with unlabeled carrier confirmed the presence of chenodeoxycholic and cholic acids in two animals. Chenodeoxycholic and cholic acids represented as much as 14% and 21%, respectively, of the total excreted radioactivity. ¶ The identification of cholic acid as a metabolite of 26-hydroxycholesterol in the hamster alters the conclusions drawn from the absence of this metabolite in the rat. The difference in metabolism may be related to the 6β hydroxylating mechanism of the rat and emphasizes the difficulties in attempting to delineate the normal pathway for bile acid synthesis in man.

Serum-Red Cell Interactions at Low Ionic Strength. DAVID E. JENKINS, JR., AND ROBERT C. HARTMANN,† Nashville, Tenn.

Paroxysmal nocturnal hemoglobinuria (PNH) erythrocytes hemolyze in isotonic, low ionic strength sucrose solutions in the presence of small amounts of serum, providing the basis for a simple and, to date, specific diagnostic test for PNH. The most important determining factor in the sucrose hemolysis system appears to be the reduction in ionic strength brought about by the sucrose media. Solutions of raffinose, mannitol, and sorbitol, which do not readily enter the red cell, can be substituted for sucrose. Solutions of sugars that readily enter erythrocytes cannot be used since osmotic lysis of normal as well as PNH cells may occur even in the absence of serum. ¶ The sucrose hemolysis reaction resembles EAC′ systems in many ways: *a*) marked erythrocyte agglutination is observed, *b*) there is significant reduction in complement activity of serum used in this system, and *c*) erythrocytes surviving hemolysis are coated with C′3 and C′4. The initiating event likely is a protein structural change at low ionic strength resulting in an "antigen-antibody-like" reaction between the red cells and serum. Further support for this supposition comes from the fact that cord serum, which has low γA and γM but normal γG and C′, does not regularly support sucrose hemolysis. Addition of a preparation of partially purified human γM renders cord serum capable of supporting sucrose hemolysis. ¶ There are additional important differences between sucrose and acid hemolysis. Sucrose hemolysis requires less serum, is less sensitive to changes in pH, occurs regularly at room temperature, is not inhibited by citrate and oxalate, and is not enhanced by thrombin. Trypsinized normal erythrocytes, which undergo marked acid hemolysis, do not hemolyze in the sucrose system. Moreover, trypsinization of PNH cells, although markedly enhancing their hemolysis in acidified serum, greatly reduces their susceptibility to sucrose hemolysis.

The Mechanism of Gene Transcription. O. WILLIAM JONES AND NANCY W. STEAD, Durham, N. C. (introduced by William S. Lynn*).

Synthesis of messenger RNA is catalyzed by the enzyme RNA polymerase. A still unanswered question is how

and where polymerase initiates transcription on DNA. Moreover, we do not know how messenger RNA is released from the DNA template. The use of homogeneous DNA molecules from bacteriophage, highly purified RNA polymerase, and a membrane filter assay for binding of polymerase to DNA provides a rapid and sensitive approach to these questions. Results show that a single phage chromosome has multiple binding sites for RNA polymerase and the number closely approximates the number of proteins encoded in the DNA molecule. All but approximately one-sixth of the sites are easily dissociable until RNA synthesis commences, and then polymerase molecules become tightly bound to the template. It is not known whether specific nucleotide sequences in DNA are part of the polymerase binding sites. However, it appears that DNA guanine is not involved, since actinomycin, at concentrations causing 95% inhibition of RNA synthesis, does not inhibit binding of polymerase to DNA. Newly synthesized RNA remains complexed to polymerase and the template. Removal of the enzyme by treatment with sodium dodecyl sulfate results in dissociation of RNA from DNA. Zone sedimentation analysis in sucrose gradients demonstrates that 70 S ribosomes dissociate up to 60% of RNA from the RNA polymerase-DNA-RNA complex. The dissociated RNA fraction forms a new peak sedimenting with 70 S ribosomes. Fifty S and 30 S ribosome subunits are not effective. We conclude that 70 S ribosomes can remove messenger RNA molecules from the template and may be a rate controlling factor in gene transcription.

The Pulmonary Echogram in the Diagnosis of Pulmonary Thromboembolism. CLAUDE R. JOYNER, JR., LEONARD D. MILLER, AND STANLEY J. DUDRICK, Philadelphia, Pa. (introduced by Calvin F. Kay†).

Reflected ultrasound lung scans have been obtained from 218 patients. A distinctive, abnormal echogram was recorded from positions on the chest overlying ischemic lung in the 50 patients of this series in whom a diagnosis of pulmonary embolism was established by the usual clinical, laboratory, and radiographic studies. Absolute confirmation with the ultrasound indication of pulmonary thromboembolism was obtained at necropsy or surgery in 10 subjects. Radioisotopic and ultrasound scans correlated precisely in 27 of 28 patients having radioisotopic scans. The radioisotopic scan failed to detect a pulmonary infarction in one patient in whom the diagnosis was established by the pulmonary echogram and confirmed at necropsy. Fourteen of the 40 subjects not having absolute confirmation by necropsy or surgery had both radioisotopic scan and radiographic confirmation. Twenty-three had either radiographic or radioisotopic scan correlation with the echogram indication of pulmonary embolism. The 3 other subjects in whom pulmonary emboli were detected by the ultrasound scan had classical clinical evidence of pulmonary infarction. No proved "false negative" pulmonary echograms have been obtained. The mass of echoes reflected from areas of ischemic lung most likely results from acoustical changes in tissue secondary to decrease in air content. As expected, 1 patient with atelectasis and 4 of 9 patients with lobar pneumonia had echogram patterns similar to those obtained from patients with pulmonary arterial occlusion. The differentiation from pulmonary infarction was easily determined in these patients by clinical and radiographic findings. The pulmonary echogram can be obtained quickly and safely at the bedside. The accuracy of this method for the detection of pulmonary ischemia appears equal to the radioisotopic scan technique.

Glucose Disappearance Rate, Serum Immunoreactive Insulin, and Dose Interrelationships during Rapid Intravenous Glucose Tolerance Tests in Man. CHARLES B. KAHN, RAY E. GLEASON, EUGENIO A. RASIO, AND J. STUART SOELDNER, Boston, Mass. (introduced by George F. Cahill, Jr.*).

To examine the interrelationships of glucose disappearance rate (K), serum immunoreactive insulin (IRI), and dose, we performed rapid intravenous glucose tolerance tests (ivGTT) at six dose levels (0.05, 0.1, 0.2, 0.33, 0.5, and 0.75 g per kg) in random order at 24-hour intervals in 10 normal subjects (aged 18 to 34). Ten levels of blood glucose (BG) and IRI were measured during each test. ¶ The mean K rates showed a progressive increase from 0.34 at the lowest dose to 2.71 at the highest. The increase between 0.5 and 0.75 g per kg, however, was not significant. ¶ Maximal IRI was achieved by 3 minutes after glucose infusion in 57 of 59 tests. IRI and BG were significantly correlated ($p < 0.01$) in 58 of 59 tests. The mean slope (IRI microunits per milliliter per milligram per cent BG) of the regression line for each test decreased as dose increased. ¶ K rate correlated with IRI output during the first 10 minutes after glucose at every dose in 9 of 10 subjects ($p < 0.05$), with maximal IRI in 8 of 10 ($p < 0.05$), and with the fraction of total IRI output occurring in the first 10 minutes in only 4 of 10 ($p < 0.05$) subjects. For each dose level no significant correlations were found between K rate and IRI output during the first 10 minutes after glucose, maximal IRI, or the proportion of total IRI output occurring in the first 10 minutes. ¶ Conclusions: Although between individuals at each dose level, K rate varies widely and fails to correlate with the insulin parameters measured, within individuals K rate increases as dose increases to 0.5 g per kg and is closely related to each IRI output. During ivGTT, BG and IRI are related irrespective of dose. This approach quantifies, within individuals, the relationship between glucose disappearance and IRI changes.

Incorporation of Glycine-^{14}C into Leukocyte Nucleic Acid. DAVID KAPLAN, HERBERT DIAMOND, MICHAEL FRIEDLAND, AND DAVID HALBERSTAM, Brooklyn, N. Y. (introduced by William Dock†).

An *in vitro* method to measure the incorporation of glycine-^{14}C into adenine and guanine of human leukocyte nucleic acid has been devised. The leukocytes are incubated with 1 μc of glycine-^{14}C for 4 hours, the nucleic acids isolated and hydrolyzed with hot perchloric acid,

adenine and guanine separated by paper chromatography, and the spots eluted and counted in a liquid scintillation counter. ¶ Twelve normouricemic individuals showed a mean incorporation into adenine of 1,218 counts per minute per 30×10^6 cells (SE = 186). The comparable figure for 9 subjects with primary hyperuricemia was 2,034 (SE − 291) ($p = 0.018$). The data for incorporation into guanine were analogous: means of 730 (SE = 126) and 1,266 (SE = 231) ($p = 0.045$) for the normal and hyperuricemic groups, respectively. Four children were also studied. One with hyperuricemia, choreoathetosis, mental retardation, and self-destructive tendencies showed incorporations of 2,998 and 2,619 into adenine and guanine. Two normal children and one child with hyperuricemia secondary to glycogen storage disease (type I) showed normal incorporations. ¶ Both iodoacetate and cyanide inhibited incorporation of glycine-^{14}C. Hypoxanthine was also an inhibitor in this system, whereas xanthine was not. This may be due to recycling of hypoxanthine back into nucleic acid synthesis, as the incorporation of hypoxanthine-^{14}C into the nucleic acids was easily demonstrated, whereas there was no evidence for the recycling of xanthine. Finally, inhibition of glycine-^{14}C incorporation by AMP and GMP was demonstrated. These observations indicate that in both primary hyperuricemia and the Lesch-Nyhan syndrome, uric acid overproduction is not due to a shunt mechanism, since the over-incorporation is into purine bases isolated from nucleic acid.

Effects of Perfusate Viscosity on Pressure-Flow Relationships and Vascular Resistance in the Dog Lung. ROBERT B. KARP, JAY A. NADEL, PAUL D. GRAF, AND JOHN F. MURRAY,* San Francisco, Calif.

Little attention has been directed to the influence of blood viscosity on pressure-flow relationships in the pulmonary circulation. The purpose of these experiments was to examine the effects of viscosity by a perfusion technique that permits control of various pressures that also affect pulmonary vascular resistance. We perfused 11 dogs' lungs *in situ* through the pulmonary artery at known flow ($\dot{Q}$). Outflow was led from the left atrium into an adjustable reservoir that could be varied to regulate left atrial pressure (P_{LA}). The lungs were ventilated between determinations. One hundred eight runs, consisting of serial pulmonary artery pressure (P_{PA}) recordings, were made while flow was varied in increments from 220 to 4,100 ml per minute and P_{LA} and alveolar pressure were held constant. We changed viscosity by varying the hematocrit ratio (Hct) of erythrocytes in dextran-saline solutions of either 70,000 or 460,000 average molecular weight. Pressure-flow relationships at 0 Hct were curvilinear at low P_{LA} and became linear as P_{LA} was raised above approximately 12 mm Hg; similar curves were obtained that became progressively displaced (higher P_{PA} at given $\dot{Q}$) when erythrocytes were added to the perfusate. At low P_{LA}, when $\dot{Q}$ was low the pressure-flow curves at different Hct (up to 53%) were close together; when $\dot{Q}$ was high the curves separated and the effect of Hct variations was more conspicuous. Resistance increased proportionately more when Hct was varied from 20 to 40% than from 0 to 20% showing a progressively greater influence of viscosity as Hct was increased. We conclude that viscosity is an important determinant of pulmonary vascular resistance, but the magnitude of its effect is variable and depends upon distending pressures, Hct, molecular weight of the perfusate, and blood flow.

Effect of Platelet Antibody Binding and Agglutination on Human Platelet Glycolysis: Comparison with Thrombin and Epinephrine. SIMON KARPATKIN AND GREGORY W. SISKIND, New York, N. Y. (introduced by Robert Silber*).

The effect of various physiologic agglutinating agents on the glycolysis of human platelets was studied. Washed platelets were aerobically incubated in a modified human Ringer solution, pH 7.1, in the presence or absence of 5 mM glucose at 37° C for 1 hour. Additions consisted of a globulin fraction of rabbit antihuman platelet antiserum or univalent fragments (Fab) of rabbit antihuman platelet antibody. Appropriate controls were included. Proof of binding of antibody fragments was established by the use of a goat antirabbit γ-globulin antiserum (Coombs' test). ¶ Binding of univalent antibody fragments had no effect on platelet glycolysis. The addition of intact antibody, thrombin, or epinephrine equally reduced ATP levels in the presence or absence of glucose. Addition of glucose alone, or intact antibody, thrombin, or epinephrine, increased lactate production. When glucose was added together with antibody, the increase in lactate production was additive. When glucose was added with thrombin or with epinephrine, the increment in lactate production was significantly greater than would be expected from a simple additive effect. However, the uptake and utilization of glucose were increased to a comparable extent in the presence of antibody, thrombin, or epinephrine. It is therefore possible that thrombin and epinephrine activate an alternative glucose-requiring glycolytic pathway. ¶ Conclusions: *1*) Coating of platelets with univalent antibody fragments does not affect glycolysis. This suggests that simple binding of antibody does not cause platelet destruction via expenditure of platelet energy stores. *2*) Intact antibody, thrombin, or epinephrine initiates platelet agglutination and increases glycogenolysis, glucose uptake, and ATP expenditure. *3*) Platelet antibody does not enhance glucose-mediated lactate production. An alternative glucose-requiring glycolytic pathway may be postulated that may be responsible for the thrombin or epinephrine enhancement of lactate production from glucose.

Failure of Aldosterone Excess to Prevent Correction of Metabolic Alkalosis. J. P. KASSIRER, F. M. APPLETON, J. A. CHAZEN, AND W. B. SCHWARTZ,* Boston, Mass.

Hypokalemic alkalosis in primary hyperaldosteronism can be distinguished from metabolic alkalosis of other types (e.g., gastric alkalosis, diuretic-induced alkalosis)

in that the acid-base disturbance is "salt resistant," i.e., that it persists despite a normal dietary salt intake. For this reason it has generally been assumed that in the alkalotic patient with secondary aldosteronism the aldosterone excess per se plays an important role in the perpetuation of the alkalotic state. On the other hand, the difficulty that we and others have had in inducing metabolic alkalosis by administration of salt-active steroids (aldosterone or DOCA) raises doubts about this conclusion. To explore the role of mineralocorticoids in the perpetuation of metabolic alkalosis in man, we have administered aldosterone (1,000 μg per day) or DOCA (40 mg per day) to nine normal human volunteers previously made alkalotic by selective depletion of HCl while ingesting a low salt diet of normal K content. After a 5- to 6-day period of steroid administration, NaCl (2 mmoles per kg) was added to the daily diet while steroids were continued. Correction of the alkalosis and marked suppression of renal acid excretion were observed within a 5- to 8-day period; plasma HCO_3 concentration thereafter remained normal despite continuation of steroid and salt for as long as 10 additional days. Correction of alkalosis was achieved despite persistent or increasing K deficits, which in some instances reached values as large as 500 to 600 mEq. When mineralocorticoids were discontinued, little further change in plasma HCO_3 concentration occurred, but in every instance there was a marked retention of K. The data indicate that aldosterone excess does not prevent restoration of normal acid-base equilibrium when NaCl is administered to subjects with gastric alkalosis, though it does prevent repair of the potassium deficit. The results of the present study, coupled with the inconsistent occurrence of metabolic alkalosis in primary aldosteronism, suggest a need for a re-evaluation of the manner in which aldosterone contributes to the genesis and maintenance of metabolic alkalosis in primary hyperaldosteronism.

Changes in Na-K-Activated Adenosine Triphosphatase of Rat Kidney Induced by Alterations in the Renal Transport of Sodium. ADRIAN I. KATZ AND FRANKLIN H. EPSTEIN,* New Haven, Conn.

An enzymatic property of cell membranes that accelerates the breakdown of ATP in the presence of Na^+ and K^+ has been described in many tissues. Evidence that Na-K-ATPase is connected with active transport of ions is strongest in red blood cells. Although the Na-K-ATPase activity of kidney cell membranes is high, its relationship to transepithelial ion transport by the kidney is not established. An attempt was therefore made to evaluate the influence of large changes in tubular reabsorption of sodium upon renal Na-K-ATPase. Glomerular filtration and tubular reabsorption of sodium per gram of kidney tissue increase progressively up to 2 to 3 weeks after contralateral nephrectomy. This is paralleled by an increase in Na-K-ATPase activity per milligram of protein in a microsomal fraction of kidney cortex. The importance of this change is underlined by the absence of simultaneous increases in other microsomal enzymes (such as glucose 6-phosphatase and Mg-ATPase) or in succinic dehydrogenase and glutaminase. Net tubular reabsorptive load of sodium can also be augmented by feeding a high protein diet and by injecting methylprednisolone. Both of these procedures induce a rise in the specific activity of renal Na-K-ATPase. On the other hand, when tubular transport of sodium is reduced by adrenalectomy, Na-K-ATPase activity is lowered. Increases in Na-K-ATPase are not an inevitable by-product of renal hypertrophy since renal ATPase activity does not rise in K deficiency, which is associated with renal hypertrophy but not with an increase in filtration or reabsorption of sodium. When the demand for net tubular reabsorption of sodium is greatly altered, an adaptive change appears to occur in the activity of Na-K-ATPase in renal tubular cells. These data suggest that Na-K-ATPase is involved in the active transport of sodium by the kidney.

Alternative Pathways of Long Chain Fatty Acid Absorption in the Absence of Chylomicron Formation. HERBERT J. KAYDEN AND MILDRED MEDICK, New York, N. Y. (introduced by Charles E. Kossmann†).

In the genetic disorder abetalipoproteinemia, chylomicron formation does not occur; nonetheless, a considerable proportion of dietary fat is absorbed. Puromycin, an inhibitor of protein synthesis, suppresses chylomicron formation in the rat. In this study, the amount of oxidation of long chain and short chain fatty acids to CO_2 in puromycin-treated rats was studied. ¶ One-^{14}C-labeled fatty acids were administered by stomach tube to fasted rats. The animals were then placed in a metabolic chamber, and expired air was collected for 18 to 20 hours. The amount of radioactive CO_2 in the expired air was measured and expressed as a per cent of the administered dose. Puromycin was given intraperitoneally for 4 hours (2.75 mg per hour) before intubation and then by continuous intraperitoneal infusion (1.1 mg per hour) for the remainder of the study. ¶ The amount of oxidation to CO_2 of long and of short chain fatty acids was not significantly different in treated or in untreated animals. After oleic acid alone, an average of 27% of the administered radioactivity was recovered; with puromycin and oleic acid, 26.9%; with octanoic acid alone, 59.5%; with puromycin, 63.3%. Similar studies using labeled triglycerides (1-^{14}C-labeled fatty acids) gave comparable data (olein, 25%; olein plus puromycin, 25.8%; octanoin, 49%; octanoin plus puromycin, 39.8%). Analysis of thoracic duct lymph in puromycin-treated animals fed fat confirmed the depression of chylomicron formation. ¶ These data indicate that despite inhibition of chylomicron formation, long chain fatty acids are absorbed and are available for oxidation and metabolism. The portal stream presumably transports the ingested fatty acids from the intestinal cells to the liver as free fatty acid-albumin complex. These studies emphasize the quantitative importance of this alternative pathway of long chain fatty acid absorption and suggest that it is probably the major route of fat absorption in patients with abetalipoproteinemia.

Antibacterial Activity of Human Urine. DONALD KAYE, New York, N. Y. (introduced by Edward W. Hook*).

Human urine is usually considered to be an excellent culture medium for many bacteria. However, the present study demonstrates that human urine is often inhibitory and sometimes bactericidal for strains of *Escherichia coli* and certain other bacteria. It is possible that the antibacterial activity of urine may be an important defense against urinary tract infection. ¶ First morning voided urine from 9 normal males and females was sterilized by filtration through a 0.45-μ Millipore filter. The fate of 13 strains of *E. coli* (6 isolated from stool and 7 isolated from urine) was studied in each of the urine specimens. One-tenth ml of trypticase soy broth containing 5×10^2 to 10^4 bacteria was inoculated into 5 ml of each urine specimen and into 5 ml of water or trypticase soy broth as controls. The number of bacteria per milliliter was determined after 3, 6, and 24 hours of incubation at 37° C. ¶ The bacteria usually multiplied faster and grew to higher titers in water or broth than in urine. All 9 urine specimens were inhibitory for some of the *E. coli* strains, and 4 of the urine specimens were inhibitory for all of the strains. Six of the urines were bactericidal for 1 or more *E. coli* strains. Urine was more inhibitory for *E. coli* strains isolated from stool than from urine, and bactericidal activity was demonstrated only against stool strains. The inhibitory or bactericidal activity of urine was not decreased by boiling or trypsinizing urine or by altering the pH but was abolished by diluting or dialyzing the urine in water. The antibacterial activity of urine for *E. coli* was correlated with urea content; concentrations of urea in water equivalent to concentrations in the urines were inhibitory or bactericidal for the strains of *E. coli*. Urine was also inhibitory for strains of *Aerobacter aerogenes, Proteus mirabilis, Pseudomonas aeruginosa,* enterococcus, *Streptococcus aureus,* and *Staphylococcus albus.*

A Specific Enzyme Defect Associated with Purine Overproduction in Adult Gout. WILLIAM N. KELLEY, FREDERICK M. ROSENBLOOM, J. FRANK HENDERSON, AND J. EDWIN SEEGMILLER,† Bethesda, Md. (introduced by James A. Shannon†).

In a large percentage of patients with primary gout accelerated purine synthesis *de novo* accounts for the hyperuricemia observed. A marked decrease in activity of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) was demonstrated in three brothers who had onset in adult life of typical gouty arthritis associated with overproduction of uric acid. Their dialyzed erythrocyte lysates showed only 2% of the enzyme activity found in normal subjects, although the activity of the closely related enzyme adenine phosphoribosyltransferase was not diminished. The mutant enzyme differed from the normal enzyme in heat stability, molecular weight, and kinetics. The finding of normal activity in eight other gouty patients who overproduced uric acid suggests that the diminished enzyme activity is not a secondary phenomenon. A similar but not identical abnormality in HGPRT activity was found in another unrelated gouty patient who overproduced uric acid. ¶ These patients showed no evidence of neurological or behavioral disorder and are thereby distinguished from the patients with the syndrome first described by Lesch and Nyhan, who showed spasticity, choreoathetosis, mental retardation, and self-mutilation in addition to marked overproduction of uric acid. We have recently demonstrated a complete deficiency of HGPRT activity in four unrelated patients with the latter syndrome. These findings in two different clinical conditions provide additional evidence that *1*) the enzyme HGPRT plays a role in the normal regulation of purine synthesis in man and its deficiency leads to excessive purine synthesis *de novo,* and *2*) several distinct biochemical aberrations can lead to excessive uric acid synthesis and clinical gout.

A Peroxidase-mediated Antimicrobial System in Leukocytes. SEYMOUR J. KLEBANOFF,* Seattle, Wash.

Saliva and milk contain an antimicrobial system that consists of lactoperoxidase, thiocyanate (or iodide) ions, and a source of H_2O_2. A peroxidase (myeloperoxidase) is present in neutrophil lysosomal granules in high concentration. Myeloperoxidase, iodide, and H_2O_2, when combined, were found to exert a strong bactericidal effect on a number of organisms (e.g., *Escherichia coli, Lactobacillus acidophilus, Staphylococcus aureus*). Myeloperoxidase could be replaced by a guinea pig leukocyte particulate preparation, H_2O_2 by a H_2O_2-generating system, and iodide by thyroxine and triiodothyronine or by other halides (chloride, bromide). The bactericidal effect was high at pH 5.0 to 6.0 and fell as the pH was increased above this range. ¶ Preincubation of myeloperoxidase, iodide, and H_2O_2 for 30 minutes before the addition of the bacteria largely prevented microbial killing. Thus the bactericidal effect was not due to toxic oxidation products, such as iodine, which accumulate during preincubation. The organisms must be present during iodide oxidation for maximal killing. Peroxidases are known to catalyze iodination reactions when incubated with iodide, H_2O_2, and an iodine acceptor. Iodination of the bacteria by the myeloperoxidase-iodide-H_2O_2 system was demonstrated chemically and radioautographically. Iodination and microbial killing were affected similarly by changes in experimental conditions in all the parameters tested. ¶ Phagocytosis of bacteria by human neutrophils was associated with the fixation of iodide in a form that was not removed by washing with methanol or water. Iodide was localized radioautographically in the cytoplasm in association with the phagocytosed bacteria. Iodide fixation was not observed in the absence of phagocytosis or in the presence of Tapazole. ¶ These considerations support the involvement of myeloperoxidase in an antimicrobial system in the neutrophil in conjunction with iodide (or chloride or both) ions and H_2O_2 generated either by leukocytes or microbial metabolism.

The Vasopermeability Response in Man to Purified C'1-Esterase. MARTIN R. KLEMPERER, VIRGINIA H. DONALDSON,* AND FRED S. ROSEN, Boston, Mass., and Cleveland, Ohio.

One of the noncytolytic pathophysiologic mechanisms mediated by the serum complement system is the development of vascular permeability. To study this reaction, we pretreated individuals with Evans blue intravenously and then gave purified human C'1-esterase intradermally. The C'1-esterase was isolated by the method of Haines and Lepow from the serum of donors judged to be free of hepatitis. This preparation did not contain any detectable Hageman factor activity. Within 10 minutes after injection an intensely blue wheal developed. The development of this wheal was not prevented by pretreatment with antihistamine (pyribenzamine 100 mg) or salicylate (salicylate level in subject = 32.5 mg per 100 ml at the time of testing). The generation of the vascular response could be evoked with a dose of C'1-esterase as small as 0.001 μ. When two individuals with a specific hereditary deficiency of C'2 were tested for their ability to generate this vascular response, it was found markedly diminished. Four-tenths μ C'1-esterase was the minimal amount capable of initiating a detectable vascular response. Their maximal response was markedly less than normal. When individuals with an acquired deficiency of serum β_{1C}-globulin (C'3) were tested for their ability to generate this complement-dependent vasopermeability response, they were found to react normally. Individuals with hereditary angioneurotic edema lack the inhibitor of C'1 esterolytic activity in their serum. They are subject to periodic attacks of localized subepithelial edema. These individuals were found to have a decreased vascular response to C'1-esterase administered within 24 hours after the termination of an attack of edema. However, when two of these individuals were given C'1-esterase intradermally, 10 days after the termination of an attack, a bout of localized angioneurotic edema was provoked. Preliminary studies employing purified C'1-esterase, C'4, and C'2 indicate that the vasopermeability factor may be derived from C'2.

The Role of Serum Factors in the Bloodstream Clearance of *Staphylococcus aureus*. M. GLENN KOENIG, M. ANN MELLY, JAY S. GOODMAN, AND DAVID E. ROGERS,* Nashville, Tenn.

Although it has been shown that serum factors play an important role in the phagocytosis of staphylococci by polymorphonuclear leukocytes, the serum factors necessary for the uptake of staphylococci by reticuloendothedial cells of the liver have not been defined. To determine the importance of serum components in bloodstream clearance, we perfused viable radiolabeled bacteria through isolated rabbit livers in buffered solutions containing various serum components. Encapsulated strains were relatively resistant to hepatic removal, but hepatic trapping of both wild-type and encapsulated staphylococci was enhanced by normal rabbit serum. Heating serum to 56° C for 30 minutes decreased but did not eliminate hepatic trapping of both types of staphylococci. The heat-stable factor enhancing removal could be partially absorbed from normal serum by coagulase positive staphylococci. Serum from animals immunized with an encapsulated staphylococcus greatly increased hepatic trapping of the encapsulated strain but not that of wild-type strains. These studies indicate that both heat-stable and heat-labile serum components play an important role in the hepatic removal of staphylococci from the bloodstream. The serum opsonic requirements for efficient reticuloendothelial uptake of staphylococci thus resemble those that promote phagocytosis by polymorphonuclear leukocytes.

Metabolic Clearance and Production Rates of Human Luteinizing Hormone in Normal and Postmenopausal Women. PETER O. KOHLER, GRIFF T. ROSS, WILLIAM W. TULLNER, AND WILLIAM D. ODELL,* Bethesda, Md., and Torrance, Calif.

The metabolic clearance rate (MCR) of human luteinizing hormone (HLH) was determined in pre- and postmenopausal women by the constant infusion technique. Highly purified HLH (Hartree) was iodinated and passed through a Sephadex G-75 column. The fraction used for infusions showed only undamaged HLH-^{131}I by chromatoelectrophoresis. This material had the same $t_{\frac{1}{2}}$ as unlabeled HLH when injected into monkeys. The HLH-^{131}I was infused into fasting subjects at a constant rate and the radioactivity precipitated from plasma by a specific double antibody technique. Plasma HLH-^{131}I levels reached equilibrium 2 to 4 hours after start of the infusion. MCR were 24.4 ± 1.8 (mean ± SE) ml per minute in 5 normal women, 23.3 ± 1.1 ml per minute in 5 normal women taking norethynodrel and mestranol, and 25.6 ± 4.1 ml per minute in 4 postmenopausal women. Since the HLH MCR were the same in each group, plasma HLH levels must be directly proportional to the secretion of HLH into plasma. Endogenous plasma HLH levels measured immediately before infusion by radioimmunoassay were 32.0 ± 9.6 mIU per ml in 5 normal women, 16.8 ± 3.2 mIU per ml in 5 normal women on oral contraceptives, and 99.2 ± 23.2 mIU per ml in the 4 postmenopausal women. The corresponding HLH PR were 733.6 ± 169.6 mIU per minute in the normal women, 387.2 ± 86.4 mIU per minute in the 5 women on norethynodrel and mestranol, and 2,402.4 ± 415.2 mIU per minute in the 4 postmenopausal women. The MCR did not change after ovariectomy in 1 woman, but the PR rose from 583.2 to 1,416.8 mIU per minute. Based on previously reported bioassay values for pituitary content and urinary excretion of HLH, the estimated turnover of HLH in the pituitary is about once per day, and only a relatively small fraction of the total HLH produced appears in the urine.

Influence of Ethinyl Estradiol-induced Cholestasis on Bile Flow and Biliary Excretion of Estradiol and Bromsulfophthalein by the Rat. M. J. KREEK, R. E. PETERSON,† M. H. SLEISENGER,* AND G. H. JEFFRIES,* New York, N. Y.

Sprague-Dawley female rats weighing 150 to 180 g were fed ethinyl estradiol, 0.5 g daily by oral tube for 9 days. Littermate control animals were similarly sham-fed. On the tenth day common bile duct cannulation and venous catheterization were performed with rats under light ether anesthesia. The animals were confined to a modified restraining cage with controlled intravenous hydration and constant temperature. Forty to 48 hours after the last dose of ethinyl estradiol and 18 to 20 hours postoperatively each animal was given 10 mg bromsulfophthalein (BSP) and a tracer dose of repurified tritium-labeled estradiol intravenously. Bile was then collected in fractions for 9 hours. At the end of the collection period the animals were sacrified and their livers examined microscopically. The volumes of bile were measured, and samples were taken for evaluation of their BSP content and radioactivity. ¶ In the ethinyl estradiol-treated animals a significant diminution of bile flow (up to 50%) was observed as compared with controls. There was a significant delay in the appearance and clearance of BSP and labeled estrogen. In control rats BSP appeared in bile within the first 10 minutes, whereas in estrogen-treated rats, BSP appearance was delayed until 25 to 35 minutes. Similarly the appearance of estradiol in the bile of treated rats was delayed, and there were both lowered radioactivity per unit volume and per unit time in the early fractions and a decreased total biliary clearance during the study period. Thus, it is concluded that cholestasis induced by ethinyl estradiol in the rat causes *1*) impaired bile flow, *2*) delayed and reduced clearance of BSP, and *3*) delayed and reduced clearance of estradiol. ¶ This demonstration that estrogen-induced cholestasis can impair biliary excretion of estrogen raises the possibility of a similarly impaired estrogen clearance in patients with idiopathic cholestatic jaundice of pregnancy and during exogenous estrogen administration.

Intraluminal Phase of Lipid Digestion in Malabsorptive Disorders. C. L. KRONE, E. THEODORE, AND G. H. JEFFRIES,* New York, N. Y.

This study was designed to investigate the significance of altered intraluminal micelle formation in the pathogenesis of steatorrhea. Proximal jejunal contents were aspirated serially through a single-lumen polyvinyl tube after a standard meal of homogenized corn oil containing polyethylene glycol (PEG) reference marker. ¶ The following analyses were performed: *1*) measurement of total lipid (gravimetric), lipid fractions [thin layer chromatography (TLC)], and fatty acid (titration) on the aspirates and the micellar and nonmicellar phases separated by high speed centrifugation; *2*) total bile acid and conjugated and unconjugated fractions (separated by TLC); *3*) quantitative bacteriologic studies (aerobic and anaerobic cultures); and *4*) measurements of pH and PEG concentration. ¶ Of four patients with ileal resection the total bile acid concentration was <1 mmole per L in three (critical micellar concentration 2 mmoles per L), micellar lipid was decreased in two, and in one decreased lipolysis was associated with gastric hypersecretion and an acid jejunal pH. In a patient with blind loop a colony count of 10^5 (*Escherichia* and *Klebsiella*) was associated with >50% unconjugated bile acid (mainly deoxycholate) and a low normal micellar lipid concentration. In scleroderma with colony counts of 10^4 (enterococcus) there was 15% unconjugated bile acid but normal micellar lipid. A patient with tropical sprue with mild steatorrhea had normal bile acid concentration and conjugation and normal micellar lipid. In total gastrectomy micellar lipid was diminished with normal lipolysis and bile acids. In four cirrhotics bile acids were normal, but in one decreased lipolysis with impaired micelle formation was associated with chronic pancreatitis. ¶ These studies in progress confirm that steatorrhea may be associated with impaired formation of lipid micelles in the intestinal lumen when there is a reduction in the concentration of conjugated bile acids or impaired lipolysis.

Catecholamine Stimulation of Myocardial Lipolysis and Fatty Acid Reesterification. FRED A. KRUGER, EDITH G. LEIGHTY, AND ARNOLD M. WEISSLER,* Columbus, Ohio.

The heart contains stored fuel in the form of glycogen and triglyceride. Although phosphorylase activation by catecholamines via 3′,5′-cyclic AMP and subsequent release of carbohydrate is well established, the mechanism for myocardial triglyceride mobilization is largely conjectural. We have previously demonstrated a labile, catecholamine-activated myocardial lipase. The present studies were designed to investigate further the control of myocardial fatty acids. ¶ Epinephrine, isoproterenol, and norepinephrine stimulated lipolytic activity in rat heart homogenates. The effect was dose dependent, and with higher concentrations fatty acid release was markedly increased during the first 5 minutes of incubation. Thereafter fatty acids declined. Cyclic AMP also stimulated lipolytic activity as measured by fatty acid and glycerol release. With higher levels of cyclic AMP, fatty acids, but not glycerol, decreased after 5 minutes. ¶ The presence of either dinitrophenol or phenethylbiguanide (which prevent ATP generation by different mechanisms) blocked catecholamine-induced lipolysis and reesterification. Addition of ATP restored catecholamine effects. Addition of glucose 6-phosphate stimulated disappearance of fatty acids. ^{14}C-labeled glucose 1-phosphate demonstrated appearance of labeled glycerides in amounts equivalent to fatty acid disappearance. ^{14}C-labeled oleic acid demonstrated epinephrine-stimulated incorporation of labeled acid into glycerides in amounts equivalent to fatty acid disappearance. Epinephrine-stimulated disappearance of fatty acids was blocked by 2-deoxyglucose without blocking stimulation of lipolysis. ¶ On the basis of these

and other experiments it is proposed that catecholamines exert a biphasic control on intracellular myocardial free fatty acids. Initially, via cyclic AMP and ATP-dependent mechanisms, fatty acids are liberated by an active, though labile, lipase. As the lipolytic activity wanes, glyceride resynthesis supervenes secondary to availability of α-glycerophosphate from glycogen via phosphorylase activation. In this way, those fatty acids that remain in excess of the energy demands of the myocardium are removed and triglyceride stores replenished.

Bronchopulmonary Lavage in Patients with Bronchial Asthma. JOHANNES A. KYLSTRA, JORGE E. BAEZ-GARCIA, KENNETH D. HALL, AND HERBERT A. SALTZMAN, Durham, N. C. (introduced by Herbert O. Sieker*).

Inspissated secretions obstructing peripheral radicles of the tracheobronchial tree often defy removal by postural drainage and bronchoscopy, but distal air passages may be cleared by bronchopulmonary lavage in patients with alveolar proteinosis. This is a report on six bronchopulmonary washings with 0.9% NaCl under general anesthesia in two patients with asthma and chronic bronchitis who did not improve with conventional therapy. The patients were intubated with a Carlen's bronchospirometry tube, and both lungs were ventilated with O_2 for 10 minutes. A volume of saline equal to one-half of the patient's FRC was then infused into one lung at a rate of 100 ml per minute. Tidal volumes of 500 ml of saline were subsequently infused rapidly and drained by gravity. At the end of the procedure, a volume of saline approximately equal to the residual volume of the lavaged lung was left in that lung. Chest X rays at various intervals after each lavage revealed gradual reabsorption of the residual liquid that was complete within 18 hours. Considerable amounts of bronchial casts with a diameter of 3 mm or less were present in the liquid returning from the lung. Improvement was apparent within 24 hours after each lavage. Three days after both lungs had been washed separately MMEFR and FEV_1 were approximately twice as large as 24 hours before lavage; FRC had decreased by approximately 1 L, and Pa_{O_2} had increased up to 15 mm Hg. Sputum production decreased and *Pseudomonas,* resistant to antibiotic therapy, was no longer present in the sputum of one patient after four lavages. Oxygen uptake through the irrigated lung, measured by differential spirometry, was normal within a few minutes after the procedure. No postlavage collapse of the lung has been observed.

Cold Reacting Antileukocyte and Platelet Antibodies in Infectious Mononucleosis. PARVIZ LALEZARI AND GEORGETTE BERNARD, New York, N. Y. (introduced by Theodore H. Spaet†).

Twenty patients with infectious mononucleosis were studied. In fourteen cases strong red cell agglutinins were identified, thirteen of which appeared to be anti-i by their preferential reaction against cord erythrocytes. Plasmas from twelve of these patients produced leukocyte agglutination in the cold, which was reversible by 37° C incubation. Although the strongest agglutination of normal and atypical leukocytes occurred at 4° C, some plasmas were effective even at 25° C. At this temperature only neutrophiles agglutinated. Cold leukocyte agglutinins were found in 20% of 200 normal donors. Leukoagglutinins in infectious mononucleosis appeared to be related to i specificity. Although platelet agglutination was not produced, the presence of both I and i determinants on leukocytes and platelets was shown by absorption and elution methods. Confirmation was obtained with antisera from patients with "viral" pneumonia and anti-i cold agglutinin disease. In addition to anti-i, three patients also had cold leukoagglutinins that did not correspond to I-i specificity, in that the antibody could not be absorbed by cord and/or adult erythrocytes. Immunochemical studies revealed these antibodies to be γM-globulins susceptible to inactivation by 2-mercaptoethanol. ¶ Varying degrees of leukopenia and thrombocytopenia were observed in these patients. The hematological abnormalities appeared to correlate with the presence of cold antibodies, the duration of illness, the temperature spectrum of the antibody activity, and the exposure of the patients to cold. When high fever necessitated use of an ice blanket, one patient developed massive leukopenia, thrombocytopenia, and hemolytic anemia. These observations suggest that similar to the hemolytic anemia observed in infectious mononucleosis, the leukopenia and thrombocytopenia may be on an immune basis.

Vasopressin and Angiotensin Interrelationship. HERBERT G. LANGFORD* AND JANICE E. REDD, Jackson, Miss.

Changes in water and salt balance will affect angiotensin (A) and vasopressin (VP) secretion. ¶ The interrelationship between the effects of the two peptides has been studied in conscious rabbits. Angiotensin in the amount used (1 μg per kg per minute) produces a profuse diuresis in the rabbit. Study I: 0.05 mμ VP was an effective antidiuretic dose in water-loaded rabbits. This amount, imposed on A diuresis, produced no significant antidiuresis. Study II: In 6 rabbits, 1 ml per kg per minute of water was administered followed by A, 1 μ per kg per minute, and VP, 10 mμ per kg per minute. Urine volume rose markedly on adding VP (as much as 5 times) or stayed constant. C_{Cr} fell (4/5); C_{osm} increased, up to 4 times. Increased C_{osm} could be accounted for by Na × 2. Study III: Water-loaded rabbits were infused with VP, 10 mμ per kg per minute. Profuse diuresis occurred, C_{Cr} decreased, and Na excretion increased up to 100-fold with urine Na well above plasma. ¶ Conclusion: Ineffectiveness of antidiuretic action of VP during A administration probably is due to abolition of the medullary gradient. (Preliminary tissue analyses support this.) Increased Na excretion produced by VP alone might be due to proximal back diffusion of Na and H_2O, with preservation of collecting duct H_2O extraction.