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ABSTRACTS

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Research Article

ABSTRACTS

Relationship between Sodium Content of Arteries and Arterial Responsiveness to Sympathetic Stimulation. FRANÇOIS M. ABBOUD* AND JOHN W. ECKSTEIN,* Iowa City, Iowa.

Forelegs of anesthetized dogs were perfused with blood at constant rates of flow. Pressures were measured in the brachial artery and vein, in a small artery and vein in the paw, in a small artery and vein in muscle, and at various points along the course of the ulnar and metacarpal arteries. Venous outflow through the cephalic vein, which drains paw, and the brachial vein, which drains muscle, were measured simultaneously. Increases in vascular resistance during sympathetic stimulation were most pronounced in large artery segments. This constriction was predominant in vessels greater than 0.5 mm o.d. immediately upstream from the metacarpal arteries; it caused significant diversion of blood flow away from the paw. Significant constriction occurred also in arteries of similar size in muscle. Sodium concentration averaged 34.3 mEq per 100 g of dried tissue at the level of the trifurcation of the ulnar artery immediately upstream from the metacarpal arteries, and 38.1 mEq in arteries of similar size in muscle. These concentrations were at least 20% greater than those in arterial segments both downstream (metacarpal arteries) and upstream (ulnar and brachial arteries) from the trifurcation ($p < 0.05$). Higher sodium content could not be attributed to a larger inulin space; this suggests an increase in the amount of bound sodium. Potassium concentration averaged 8.1 mEq in the brachial artery. This amount was 40% lower than that found in arterial segments downstream in the foreleg ($p < 0.01$). Administration of ethacrynic acid (a potent natriuretic), 1 mg per kg for 1 week, failed to alter either sodium content or responsiveness of arterial segments. We conclude that *1*) sympathetic nerve stimulation caused more constriction of arterial than venous or small vessel segments, *2*) the arterial constriction was segmental and not uniform, and *3*) segments containing the highest sodium concentration exhibited the greater constrictor responses.

The Effect of 3′,5′-Cyclic AMP and Other Nucleotides on Urine Flow and Hemodynamics in the Rat. CARL S. ALEXANDER, Minneapolis, Minn. (introduced by Wendell H. Hall †).

On the basis of studies with the urinary bladder of the toad done by others, adenosine 3′,5′-monophosphate (3′,5′-cyclic AMP) had been proposed as an intermediate in the antidiuretic action of antidiuretic hormone on the mammalian kidney. In rats underdoing steady-state perfusion and diuresis the effect of intravenous administration of 1 to 10 μmoles of adenine, adenosine, AMP, ATP, and 3′,5′-cyclic AMP was observed on urine flow, heart rate, and blood pressure. Adenosine, AMP, and ATP caused an immediate antidiuretic effect that was characteristically accompanied by profound hypotension and bradycardia. Injection of 15 to 50 μU of Pitressin also caused antidiuresis but without significant hemodynamic response. Neither antidiuresis nor hemodynamic effects were produced by the injection of adenine, 3′,5′-cyclic AMP, or theophylline. Results after injecting ATP into a mesenteric vein paralleled results after peripheral vein injection, but injection into the abdominal aorta above the renal artery decreased the hemodynamic response five- to tenfold. At times theophylline enhanced the antidiuretic effect of ATP in the rat, but when injected by itself it had no consistent action. Absorption spectrophotometry in the 220- to 320-mμ range of urine collected immediately after injection of adenosine or nucleotides failed to demonstrate their presence. Except for the absence of bradycardia similar antidiuretic and hemodynamic responses were observed following intravenous injection of 25 μmoles of ATP into a dog. In contrast, 100 μmoles of 3′,5′-cyclic AMP caused neither hemodynamic nor antidiuretic effects. In the same dog 50 mg of theophylline produced moderate hypotension and diuresis. In these experiments the antidiuretic action of adenosine and active nucleotides cannot be divorced from their vasodepressor action. Although these results do not invalidate the theory implicating 3′,5′-cyclic AMP as an intermediate of ADH action on the mammalian nephron, *in vivo* experimental evidence is required to support it.

The Metabolism of an I^{131}-labeled Human Complement Component. CHESTER A. ALPER, ALAN S. LEVIN, AND FRED S. ROSEN, Boston, Mass. (introduced by Charles A. Janeway †).

β_{1C}-Globulin, a component of C′3, was isolated by the method of Müller-Eberhard, Nilsson, and Aronsson from the serum of normal donors carefully screened for hepatitis and was labeled with I^{131}. The radioiodinated protein was fully active in the lysis of $EAC'_{1,4,2}$ in the presence of sublytic amounts of human serum. Immunoelectrophoresis with antiserum to human serum showed a single band. Radioimmunoelectrophoresis indicated that all the I^{131}-β_{1C}-globulin was converted to I^{131}-β_{1A}-globulin by treatment with hydrazine and revealed a minor contaminant (β_{1F}-globulin, another component of C′3). The sterile labeled β_{1C}-globulin was injected intravenously in five normal subjects and ten patients with diseases in which complement is suspected to be involved in pathogenesis: paroxysmal nocturnal hemoglobinuria (PNH)

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(two), systemic lupus erythematosus (SLE) (four), hereditary angioneurotic edema (HANE) (two), acquired hemolytic anemia (AHA) (one), and acute glomerulonephritis (AGN) (one). Radioactivity was followed in plasma and urine for 4 to 7 days, and the data were analyzed by the method of Matthews. In no patient was there significant protein-bound radioactivity in the urine, nor radioactivity on their washed red blood cells. The catabolic rate in five normal subjects was found to be $2.5 \pm 0.8\%$ of the plasma pool per hour, and their synthetic rate was 1.5 ± 0.6 mg per kg per hour. In four of the ten patients catabolic rates higher than normal were found: SLE (two), HANE (one), and AGN (one). The extravascular/plasma pool ratio averaged 0.7 and was the same in normals and patients. Synthesis was markedly decreased in the patient with AGN, but was normal in all other patients. These results provide *in vivo* evidence for increased disappearance of at least one complement component in some patients with SLE, AGN, and HANE. That decreased synthesis may also contribute to low plasma β_{1C}-globulin concentration and diminished complement activity is suggested by the results in one patient with AGN.

In Vitro Inhibition of Brain Protein Synthesis: An Approach to the Molecular Pathology of Maple Syrup Urine Disease and Phenylketonuria. STANLEY H. APPEL, Durham, N. C. (introduced by J. B. Wyngaarden *).

Rat brain ribosomes can be isolated as aggregates (polysomes). Their sedimentation in sucrose and their electron microscopic appearance can be altered by ribonuclease but not by deoxyribonuclease or pronase. These ribosomes will incorporate radioactive amino acids into protein *in vitro* in the presence of pH 5 fraction, ATP or ATP generating system, GTP, Mg^{++}, and NH_4^+. Under these conditions chromatographically pure leu-C^{12} has been found to inhibit val-C^{14} and ileu-C^{14} incorporation, and ileu-C^{12} to inhibit val-C^{14} and leu-C^{14} incorporation. The α-keto acid derivatives of these branched chain amino acids are also inhibitory. Furthermore, in brain slices inhibitory effects were noted by these α-amino and α-keto acids on net uptake into the cell and incorporation into protein. Similarly phenylalanine was found to inhibit the intracellular accumulation of tyrosine-C^{14} in tissue slices and its incorporation into protein in both cell-free and brain-slice preparations. The incorporation of amino acids into protein consists of three general reactions: *1*) the transport of extracellular amino acids into the cell; *2*) the formation of aminoacyl-s-RNA; and *3*) the transfer of aminoacyl-s-RNA to the ribosomes and the assembly of polypeptides on a messenger RNA template. Inhibition occurs at step 1 in our slice preparation, and an independent inhibition occurs at step 2, but not at step 3 in the cell-free system. This inhibition may reach 70% *in vitro* with levels of extracellular amino acids that are found *in vivo* in maple syrup urine disease and phenylketonuria.

Black Liver Disease in Corriedale Sheep: Metabolism of Tritiated Epinephrine and Incorporation of Isotope into the Hepatic Pigment _In Vivo_. IRWIN M. ARIAS,* LESLIE BERNSTEIN, ROBERT TOFFLER, AND JUDITH BEN-EZZER, New York, N. Y.

We have previously described photosensitive Corriedale sheep in which biliary excretion of conjugated bilirubin, phylloerythrin, sulfobromophthalein, and iodopanoic acid is defective. The liver is black. Histochemical studies of liver and physical chemical studies of the isolated pigment were identical to those observed in the Dubin-Johnson syndrome and were consistent with a melanin. The pigment was postulated to result from defective hepatic excretion of a metabolite of tyrosine, tryptophane, or phenylalanine. A complete biliary fistula was prepared in a normal and a mutant sheep. Bile and urine were separately collected for 20 days after the intravenous administration of DL-epinephrine-7-H^3-D bitartrate. Tritium-labeled metabolites were identified by chromatography and radio-scanning before and after enzymatic hydrolysis. Pigment was serially isolated from mutant sheep liver by density gradient centrifugation, hydrolysis, and organic extraction. The mutant sheep excreted less than 1% of administered radioactivity in bile (normal sheep: 18%) and 73% of administered radioactivity in urine (normal sheep: 62%). All of the radioactivity in bile of both sheep was accounted for as metanephrine glucuronide (MTG). Isotope counting and radioautography revealed incorporation of radioactivity into the hepatic pigment by the third day, and subsequent turnover was slow. Bile containing MTG-H^3 was injected intravenously into a normal and a mutant sheep. The mutant sheep excreted 0.5% of administered radioactivity in bile (normal sheep: 4.5%) and 88% of administered radioactivity in urine (normal sheep: 85%). Incorporation of radioactivity into the hepatic pigment was observed within 12 hours. These studies demonstrate a defect in hepatic excretion of MTG-H^3 in mutant Corriedale sheep and the incorporation of MTG-H^3 into the hepatic pigment *in vivo*.

The Fate of Intravenously Administered Tritiated Vitamin D_3 in Normal Subjects and in Patients with Sarcoidosis. LOUIS V. AVIOLI, JOSEPH E. MCDONALD, JUDITH LUND, AND HECTOR DELUCA, Jersey City, N. J., and Madison, Wis. (introduced by Philip H. Henneman *).

After the intravenous injection of 5 μc of tritiated vitamin D_3 (D_3-H^3) with specific activity > 1,200 dpm per U to five normal adults and four patients with sarcoidosis, serum, urine, and stool collections were made for the subsequent 48-hour period. Total radioactivity in plasma, lyophilized urine, and homogenized stool was determined by liquid scintillation counting of combusted samples. D_3-H^3 and lipid soluble metabolites thereof were separated by silicic acid thin layer and gradient elution chromatography. In the normal subjects the plasma levels of D_3-H^3 fell rapidly for 3 hours, rose gradually during the subsequent 5 hours, then decreased exponentially

with a mean half-time of 36 hours. By contrast, in the sarcoid patients plasma re-entry was maximal within 4 hours, and the plasma half-time was 16 hours. The rapid turnover of D_3-H^3 in sarcoidosis was associated with more rapid appearance of plasma lipid soluble metabolites. Of the 4.9% recoverable stool radioactivity in normals, 50.1% was lipid soluble. 6.5% of this lipid soluble material was identified as unaltered D_3-H^3. Sarcoid patients excreted 9.3% of the injected dose in the stool, 99.2% of which was lipid soluble. Only 0.6% of the lipid soluble radioactivity was identified as unaltered D_3-H^3. 2% of the injected dose appeared in the cumulative 48-hour urine in both normal and sarcoid subjects, but the lipid soluble urine radioactivity in sarcoidosis was twice that of the normals. These results indicate that the metabolism of vitamin D_3 is abnormal in sarcoidosis as manifested by *1*) a fractional turnover rate 2.2 times normal, *2*) an abnormally rapid appearance of lipid soluble vitamin D metabolites in plasma, and *3*) increased excretion of lipid soluble metabolites in urine and stool. These abnormalities of tritiated vitamin D_3 metabolism may be related to the known increased sensitivity to vitamin D in this disorder.

Individual Nephron Function in Experimental Pyelonephritis. NORMAN BANK AND HAGOP S. AYNEDJIAN, New York, N. Y. (introduced by Saul J. Farber †).

The micropuncture experiments described here were carried out to study the function of individual intact nephrons in rats with experimentally induced bilateral pyelonephritis. Approximately 10^8 organisms of *Proteus mirabilis* were injected into the exposed urinary bladder and the animals studied 4 to 6 weeks later. The resulting pathology included flat, sharply demarcated, depressed areas over the surface of both kidneys. When viewed through the microscope, the superficial tubules were necrotic in these areas, and underlying perfused glomeruli could frequently be seen. Medullary and cortical abscesses and multiple calculi were also common. Urine cultured from ureteral catheters grew out $> 10^5$ organisms per ml in every case. Glomerular filtration rate (inulin clearance) was reduced in all animals as were maximal urine concentration (U_{max}) and solute-free water reabsorption ($T^c_{H_2O}$) during mannitol diuresis. Urinary protein excretion was increased. Tubular fluid was collected from normal-appearing surface nephrons. The measurements made during mannitol diuresis were TF flow rate, TF/P_{In}, TF/P_{Na}, and TF/P_K ratios. Glomerular filtration rate per nephron was calculated from TF flow $\times$ TF/P_{In} and absolute rates of Na and K reabsorption were calculated for individual nephrons. It was found that GFR/nephron ranged from high normal to markedly supernormal values due to increases in TF flow. TF/P_{In} ratios were approximately normal, and TF/P_{Na} and TF/P_K ratios were less than 1.0 due to the mannitol. In the hyperfunctioning tubules, the absolute amounts of Na and K reabsorbed were increased above control values. These observations support the view that surviving nephrons retain their functional integrity in pyelonephritis. Furthermore, they demonstrate that intact nephrons may undergo a striking adaptation that includes an increase in GFR/nephron and enhanced ability to reabsorb sodium and potassium.

Bilirubin Turnover Studies in Normal and Pathologic States Utilizing Bilirubin-C^{14}. PETER V. D. BARRETT AND NATHANIEL I. BERLIN,* Bethesda, Md.

Turnover studies utilizing tracer quantities of bilirubin (BR) have not previously been reported in patients with normal BR levels. A new technique employing BR-C^{14} (10,000 dpm per μg) and an efficient solvent system (Weber and Schalm, 1962) for separating free and conjugated plasma BR was used in nine patients. Such studies are desirable to define the mechanisms involved in the production and excretion of BR in the normal individual and to learn how these mechanisms are altered by disease. The plasma disappearance of free BR-C^{14} took the form of the sum of two exponential functions with half-times of 15 to 35 and 61 to 194 minutes, respectively. Assuming a compartmental model with bidirectional flux of BR between the plasma and compartment x, and a unidirectional flux after BR conjugation, the BR turnover value in three patients with normal hepatic and hematologic function was 226 to 272 mg per day. Two patients with rapid rates of hemoglobin catabolism had daily BR turnovers of 447 and 601 mg. Reported studies using large amounts of nonradioactive BR have also demonstrated a second plasma disappearance component; this second component could represent feedback of free BR from the liver or any other tissue. The possibility was investigated that this feedback could arise from BR transiently bound to red cells or re-entry from lymph. In one patient no binding of BR-C^{14} to red cells was found *in vivo*, despite reports of binding *in vitro* up to 3 mg per 100 ml of red cells. In another patient with a lymph fistula, a peak of radioactivity was found at 1¼ hours, but the amount present in the first 4 hours was only 0.4% of the injected dose. Thus, the source of the second component remains unclear. It is felt that this technique may prove of considerable value in studying liver function.

On the Formation of Bile Acids from 4-C^{14}-Vitamin D_3 in the Rat. NORMAN H. BELL AND PHYLLIS BRYAN, Chicago, Ill. (introduced by David P. Earle †).

Very little is known of the mechanism for excretion of vitamin D. Vitamin D_3 differs in structure from cholesterol in having *a*) an open B ring, *b*) the hydroxyl group oriented in the 3 α instead of 3 β position, and *c*) unsaturated bonds at carbons 7 and 10 to 19. It has been shown in the rat that the formation of bile acids from cholesterol labeled appropriately with either tritium or carbon^{14} involves saturation of the double bond at carbon 5, hydroxylation at positions 7 α and 12 α, epimerization of the hydroxyl group from 3 β to 3 α, removal of the terminal isopropyl group, oxidation of carbon 24 to a carboxyl group, and conjugation of this group with either taurine or, to a lesser extent, glycine.

The principle bile acids formed are cholic acid, a trihydroxy bile acid, and chenodeoxycholic acid, a dihydroxy bile acid. To determine whether vitamin D_3 is similarly acted upon, 4-C^{14}-vitamin D_3 has been administered to rats with bile fistulas. Radioactivity was readily excreted in bile, which was then fractionated by column chromatography. The largest peak of radioactivity was associated with taurocholic acid with smaller peaks with taurochenodeoxycholic and glycocholic acids. After hydrolysis, two peaks were observed, a large one coinciding with cholic acid and a very small one with chenodeoxycholic acid. Studies are in progress to determine if these are the same or different bile acids. The results indicate for the first time that in the rat vitamin D_3 forms trihydroxy and dihydroxy bile acids that are conjugated with taurine or glycine. It appears likely that the pathways utilized in the formation of these bile acids are similar to those involved in the metabolism of cholesterol.

An Effect of Ethanol on Folate Metabolism. J. R. BERTINO,* J. WARD, A. C. SARTORELLI, AND R. SILBER, New Haven, Conn., New York, N. Y., and Saranac Lake, N. Y.

Inhibition of hematopoiesis by ethanol in folate-deficient subjects has been recently demonstrated by Sullivan and Herbert. To define the effect of ethanol at the enzymatic level, studies of folate-dependent transfer of one-carbon units in rat bone marrow and liver were undertaken. The incorporation of C^{14}-formate, but not of H^3-thymidine, into nucleic acids of bone marrow cells *in vitro* was significantly inhibited by 15 mg per ml of ethanol. A survey of enzymes catalyzing the transfer of one-carbon units in liver revealed that only tetrahydrofolate formylase (the formate-activating enzyme) was inhibited by ethanol at a comparable concentration. This enzyme was partially purified by ammonium sulfate fractionation, treatment with calcium phosphate gel, and gel filtration on Sephadex G-200. The purified enzyme, free of alcohol dehydrogenase activity, was inhibited by ethanol in a competitive manner with respect to formate. The inhibition of the formate-activating enzyme at concentrations of ethanol that could account for the impairment of the biosynthesis of nucleic acids observed with intact bone marrow cells allows the suggestion that the suppression of hematopoiesis by ethanol observed in man may be mediated, at least in part, through the reversible inhibition of this enzyme activity.

The Duarte Variant of Galactose-1-Phosphate Uridyl Transferase: A Prevalent Allele of the Gene for Galactosemia. ERNEST BEUTLER,* MARYELLEN C. BALUDA, PHILLIP STURGEON, AND ROBERT W. DAY, Duarte, Los Angeles, and Berkeley, Calif.

The red cells of galactosemia carriers have approximately ½ normal galactose-1-phosphate uridyl transferase activity. In screening over 2,000 subjects, 1.53% were found to have transferase activity of 12 U per g hemoglobin or less (normal, 18 to 26 U). Family studies revealed it to be highly unlikely for certain of these subjects to be heterozygous for galactosemia: neither parent had normal or less than 12 U of red cell enzyme activity. Instead, parents of such subjects had ¾ normal (12 to 18 U) of enzyme activity. All offspring of such subjects had ¾ normal enzyme activity, suggesting that a single gene pair was involved. Two of the 31 subjects with less than 12 U of enzyme activity appeared unique in having less than 6 U of enzyme (¼ normal). It was postulated that such subjects carried both the gene for galactosemia and the new (Duarte) variant. Two parents and six children of these subjects have been examined; all had either ½ or ¾ normal enzyme activity. None were normal or ¼ normal. It is highly probable, therefore, that the Duarte variant and galactosemia genes are allelic. Enzyme from subjects homozygous for the Duarte variant have been studied with regard to the electrophoretic mobility of the enzyme on starch gel, its thermal stability, and its pH optimum. No abnormalities have been found. On the basis of *1*) the incidence of homozygotes as determined by kindred studies; *2*) the prevalence of ¼ normal enzyme activities among heterozygotes for the gene for galactosemia; and *3*) a population survey, we estimate that approximately 15% of the U. S. population are carriers of the Duarte variant. The effect of this variant on the health of homozygotes and carriers is not yet apparent.

The Mechanism of Formation of Secondary Fat Particles from Lymph Chylomicrons. EDWIN L. BIERMAN * AND D. E. STRANDNESS, JR., Seattle, Wash.

During alimentary lipemia in normal man, turbid plasma results from the presence of two different kinds of fat particle (density <1.006, $S_f > 400$) in the circulation, only one of which ("primary particle") appears to be identical with the lymph chylomicron. The other type ("secondary particle"), differing in fatty acid composition and physical properties (electrophoresis, PVP flocculation), was thought to originate in the liver, since Kay and Entenman had shown that the perfused rat liver can produce "chylomicron-like" material. To test this hypothesis, anesthetized dogs were given constant intraportal infusions of fresh unrefrigerated C^{14}-labeled lymph obtained during peak fat absorption from the thoracic duct of donor dogs fed C^{14}-palmitate in corn oil. Hepatic vein blood was sampled during and after 60- to 90-minute infusions (2 to 4 mg triglyceride per kg per minute). Particulate fat in plasma samples and the infused lymph were fractionated by flocculation in 3% PVP solutions. Although 98 to 100% of the fat particles in the infused lymph were primary particles, most particles in early hepatic vein samples were the secondary type. A progressively larger proportion of unaltered primary particles was obtained as the infusion continued. Specific activity of secondary particle triglyceride (TG) ranged from 50 to 70% of that of the infused primary particle TG; however, in each dog secondary particle TG specific activity remained constant throughout the experiment. These results indicate that secondary particle formation results from instantaneous mixing of lymph chylomicrons

with plasma rather than from gradual mixing with hepatic TG. The essential role of a plasma lipoprotein acceptor in secondary particle formation was supported by evidence that secondary particles were produced *1*) in a peripheral artery, during infusion of lymph into the artery proximally in a hepatectomized, partially eviscerated dog; *2*) in lymph, during the earliest phase of fat absorption following a 72-hour fast; and *3*) *in vitro,* as a result of incubation of lymph with plasma.

Precipitating Antibodies against a Serum Protein ("Australia Antigen") in the Serum of Transfused Hemophilia Patients. BARUCH S. BLUMBERG * AND HARVEY J. ALTER, Philadelphia, Pa., and Seattle, Wash.

Patients who receive many blood transfusions frequently develop precipitating antibodies against low density (beta) lipoproteins. Approximately one-third of transfused patients with thalassemia and 10% of transfused patients with other diseases will develop these antibodies. Studies with two of the human antisera indicate that the antigenic specificities of the beta lipoproteins are inherited as simple autosomal traits. Additional experiments have shown that the low density lipoprotein group contains several inherited antigenic specificities that constitute a complex serum polymorphic system. Approximately 30% of patients with hemophilia may develop antibodies against a serum protein that has not as yet been identified with any of the known human serum proteins. The protein is tentatively referred to as "Australia antigen," since it was first studied in the blood of an Australian aborigine. The protein contains a relatively small amount of lipid, has an electrophoretic mobility in the alpha region, and can be isolated in the 1.063 to 1.21 and 1.21 to 1.30 specific gravity fractions by ultracentrifugation in a high density salt medium. Preliminary studies indicate that it is of high molecular weight and may represent a form of delipidated lipoprotein. The Australian antigen has not been detected in any of approximately 700 normal U. S. white and Negro sera that have been tested. This implies that the fresh freezing of plasma given to hemophilia patients in some way alters a normal serum constituent to produce the anti-Australia antigen precipitin in the transfused patients. The antigen is found in from 5 to 15% of various foreign populations including Australian aborigines, Vietnamese, Samaritans, and Greeks. It is also found in approximately 10% of patients with leukemia and 25% of patients with mongolism (Down's syndrome) but not in approximately 1,000 patients with other diseases. The epidemiological findings are consistent with the explanation that the protein is associated with some factor common to leukemia and mongolism.

Successful Long-Term Peritoneal Dialysis in Terminal Renal Failure. S. T. BOEN, H. TENCKHOFF, AND C. M. MION, Seattle, Wash. (introduced by B. H. Scribner *).

Peritonitis and adhesions observed with indwelling devices have been the main causes of failure in periodic peritoneal dialysis of patients with end-stage renal disease. We have now reached the point that long-term peritoneal dialysis is beyond the experimental phase and can be carried out successfully without infection. The reasons for success are *1*) the use of repeated puncture technique, eliminating indwelling devices, and *2*) a closed sterile dialysis system with fluid in large containers and a cycling machine. Two female patients have been on once weekly dialysis for 21 and 10 months, respectively. Their creatinine clearances are 1.5 and 2.7 ml per minute, respectively. Both were comatose before dialysis treatment. Dialyzing rate is 3 L per hour and duration of dialysis 14 to 24 hours. In the first patient peritoneal urea clearances did not decrease after 21 months, ranging from 20 to 35 ml per minute. Protein removal was up to 75 g per dialysis, of which 40 g was due to removal of ascitic fluid before dialysis. Serum proteins fell to 5.5 g per 100 ml, rising to 6.7 g per 100 ml recently after institution of intravenous reinfusions of ascitic fluid; the electrophoretic pattern is normal. The average pre- and postdialysis BUN were 110 and 60, creatinine 16 and 9. Clincally she is remarkably well, has gained weight, and is able to do normal housework. Low sodium diet and removal of fluid with dialysis have resulted in maintenance of normal blood pressure. Nerve conduction times became normal. The second patient is dialyzed at home with a machine without professional attendance. Puncture wound bleedings have occurred several times. She is carrying out housework and enjoys camping and fishing. The results call for reassessment of periodic peritoneal dialysis in the management of terminal renal disease; it is suggested that a community peritoneal dialysis center is feasible.

Metabolic Aspects of the Pathogenesis of Osteoarthritis: Polymerization of Cartilage Chondroitinsulfate. ALFRED JAY BOLLET,* Charlottesville, Va.

Decreased chondroitinsulfate concentration but increased S^{35} uptake in osteoarthritic cartilage suggested increased polysaccharide loss as the mechanism of chondromalacia, a primary aspect of the pathogenesis of osteoarthritis. Loss of chondroitinsulfate but not protein from the cartilage protein-CS suggested hyaluronidase rather than proteolysis as the underlying mechanism; subsequently hyaluronidase activity was found widely distributed among animal tissues. As further test of the hypothesis that hyaluronidase was responsible for the loss of chondroitinsulfate, the polymerization of this polysaccharide was determined in normal and osteoarthritic human knee cartilage obtained at autopsy, since protease would have no effect, but hyaluronidase should leave partially degraded chondroitinsulfate in the lesions. Reducing sugar (as end group analysis), uronic acid, and hexosamine were determined, and the formula of Partridge was used to calculate average chain length. In normal areas molecular weights averaged 27.9 ± 2.57 ($\times 10^3$); osteoarthritic areas averaged 12.1 ± 1.35 ($p = <0.001$). In abnormal sites chondroitinsulfate concentration averaged one-third that of normal areas, and there was cor-

relation between the decrease in concentration and in polymerization ($r = 0.61$, $p = <0.01$). Lower molecular weight chondroitinsulfate in osteoarthritic cartilage was also evident by an increased proportion soluble in 40% ethanol, and held up on dextran gel. Synthesis of less polymerized polysaccharide in the osteoarthritic lesions could give similar findings; accordingly, cartilage samples were incubated *in vitro* with SO_4^{35} or C^{14}-acetate, and the chondroitinsulfate was fractionated at varying ethanol concentrations. A similar distribution of counts was found in normal and osteoarthritic sites, although specific activity was higher in all fractions in the osteoarthritic samples. These data support the concept that hyaluronidase activity is responsible for the increased loss of chondroitinsulfate in the cartilage lesions of osteoarthritis, and help to design analyses of controls of the rate of cartilage matrix degradation.

Lysosomal Hydrolase Activity in Ischemic Myocardium. NORMAN BRACHFELD AND TERUO GEMBA, New York, N. Y. (introduced by Robert F. Watson †).

Release of lysosomal hydrolases following cell damage may precede final cellular destruction. Myocardium is often severely challenged by ischemia, a state shown to promote lysosomal enzyme release in liver and kidney. An *in vivo* experiment was designed to study the effects of acute, myocardial ischemia on the integrity of the lysosomal particle and on the cellular distribution of its hydrolases. The left coronary artery (dog) was ligated, and animals were sacrificed at 2, 6, 12, 24, and 48 hours. Samples were homogenized and separated by ultracentrifugation into supernatant-free (S), large granule (L), and nuclear (N) fractions. Antemortem sequential arterial (A) and coronary sinus (CS) samples, the total homogenate and its fractions were assayed for cathepsin (Cath), β-glucuronidase (β-Gluc), acid phosphatase (AP), cytochrome oxidase (Cyto) (mitochondrial), and nitrogen content. Mean enzyme recovery = 96%; nitrogen recovery = 94%. Uninvolved left ventricle served as control. Mean total nitrogen loss = 17.8%; water content increased 1.96%. An ECG monitor verified ischemia. There was a temporally related fall in total homogenate activity (units per milligram N or per gram wet weight) for Cath, AP, and Cyto (−45%, −63%, −60% per g wet weight) noted with 1½ hours. Total homogenate activity of β-Gluc rose 46%. When expressed in terms of fractional activity (per cent), there was an increase in the S and L fractions for Cath (+50%, +24.6%), β-Gluc (+28%, +55%), and Cyto (+181%, +70%) with reciprocal changes seen in fraction N. Per cent total AP activity decreased in fraction S (−41.7%) and increased in fractions L and N (+220%, +55%). The CS concentration of AP and β-Gluc was elevated, supporting these changes as being a true intracellular ischemic response. Serum GOT, LDH, and LD-5 titers remained elevated and varied reciprocally with their concentration in fraction S. Tissue lactate and α-glycerophosphate concentration was elevated (+797%, +339%) in early samples but decreased after 2 hours. The study demonstrates myocardial lysosomal activity. *In vivo* ischemia caused profound variations in total and fractional enzyme distribution which differed from that seen in other tissues. Myocardial lysosomal enzyme behavior was complex and nonhomogeneous in terms of release and selective retention of activity.

Treatment of Circulatory Shock with Adrenolytic (Vasodilator) Drug. EDWARD C. BRADLEY AND MAX H. WEIL, Los Angeles, Calif. (introduced by George J. Friou *).

In dogs, phentolamine (Regitine) consistently increased venous return while it lowered arterial pressure and peripheral resistance. It appeared likely that this drug had promise for increasing venous return and cardiac output in patients with shock. A total of 37 observations was made on 13 patients with clinical features of shock. Shock was related to bacterial infection in six, fluid loss in two, a cardiac cause in three, and neurological injury in two cases. Phentolamine was infused intravenously at an average rate of 0.6 mg per minute for an average duration of 22 minutes. In each trial, cardiac output increased during infusion of phentolamine. Cardiac index increased from an average of 2.8 to 3.6 L per minute per m^2 on 15 trials in eight patients ($t = 6.5$, $p < 0.001$), and arterial pressure declined from a mean of 73 to 59 mm Hg ($t = 6.45$, $p < 0.001$). When phentolamine was administered on 22 occasions to five patients in whom arterial pressure had been maintained by a constant infusion of metaraminol, cardiac index increased from 2.1 to 3.4 L per minute per m^2 ($t = 3.26$, $p < 0.001$). Arterial pressure declined from a mean value of 73 to 62 mm Hg. Improvement in systemic blood flow was manifested by a reduction in mean circulation time, an increase in peripheral skin temperature, and an increase in urine flow. We conclude that in selected instances an adrenolytic drug may be used in conjunction with, or in preference to, a pressor amine to improve effective blood flow in circulatory shock.

The Effects of Alcohol on Host Defenses. ROBERT G. BRAYTON, PETER STOKES, AND DONALD B. LOURIA,* New York, N. Y.

Alcohol increases susceptibility to certain infections in man. Animal studies suggest that increased severity of infection is related to diminished polymorphonuclear leukocyte mobilization, defective phagocytosis, and intracellular bacterial destruction. To study leukocyte mobilization in man, 50 to 75 ml of 95% ethyl alcohol was given intravenously over 1 hour to 16 normal persons. Simultaneously with the infusion, a 1-cm^2 forearm area was abraded superficially; a 5-ml plastic cup was placed snugly over the area and filled with a Hanks-Varidase solution. At 2, 4, 6, and 8 hours, the number of polymorphonuclear leukocytes (PMN) entering the traumatized area was counted. Compared to 54 control subjects, PMN mobilization was strikingly decreased in alcohol-treated individuals, being 200-fold less at 2 hours, 50-fold less at 4 hours, and 13-fold less 6 hours after initiation

of trauma. In 16 patients in shock, mobilization was reduced at 2 hours, but by 6 hours, normals and shock patients were indistinguishable. Twenty-three diabetics mobilized more leukocytes than those receiving alcohol, but less than controls. In 10 patients with azotemia, 16 with cirrhosis, 19 in coma from cerebrovascular accidents, and 15 undergoing general anesthesia PMN mobilization was normal. Leukocyte mobilization was not altered in normals by lactate infusions. *In vitro* differential centrifugation studies using human PMN from venous blood, rotated at 37° C for up to 4 hours, showed that addition of alcohol, up to 400 mg per 100 ml, did not impair phagocytosis or intracellular killing of staphylococci. Homogenates of peripheral portions of human lung obtained at thoracotomy, consisting of more than 90% mononuclear cells, were also studied by differential centrifugation techniques; phagocytosis and intracellular killing of staphylococci were unaltered by addition of up to 400 mg per 100 ml alcohol. These studies suggest that the major cellular abnormality in host defenses in man following alcohol ingestion is defective diapedesis.

A Possible Mechanism for Decreased Oxygen Affinity of SS Blood. PHILIP A. BROMBERG AND WALLACE N. JENSEN,* Pittsburgh, Pa.

The oxygen affinity of sickle cell blood is generally subnormal. However, most investigations of the oxyhemoglobin dissociation curve of S hemoglobin solutions have shown no abnormality. The abnormal oxygen binding characteristics of intraerythrocytic S hemoglobin might be related to polymerization of concentrated deoxygenated S hemoglobin within the cell. To investigate this possibility, *in vitro* oxygen dissociation curves were constructed by tonometry using SS blood to which was added sufficient urea to obtain concentrations up to 1.0 M. One molar urea inhibits gelling of concentrated solutions of deoxygenated S hemoglobin (Allison), penetrates the red cell membrane, and is well below the concentrations employed to dissociate the hemoglobin molecule. In this concentration, urea caused marked inhibition of the sickling phenomenon in bisulfite preparations, in oxygen exclusion preparations, and in blood equilibrated with gas of low oxygen tension examined after formalin fixation. Intermediate degrees of inhibition were observed at lower urea concentrations. One molar urea caused a marked increase in oxygen affinity of SS blood. In 5 SS bloods the $P_{50}O_2$ values (pH 7.40, 37° C) fell from 41, 41, 42, 38, and 30 mm Hg to 18, 26, 26, 26, and 18 mm Hg, respectively. Intermediate urea concentrations produced intermediate decreases in $P_{50}O_2$ values. In blood from 3 normal individuals 1.0 M urea decreased $P_{50}O_2$ values from 25, 25, and 27 mm Hg to 20, 19, and 18 mm Hg, respectively. The ability of urea to inhibit the intraerythrocytic polymerization of S hemoglobin (as evidenced by inhibition of sickling) and to strikingly increase oxygen affinity of SS blood is consistent with the hypothesis that intraerythrocytic polymerization of deoxygenated S hemoglobin is related to the decreased oxygen affinity of SS blood.

Regulation of Proximal Tubular Fluid Reabsorption as Studied by Stopped Flow Microperfusion in the Rat Kidney. FELIX P. BRUNNER, FLOYD C. RECTOR, JR.,* AND DONALD W. SELDIN,* Dallas, Texas.

Proximal tubular reabsorption of salt and H_2O appears closely related to glomerular filtration rate (GFR). Whether this is due to changes in linear velocity of tubular fluid, tubular geometry, peritubular blood flow, or other factors is unknown. To examine the effect of altered tubular geometry independent of linear velocity, isotonic NaCl reabsorption was measured in individually perfused tubules, using the shrinking drop technique of Gertz. Proximal tubules were dilated by raising hydrostatic pressure in the renal pelvis; measurements were made at 0, 25, 40, and 60 mm Hg of intrapelvic pressure. Mean luminal radii of perfused tubules measured 16.4, 17.5, 19.8, and 19.2 μ, and mean half-times of reabsorption were 10.0, 9.1, 10.4, and 14.6 seconds, respectively. In further experiments renal arterial pressure was lowered by aortic constriction. Decrease in luminal radius was not associated with changes in reabsorptive half-time; only when blood pressure was dropped below 80 mm Hg did half-time increase significantly. The constant reabsorptive half-times at different luminal radii indicate that proximal reabsorption is not constant for a given unit of either tubular length or tubular surface area but varies proportionately with tubular volume. This relation was disrupted only at blood pressures below 80 mm Hg or at intrapelvic pressures greater than 40 mm Hg. Alteration in reabsorptive rate proportionate to tubular volume might be explained by altered permeability of the luminal membrane, possibly by changing pore size as the membrane is either stretched or relaxed. The following simple mechanism for glomerular-tubular balance is proposed. Increased GFR, by augmenting transiently proximal tubular inflow relative to outflow because of the known resistance to flow through the loop or collecting duct, will result in higher intratubular pressure and tubular dilatation; the latter will increase reabsorptive capacity proportionately to tubular volume. Conversely, diminished GFR will narrow tubular lumen and thereby diminish reabsorptive rate.

Reticulocyte Response in Treated Megaloblastic Anemias: A Biochemical Analysis. EDWARD R. BURKA, ARTHUR BANK, AND PAUL A. MARKS,* New York, N. Y.

Treatment of megaloblastic anemias (MA) is generally associated with a prompt reticulocytosis, but a relatively slow rise in hemoglobin levels. The basis of this phenomenon was investigated with blood from 10 patients with MA before and at intervals following vitamin B_{12} or folic acid therapy and from 11 subjects with acquired hemolytic (AHA) or sickle cell anemia (SS). The following were determined: reticulocytes, hemoglobin level, rate of hemoglobin synthesis by reticulocytes (C^{14}-amino acid incorporation), and ribosome and polyribosome content (sucrose density gradient techniques). Subjects with AHA or SS had hemoglobin

synthetic rates averaging 33.5 ± 18.3 mμmoles per mg ribosomes per hour, which varied little over a range of reticulocytosis of 5 to 40%. In contrast, the average value for hemoglobin synthesis by cells of treated MA patients, at peak reticulocytosis (6 to 30%), was 6.6 ± 4.9 mμmoles per mg ribosomes per hour, and, thereafter, expressed per reticulocyte or milligram ribosomes, fell progressively. However, the decline in hemoglobin synthetic rate of these cells was proportional to the fall in polyribosomes. Previous studies established that the loss in capacity to synthesize hemoglobin occurring as rabbit reticulocytes mature *in vitro* is related to a fall in polyribosomes. The decreased protein synthetic capacity of reticulocytes after therapy in MA can be explained by the fall in polyribosomes in these cells. The activities of ribosomes and supernatant fractions from cells of treated MA patients and those of AHA and SS were assayed in a cell-free system for protein synthesis. These results substantiate that the decreased capacity for protein synthesis of MA cells is primarily due to alterations in ribosomes. Thus, the reticulocytosis following therapy of MA represents a uniform population of maturing cells that are relatively ineffective in hemoglobin formation. This may reflect either their advanced state of maturation when released or a defect in their protein synthesizing elements related to the fact that the precursor cells were formed during the period of nutritional deficiency.

Inhibition of Aldosterone Biosynthesis by Puromycin. GERARD N. BURROW AND PATRICK J. MULROW,* New Haven, Conn.

In the rat puromycin blocks the incorporation of amino acids into adrenal protein and the stimulation of corticosterone production by ACTH. Basal corticosterone production is not inhibited. In the present study the effect of puromycin on aldosterone production *in vitro* by the rat adrenal was investigated. Adrenal quarters from rats on normal and sodium-deficient diets were incubated with puromycin for 3 hours after a 1-hour preincubation. The production of aldosterone and corticosterone and the incorporation of leucine-C^{14} into adrenal protein were determined. In rats on both normal and sodium-deficient diets, protein synthesis was inhibited 96% and aldosterone production 83%, whereas corticosterone production was only inhibited 27%. After a 30-minute preincubation with puromycin, adrenal quarters were incubated for 60 minutes and 90 minutes with puromycin and leucine-C^{14}. Aldosterone production and protein synthesis were inhibited throughout the experiment, whereas in control adrenals both variables increased with time. Addition of TPN and glucose-6-PO_4 to the incubation medium markedly stimulated corticosterone production in both control and puromycin-treated adrenals, despite inhibition of protein synthesis by puromycin. A stimulating effect on aldosterone was striking in the control adrenals, but was inhibited 43% by puromycin. Progesterone-C^{14} was incubated with rat adrenal quarters. There was a 59% reduction in the conversion of progesterone-C^{14} to aldosterone and a 37% reduction in the conversion to 18-OH corticosterone by puromycin-treated adrenals. Conversion to corticosterone and 18-OH desoxycorticosterone was the same in both groups. Incubation of corticosterone-C^{14} in the presence of puromycin resulted in a 46% inhibition of its conversion to aldosterone. These data demonstrate that puromycin inhibits protein synthesis and aldosterone production by rat adrenals. The block in aldosterone biosynthesis is mainly in the pathway between corticosterone and aldosterone. Puromycin may be blocking the synthesis of an enzyme or cofactor that has a rapid turnover and is critical for the production of aldosterone.

The Effects of Serum Factor on Erythrocyte Carbohydrate Metabolism. JOHN J. BUTLER, Jersey City, N. J. (introduced by Max Michael, Jr. †).

Human serum was dialyzed against 2 vol of Krebs-Ringer (KRB) bicarbonate buffer, and the dialyzate was tested for its effect on erythrocyte metabolism *in vitro*. Incubation mixtures contained 1.35 ml washed cells, 1.65 ml dialyzate, and 7.0 mg of glucose-U-C^{14}. Control vessels were identical except that the dialyzate was heated to 60° C for 30 minutes (other controls utilizing KRB gave similar values). In a typical experiment glucose consumption was 7.4 ± 0.13 μg per ml per minute (mean ± SD); control was 2.84 ± 0.05. Of the glucose consumed in the control 92.9 ± 3.15% was accounted for as lactic acid (determined enzymatically) and 7.91 ± 0.35% as $C^{14}O_2$. In the experimental flasks 35.7 ± 1.21% was accounted for as lactic acid; 14.18 ± 0.25% as $C^{14}O_2$, and 50.1% was unaccountable. Since pentose phosphate shunt activity is thought to be dependent on the availability of NADP, tests for the ability of dialyzate to act as an electron acceptor for NADPH were performed. When dialyzate was incubated with 0.25 μmoles of NADPH and 1.8 U of diaphorase, Δ OD_{340} was 0.010 U per minute. When 1×10^{-2} M ascorbic acid replaced dialyzate in this experiment, Δ OD_{340} was 0.040 U per minute. In experimental vessels incubated with 2.5×10^{-5} M ouabain there was a 37% decrease in glucose consumption, but the amount of $C^{14}O_2$ produced was unchanged. In control vessels ouabain had no effect. These observations indicate that serum contains a dialyzable, heat labile factor that affects carbohydrate metabolism in erythrocytes. This factor *1*) increases glucose consumption, *2*) increases pentose phosphate shunt activity, and *3*) decreases the proportion of glucose consumed that appears as lactic acid. Evidence is presented which suggests that this factor acts in part by increasing NADP. Partial inhibition by ouabain of the unaccountable glucose consumption suggests that stimulation of ATPase may be involved in this pathway.

Metabolism in Starvation Diabetes and Diabetes Mellitus. GEORGE F. CAHILL, JR.,* M. GUILLERMO HERRERA, ALFRED P. MORGAN, AND GEORGE A. REICHARD, JR., Boston, Mass., and Philadelphia, Pa.

Totally fasted normal males (ages 22 to 39) require 5 to 7 days to achieve steady and maximal release of

fat from adipose, ketones from liver, and presumably uptake of both by peripheral tissues, sparing in turn glucose for utilization by glucose-dependent tissues (primarily nerve), thereby minimizing gluconeogenesis from amino acids and ultimately sparing body protein. Since glycogen reserves are both limited and conserved for emergency needs, days 3 to 5 are associated with increased gluconeogenesis (total gas exchange and nitrogen balance) to supply glucose until peak glucose-sparing is achieved. Glucose turnover studies (glucose-1-C^{14}) after 7½ days complete fast show basal values of ~60 mg per kg per hour, totally accounted for by uptake by nervous tissue and red cells (calculated from published data) and by synthesis from calculated body protein breakdown (nitrogen balance), plus adipose glycerol, plus lactate and pyruvate (recovery of C^{14} recycled in glucose carbon-6) all determined in these experiments. Patients with maturity-onset diabetes and without glycosuria are metabolically identical to normals fasted 5 to 7 days, and thereby, on fasting, they are already at optimal glucose-sparing efficiency and maximally conserve nitrogen from the start of the fast, a distinct survival advantage for intermittent starvation-refeeding regimens. Normals ($n = 6$) lost 13 ± 2 g extra nitrogen each adaptation to starvation (diabetic-like) metabolism. These data provide another possible explanation for the genetic predisposition to diabetes, particularly in certain populations (e.g., Natal Indians) previously exposed to centuries of intermittent starvation. Similar and parallel results have been found in this laboratory and others in the sand rat, *Psammomys obesus,* and in other experimental animals (e.g., the Wellesley mouse) which, like certain men, are better adapted to starvation regimens and develop overt diabetes mellitus on exposure to excess food.

Acute Hemolytic Anemia Due to Anti-i; Frequent Cold Agglutinins in Infectious Mononucleosis. ROBERT CALVO, WILLIAM STEIN, SHAUL KOCHWA, AND RICHARD E. ROSENFIELD, New York, N. Y. (introduced by Louis J. Soffer †).

Investigation of strong cold agglutinins in the serum of a patient having both acute acquired hemolytic anemia (AHA) and infectious mononucleosis (IM) revealed anti-i specificity. These antibodies were separable from heterophile agglutinins by absorption and elution. Eluates of anti-i did not react with sheep or ox cells; eluates of heterophile agglutinins did not react with human cells. Both anti-i and heterophile agglutinins had characteristics of γ_M-globulins. Similar anti-i agglutinins were found in the sera of 23 of 38 unselected patients with IM. Anti-i antibodies, in contrast to the more frequently observed anti-I cold agglutinins, display preferential binding with and agglutination of cord blood RBC, the cells of I-negative adults (rare genotype), and RBC of certain patients under chronic erythropoietic stress. The patient studied was a 16-year-old white female with thalassemia trait (3% alkali-resistant and 5.5% A_2 hemoglobin) whose RBC, while she had active AHA and IM, were strongly agglutinated with anti-C′ (anti-β_{1E}[C′4] and anti-β_{1C}[C′3a] antiglobulin sera. Tests with standard reagents showed the patient to be both I- and i-positive. The degree of i-positivity became stronger during convalescence in parallel with the disappearance of the positive C′ antiglobulin reaction and simultaneous recovery from both AHA and IM. These findings may explain the etiology of at least some instances of AHA complicating IM.

Intestinal Absorption of Bile Salts in Man and Rabbits with Ileal Bypass. JAMES B. CAREY, JR.,* HENRY BUCHWALD, AND RICHARD L. VARCO, Minneapolis, Minn.

The ileum is regarded as the major site of bile salt absorption. Evidence derived from experiments with gut sacs, gut slices, and isolated intestinal segments in animals and sampling intestinal contents in man supports this concept and further implies that very little bile salt absorption occurs elsewhere in the intestine. A rare opportunity to test this hypothesis in a human subject arose when a 30-year-old woman with familial hypercholesterolemia had a 200-cm ileal bypass and 8 months later cholecystectomy (for cholelithiasis) with T-tube drainage of the common bile duct. Approximately 10 μc of C^{14}-sodium cholate in 30 mg sodium cholate carrier was administered orally and all bile collected for 4½ days. Over 80% of the administered radioactivity was excreted as cholic acid in the bile in 12 hours, 85% in 48 hours. The remainder (14%) was recovered in the feces. Bile radioactivity was assayed in ethanol diluted samples on planchets in a gas flow counter. Fecal radioactivity was assayed by combustion and liquid scintillation counting of $C^{14}O_2$. Cholic acid was isolated by column chromatography and crystallized to constant specific activity. Similar experimental conditions were established in seven rabbits with ileal bypass and bile fistulas. In these animals, an average of 68% of C^{14}-cholic acid administered by stomach tube was recovered in the bile in 24 hours. A control group of five rabbits with a bile fistula but intact intestines excreted 73% of the administered C^{14}-cholic acid in 24 hours. These results do not contradict the idea that bile salts are absorbed most efficiently by the ileum. Consideration must be given, however, to modifying the current concept of ileal specificity for bile salt absorption to account for the observation that other intestinal segments participate effectively in the absorption of bile salts.

Atrial Contribution to Ventricular Performance. RICHARD A. CARLETON AND JOHN S. GRAETTINGER, Chicago, Ill. (introduced by Robert M. Kark †).

This study of human atrial function was prompted by the observation that cardiac output did not increase in patients converted from atrial fibrillation to sinus rhythm if their ventricular rates (VR) were below 100 per minute. Ventricular pacing was performed in 12 patients with complete A-V block during hemodynamic studies. The intervals between sinus node-induced atrial systoles and pacemaker-stimulated ventricular systoles varied

randomly and were estimated from the P-R intervals in the electrocardiograms. At any VR (35 to 125), the average of peak aortic systolic pressure (PASP) was significantly related to average stroke output (Fick); therefore changes in PASP were used as the index of beat-to-beat changes in stroke output. Scattergrams of PASP as a function of P-R at each rate appeared parabolic; PASP was lowest at very short or very long P-R and highest at an intermediate P-R. The P-R corresponding to the highest PASP, at which the maximal atrial contribution occurred, was defined as the optimal P-R; it was computed by least squares parabolic regression analysis for each of the 34 scattergrams. These optimal P-R were inversely related to VR ($r = -0.89$). At rates under 60 they were over 0.21 second; at rates over 90 they were under 0.19 second. This change in optimal P-R approximates the physiologic change in P-R with rate. The magnitude of atrial contribution was expressed as the percentage increment of PASP, at each optimal P-R, above the lowest PASP values. These maximal contributions were also significantly rate related ($r = +0.79$); at VR 35 to 59, 9.8% (SE $\pm$ 1.3%); at 60 to 89, 18.1% ($\pm$ 1.7%); and at 90 to 125, 27.4% ($\pm$ 2.9%). These results suggest that, although atrial systole is of relatively little importance at slower VR, both the occurrence and timing of atrial systole become increasingly important at higher heart rates.

Acid Mucopolysaccharides in the Kidney and Urine of the Dog. C. WILLIAM CASTOR,* JAMES A. GREENE,† AND ROBERT K. PRINCE, Ann Arbor, Mich.

Analysis of the acid mucopolysaccharides (AMPS) found in kidney and urine was undertaken to assess their relationship to medullary hypertonicity, and to examine the kidney itself as a possible source of AMPS found in urine. The concentration of AMPS in the medulla was found to be 4 to 5 times greater than in the cortex. Crude medullary AMPS is very viscous ($[\eta] = 15$ to 20) and labile to testicular hyaluronidase. In this crude medullary AMPS, hexosamine, uronic acid, and sulfate were found in a ratio of 1:1:1. Medullary AMPS was separated into two fractions, the viscous material accounting for 77% of the total. This fraction contained 17% protein and had some of the chromatographic characteristics of hyaluronic acid, but differed in being intensely metachromatic. The minor component had virtually no viscosity and resembled heparitin sulfate. In contrast, the cortical AMPS had little viscosity, 85% resembling heparitin sulfate and containing 50% protein. The minor cortical component was similar to the viscous material of the medulla. Analysis of crude cortical AMPS showed hexosamine, uronic acid, and sulfate in a ratio of 1:0.8:2. Medullary and cortical AMPS each contained both glucosamine and galactosamine in significant amounts. Urinary AMPS were not viscous and chromatographically resembled chondroitin sulfate A. Galactosamine was the predominant hexosamine. Injection of hyaluronidase into the renal artery led to prompt impairment of renal concentrating ability and concomitant diuresis of low molecular weight AMPS. In summary, the renal medulla is distinguished from the cortex by a difference in the type and concentration of AMPS, a finding that may have relevance with respect to maintenance of medullary hypertonicity. Urinary AMPS appeared distinct from those found in renal parenchyma, supporting the view that they are excretory products of nonrenal origin.

Polycythemia Associated with a Hemoglobinopathy. SAMUEL CHARACHE, DAVID J. WEATHERALL, FREDERICK C. BATTAGLIA, AND ANDRE E. HELLEGERS, Baltimore, Md. (introduced by Dudley P. Jackson *).

Polycythemia was encountered in a family heterozygous for an abnormal hemoglobin with an unusual affinity for oxygen. The propositus was an 81-year-old white man with a hematocrit value of 58%. In three generations of his family, five men with the hemoglobin variant had a mean hematocrit value of 53.6%, and nine women similarly affected a mean value of 50.2%; six men and three women without the abnormal hemoglobin had mean hematocrit values of 47.1% and 46.0%, respectively. The red cells were normal in appearance. The abnormal hemoglobin comprised 28 to 35% of hemolysates from affected individuals. At pH 8.6 it migrated between hemoglobins A and I. The visible absorption spectra of its oxy-, deoxy-, met-, and cyanmethemoglobin derivatives were identical to those of hemoglobin A. Its amino acid substitution was localized to peptides αTp 11 to 13, which include amino acids 91 to 138, but not the "heme-linked" (α 87) or "distal" (α 58) histidines. Blood from affected individuals had an oxygen affinity which was much greater than that of normal adult blood, and greater than that of umbilical cord blood. Female heterozygotes were capable of bearing normal children, with normal hemoglobin, suggesting that fetal oxygenation was adequate. In contrast to umbilical cord blood, blood containing the abnormal hemoglobin was neither resistant to alkali nor sensitive to nitrite oxidation. The familial polycythemia is thought to be a consequence of the unusual oxygen affinity of the blood of affected members. The hemoglobin variant tentatively has been named hemoglobin$_{\text{Chesapeake}}$.

Biochemical Studies of Energy Metabolism in the Failing Human Heart. CHARLES A. CHIDSEY, EUGENE C. WEINBACH, PETER E. POOL, AND ANDREW G. MORROW, Bethesda, Md. (introduced by Robert S. Gordon *).

Observations in experimental heart failure in animals have indicated that there may be a bioenergetic defect in the myocardium involving an impairment of oxidative phosphorylation. In this study the possibility that such a defect is present in heart failure in man was examined with myocardial tissue obtained from patients at the time of cardiac surgery. Mitochondria were isolated from papillary muscles removed from the left ventricle during replacement of the mitral valve in 11 patients with left ventricular failure. Measurement of oxidative

phosphorylation with pyruvate/malate as substrate gave P/O ratios of 2.8 (2.2 to 3.3). Respiratory control ratios (oxidation with ADP/oxidation without ADP) determined both manometrically and polarographically were 6.6 (4.4 to 10.0) with pyruvate/malate and 5.9 (5.5 to 6.3) with α-ketoglutarate. Endogenous ATPase activity was 0.14 (0 to 0.28) μmoles P_i liberated per minute per mg mitochondrial N; it was 0.36 (0.07 to 0.56) with Mg^{++}, 1.79 (1.66 to 2.46) with 2,4-dinitrophenol, and completely inhibited by oligomycin. These values are comparable to those reported for normal mitochondria obtained from experimental animals. Examination of myocardial tissue by electron microscopy revealed no apparent abnormality of mitochondrial size or crystal pattern. Creatine phosphate was determined in rapidly frozen ventricular biopsies from 15 patients with heart failure undergoing valve replacement. The mean tissue concentration was 4.0 (0.7 to 13.4) μmoles per g compared to 4.1 (1.8 to 5.2) μmoles per g in similar biopsies from 5 patients without heart failure. These biochemical studies indicate that electron transport and coupled phosphorylation appear to be normal in mitochondria isolated from failing human hearts and that there is no consistent reduction of the myocardial store of high energy phosphate. It is concluded that the formation of chemical energy is not impaired in the failing heart, and it is suggested that the biochemical abnormality responsible for defective myocardial function involves utilization of energy in the contractile process.

Studies of Protein and Lipoprotein Synthesis in Human and Canine Blood Vessels. ARAM V. CHOBANIAN, SIDNEY R. COOPERBAND, AND WILLIAM HOLLANDER,* Boston, Mass. (introduced by Robert W. Wilkins †).

The local intimal synthesis of protein and lipoprotein was studied in intact segments of human and canine blood vessels that were incubated with C^{14}-leucine, C^{14}-acetate, and P^{32}-phosphate. Arterial lipoproteins were isolated by density gradient ultracentrifugation, subcellular fractionation of tissue was performed by differential ultracentrifugation, and tissue lipids were analyzed by chromatographic techniques. The results indicate: *1*) Active intracellular microsomal synthesis and gradual release into the extracellular medium of protein and lipoprotein occurred in both human and canine arteries. *2*) In human atherosclerotic vessels, most of the newly synthesized lipoproteins were of low-density type ($D < 1.063$). In contrast, the lipoproteins synthesized by the dog intima were predominantly high density lipoproteins ($D = 1.063$ to 1.210). *3*) Total protein synthesis was reduced in atherosclerotic as compared with normal intima. *4*) Addition of puromycin to the incubation medium (10^{-7} moles per ml) was associated with a reversible inhibition of total protein synthesis (62 to 96% inhibition) in both human and canine intima. Actinomycin D (10^{-8} moles per ml) also had a significant though lesser inhibitory effect on protein synthesis. Despite the inhibition of protein synthesis, there was persistent synthesis of lipids within the arterial wall. *5*) Inhibition of protein synthesis was associated with a reduction in the rate of release of newly formed phospholipids from intracellular microsomal sites. These *in vitro* studies indicate that intracellular synthesis and secretion of protein and lipoprotein occur in the arterial intima. The accumulation of lipids in atherosclerotic lesions may be due in part to an intrinsic defect in protein and lipoprotein synthesis.

Effect of Caval Ligation on Response of Proximal Tubular Sodium Reabsorption to Saline Infusion. WILLIAM J. CIRKSENA, JOHN H. DIRKS, AND ROBERT W. BERLINER,† Bethesda, Md.

Infusion of isotonic saline has previously been shown to decrease fractional sodium reabsorption in dog proximal tubule by an effect independent of filtration rate. Effect of partial suprahepatic inferior vena cava ligation (IVCL) on proximal tubular sodium reabsorption during saline infusion was studied in dogs infused with supramaximal doses of mineralocorticoids and vasopressin. Micropuncture collections were made from the same tubular segments before and after interventions. Controls were 63 paired collections in continued hydropenia with mean change in tubular fluid to plasma inulin ratio (TF/P) of $-0.04 \pm .021$, and 43 paired collections comparing TF/P in hydropenia with that during constriction of renal artery to reduce GFR by 30 to 70% during continued hydropenia (Δ TF/P $= -0.07 \pm .047$). IVCL prevented or greatly reduced diuresis, and mean TF/P rose from hydropenia $+0.32 \pm .104$, $N = 29$. After release of IVCL, diuresis ensued with mean Δ TF/P $= -0.31 \pm .085$, $N = 21$, compared with control hydropenia. Filtration rate decreased after IVCL, but changes in TF/P were not related to degree of GFR change, and GFR change alone does not affect TF/P. Results indicate that depression of proximal sodium reabsorption induced by infusion of isotonic saline is prevented and reversed by IVCL. A similar mechanism is probably responsible for failure of sodium excretion leading to edema.

Studies of Cellular Proliferation in Acute Leukemia. BAYARD CLARKSON, TAKESHI OHKITA, KUZUO OTA, AND ANNABEL O'CONNOR, New York, N. Y. (introduced by David A. Karnofsky †).

Nine acute leukemia patients were given H^3-thymidine (H^3-T) by single injection or continuous infusion to study their cellular proliferation by radioautographic methods and grain count analysis. An average of only 10.1% (4.0 to 18.4%) blasts in marrow and 4.4% (0.2 to 11.0%) in blood incorporated H^3-T immediately *in vitro* and *in vivo*, although almost 100% were viable as shown by H^3-uridine and H^3-leucine incorporation *in vitro*. The mitotic index of the marrow blasts was 0.65% (0.20 to 0.95%) and 0 to 0.02% in blood. In five untreated patients, the blasts' average mean generation time (T_G) was 132 hours (105 to 178), and their average mean blood transit time, 43 hours (38 to 46).

The average total number of dividing blasts in marrow and other sites from which they could enter the blood was 5.3×10^9 (2.1 to 12.1×10^9) per kg body weight. In three patients started on chemotherapy during the studies, T_G was 133 hours (76 to 215), whereas in one who had a spontaneous remission in midstudy, the blasts stopped proliferating. In one patient with monocytic leukemia, some blasts matured into monocytes which no longer divided. In two untreated patients the per cent labeled blast mitoses were determined in serial marrow samples and the mean durations of the mitotic cycle phases measured: DNA synthesis (S) = 19 and 22 hours; premitosis (G_2) = 3 hours; mitosis (M) = 0.6 and 0.7 hours; and postmitosis (G_1) = 80 and 89 hours. After 8 to 10 days' continuous infusion of H^3-T in four patients, 82 to 93% of blasts in marrow and blood were labeled. Analysis of the data indicated the blasts in individual patients had variable generation times. Normal myeloid or erythroid cells, when present in sufficient numbers for accurate counting, showed rates of proliferation, maturation, and emergence into and disappearance from blood similar to those previously found in hematopoietically normal subjects. The duration of S is reported to be 9 to 12 hours in erythroid and myeloid precursors and T_G 1 and 2 days, respectively. The slower rate of proliferation of leukemic cells and their variability in generation time will be considered in relation to the effects of antileukemic drugs that act principally by killing actively proliferating cells.

Ribonucleic Acid Biosynthesis in Human Leukocytes. MARTIN J. CLINE, Bethesda, Md. (introduced by Hugh Fudenberg *).

The patterns of ribonucleic acid biosynthesis were examined in granulocytes and lymphocytes from normal individuals and from patients with hematologic diseases. Three major components constitute the bulk of phenol-extractable leukocyte RNA. In sucrose gradients these components sediment as 28 S and 18 S (ribosomal RNA) and as 4 S (transfer RNA), a pattern identical to that of human liver. When leukocytes are incubated *in vitro* with H^3-uridine, the radioactive label is incorporated into different RNA fractions at different rates. That fraction most tenaciously bound to DNA is most rapidly labeled. It is separable from DNA only at elevated temperatures and pH. This fraction sediments either as a high molecular weight component (35 to 45 S) or as a broad-based peak (14 to 16 S). With prolonged incubation, labeled uridine is distributed throughout all classes of cellular RNA. The patterns of RNA labeling in normal and neoplastic granulocytes and lymphocytes and in leukocytes from individuals with infectious mononucleosis and agammaglobulinemia are qualitatively similar; however, striking quantitative differences in the rates of uridine incorporation were observed. RNA synthesis was greatest in leukemic myeloblasts and in the mononuclear cells of infectious mononucleosis. Human leukocyte RNA can be shown to bind specifically to human DNA. The kinetics of the interaction between these nucleic acids under annealing conditions were defined and found to be similar to those described for certain nonmammalian RNA-DNA systems. Leukocyte RNA synthesis *in vitro* is not fixed but is sensitive to the constituents of the suspending medium. RNA biosynthesis in normal and abnormal granulocytes is profoundly altered by phagocytosis. For several hours after ingestion of inert particles there is enhanced uridine incorporation into RNA, despite the concomitant release of nucleases from ruptured granules. Leukocyte RNA synthesis is sensitive to actinomycin D and can be almost totally suppressed by this antibiotic.

Maximal RBC and Hemoglobin Catabolism in Dogs. R. F. COBURN AND P. KANE, Philadelphia, Pa. (introduced by A. B. DuBois *).

Suspensions of red blood cells (RBC), damaged by incubation with 46 μmoles per ml RBC *N*-ethylmaleimide, were injected iv in varying amounts into 16 14- to 21-kg anesthetized dogs. The rate of Hb catabolism during each hour was calculated from measurements of rate of CO production. When relatively small numbers of RBC containing 1.05 to 2.34 g Hb were injected (group A), the highest hourly rate at which Hb was catabolized to CO ($\dot{V}hb$ *) was proportional to the quantity of Hb injected. When the RBC injection was increased to 3.00 to 7.62 g Hb (group B), $\dot{V}hb$ * did not increase further and averaged 1.14 ± 0.28 (SD) g per hour. This value appears to represent the average maximal hourly rate at which Hb in RBC can be catabolized to CO in these animals. The injected "damaged" cells were rapidly sequestered in the liver and spleen as measured with Cr^{51}. The half-time ($t_{\frac{1}{2}}$) in group A averaged 9.1 ± 2.30(SD) minutes; the average of RBC sequestration in group B remained unchanged from group A until more than 5 g Hb was injected when $t_{\frac{1}{2}}$ increased (to 15 and 22 minutes in 2 experiments). Sequestration of RBC, therefore, did not appear to be a limiting parameter to maximal Hb catabolism until quantities of RBC containing more than 5 g Hb were injected. The total number of sequestration sites, assuming one site per RBC, appears to be equal to or greater than 1.55×10^5 per kg body weight. In the experiments where maximal $\dot{V}hb$ * was achieved (group B) and the reticuloendothelial system presumably overloaded, large quantities of Hb entered the plasma reaching levels as high as 192 mg per 100 ml. There was evidence that RBC were not escaping into the general circulation following completion of cell sequestration, since blood RBC radioactivity did not increase significantly for the remainder of the experiments. The maximal rate of $\dot{V}hb$ * found here is about 20 times the presumed normal rate of RBC and Hb catabolism in these dogs.

The Use of Selenomethionine (Se^{75}) as a Label for Canine and Human Platelets. PHIN COHEN, MARK H. COOLEY, AND FRANK H. GARDNER,* Boston, Mass.

Fifty microcuries of selenomethionine (Se^{75}) was injected intravenously into three 20-kg dogs. For 25 con-

secutive days, 12-ml blood samples were obtained from the jugular vein, mixed with 10 ml EDTA-saline, and centrifuged at 200 g for 15 minutes. The platelet rich plasma-EDTA supernatant containing 200,000 to 400,000 platelets and less then 50 leukocytes and 300 erythrocytes per mm^3, respectively, was centrifuged at 1,000 g for 30 minutes. The platelet button was washed twice with 10 ml saline and counted in a gamma well scintillation counter. Results were graphed as per cent of highest platelet button radioactivity. Curves exhibited an increase of radioactivity until day 5 in two animals and day 6 in a third, followed by a decline to 20% of the peak value at day 12. No plateau of radioactivity was apparent. Fifty per cent of peak radioactivity was reached on day 2 on the ascending limb and day 10 or 11 on the descending limb of the curve, suggesting an 8- to 9-day lifespan. Two smaller secondary peaks of radioactivity were noted during the next 10 days, perhaps indicating reutilization of the label. The study was repeated in the same animals with identical results. Four male human volunteers were studied, using a 200-μc labeling dose and 15-ml whole blood samples mixed with 5 ml EDTA-saline. Leukocyte and erythrocyte free (0 to 50 and less than 100 per mm^3, respectively) platelet buttons were obtained. In the human studies the radioactivity rose slowly until day 3 and then briskly from day 4 to 5. A plateau was maintained from day 5 until day 9 or 10, following which radioactivity declined to 20% of the peak value at day 18 to 20. Fifty per cent of peak radioactivity was reached on day 4 on the ascending limb and day 13 to 15 on the descending limb of the curve, suggesting a 9- to 11-day lifespan.

The Sulfation of Thyroid Hormone(s) by Beef Thyroid *In Vitro*. GEORGE L. COHN,* New Haven, Conn.

Sulfokinases transfer "'active sulfate" from 3'-phosphoadenosine-5'-phosphosulfate (PAPS) to aliphatic and aromatic hydroxyls and to amines. The sulfate esters *1*) facilitate transport or utilization of substances, *2*) are detoxications of normal metabolic products, and *3*) produce normal metabolic tissue constituents such as steroid sulfates. Since the latter metabolic function is well demonstrated in mammalian ovarian and adrenal tissues, an investigation of thyroid gland sulfokinases and sulfation of thyroid hormone(s) and iodinated amino acid intermediates was undertaken. Fresh beef thyroid glands were homogenized and fractionated by differential centrifugation into fractions, *1*) filtered crude homogenate, *2*) $700 \times g$ supernatant, *3*) $12{,}000 \times g$ supernatant, and *4*) $105{,}000 \times g$ supernatant. The sulfokinase reaction mixture to which was added a yeast sulfate ATP activating enzyme was incubated in a metabolic shaker for 35 minutes in air at 37° C. The usual control flasks were run concomitantly. The ammonium salts of the sulfate esters were measured by a methylene blue colorimetric method after the addition of NH_4OH and extraction with a *n*-butanol. The highest sulfokinase activity, 21.6% conversion per mg of protein, was observed in Fraction IV compared to Fractions I to III (12.6 to 16.2%). The most active substrates were the sodium salts of L-thyroxine (T_4) and L-triiodothyronine (T_3), and progressively lesser activity was observed with 3:5 diiodothyronine, 3,5-diiodo-L-tyrosine, and 3-iodo-L-tyrosine. L-Tyrosine, dehydroepiandrosterone, *p*-nitrophenol, and estradiol were not sulfated in this system. The iodinated amino acid sulfates were identified by characteristic mobilities in paper chromatographic and electrophoretic systems relative to the mobilities of the standard ammonium salts of T_3S and T_4S. The data indicate that the beef thyroid contains sulfokinases that readily sulfate T_3, T_4, and iodinated amino acid intermediates. The physiological importance of these thyroid hormone derivatives remains to be determined.

A New Perspective of the Cellular Basis of Immunity and Lymphocytic Malignancies. M. D. COOPER, R. D. A. PETERSON, AND R. A. GOOD,* Minneapolis, Minn.

Recognition of two distinct components of the lymphoid system provides the basis for new insight into normal immune mechanisms and permits more meaningful classification of immunological deficiency states and lymphocytic malignancies in man. In recent experiments, we have accomplished clear morphological and functional delineation of two distinct components to the chicken lymphoid system. The thymus is the embryological source organ for the cell population made up largely of small lymphocytes. This radiosensitive system appears responsible for recognition of foreignness, immunological memory, and immune responsiveness characterized by graft rejection, graft-vs.-host reactivity, and delayed hypersensitivity. The bursa of Fabricius is the source organ of the large pyroninophilic lymphocytes that compose the germinal centers and of plasma cells. This relatively radioresistant system is responsible for immunoglobulin synthesis but requires an intact thymus system for efficient antibody production. These observations have enabled us to separate the mammalian lymphoid system into two distinct components that are morphologically and functionally similar to the avian systems. In rabbits the immunoglobulin-producing (bursa-equivalent) system is most clearly seen following thymectomy and irradiation. Germinal centers remain intact but lack the surrounding cuff of thymus-dependent small lymphocytes. Plasma cells continue to synthesize immunoglobulins, but antibody response is defective. In these animals, "autoimmune" phenomena and amyloid disease are frequently seen. Sex-linked agammaglobulinemic patients (Bruton's type) present an incisive clinical parallel to bursa-less chickens in their lack of germinal centers, plasma cells, immunoglobulins, and antibody. These patients also lack a prominent gut associated lymphoepithelial organ similar in morphology and ontogeny to the bursa of Fabricius—the tonsils. Lymphocytic malignancies in animals involve but one lymphoid system, never both. Preliminary studies suggest that human lymphocytic malignancies also involve

only one system, an observation that should permit new insight into their pathogenesis.

Recovery of Lymphatic System from Cortisol Depletion in the Rat. CHARLES G. CRADDOCK * AND ALAN WINKELSTEIN, Los Angeles, Calif.

Studies by Miller and others have ascribed an important role to adult thymus in restoration of lymphatic tissue and immunological capacity following whole-body radiation. Evidence favors a thymic hormone produced by epithelial elements as responsible, although seeding of thymus lymphocytes to peripheral nodes remains a possible mechanism in development of the lymphatic system. Present observations concern recovery of lymphatic tissue from depletion following a single large dose of hydrocortisone (10 mg per 100 g) in normal rats and rats thymectomized at weaning. Rats were given tritiated thymidine at intervals after cortisol, serially sacrificed 1 hour or 24 hours later, and proliferative activity and cellularity of various parts of lymphatic system determined. Observations were: *1*) Cortisol depletion of lymphatic tissue, unlike whole body X ray, does not interfere with erythropoiesis or myelopoiesis. *2*) Increased proliferative activity begins in peripheral lymphoid follicles earlier than in thymus or bone marrow lymphatic tissue. *3*) Recovery of peripheral lymphatic tissue occurs as rapidly in thymectomized rats as in intact animals. *4*) Active myelopoiesis was observed in the depleted thymus before return of lymphatic tissue. This was thought possibly due to acute alterations in thymus substance allowing localization of circulating marrow elements in the damaged thymus. Transfusion of labeled marrow elements to rats with cortisol depletion of thymus failed to provide evidence of this. It is suggested that myelopoiesis may occur in thymus *de novo*. These data do not favor a "seeding" role for adult thymus in recovery from cortisol-induced lymphocytic depletion. Whereas adult thymus appears necessary for lymphocytic recovery from X-ray injury, it did not influence recovery from cortisol depletion. The similarity of recovery pattern in thymus and bone marrow lymphocytes and the presence of myelopoiesis in depleted thymus support other evidence of a close relationship between thymus and hemopoiesis.

Cytomegalovirus Infections after Renal Homotransplantation. JOHN E. CRAIGHEAD, ROBERT E. KANICH, AND GUSTAVE J. DAMMIN,† Boston, Mass.

Eight of 12 recipients of renal homotransplants who died at the Peter Bent Brigham Hospital during the past 2 years had pulmonary cytomegalic inclusion disease. These patients had received azathioprine, azaserine, actinomycin C, and prednisone, in varying combinations. A review of all recipients of renal homotransplants studied here since 1950 showed that the disease occurred only among those who received these immunosuppressive drugs. The etiology was established by the isolation of cytomegalovirus (salivary gland virus) from the lungs of 5 patients. Substantial quantities of virus (10^4 to 10^6 tissue culture infectious doses) were recovered in each instance. The relative numbers of inclusion-bearing cells and the extent of the histopathological alterations in the lungs could not be correlated with the virus titers of the tissues. Subclinical cytomegalovirus infections were demonstrated in 7 of 11 homograft recipients maintained on immunosuppressive drugs as outpatients. A few of these individuals were shown to yield virus in the oropharynx or urine or both for periods of several months. Members of a control group comprised of healthy adults and azotemic, hospitalized patients were uniformly negative. The urine from the transplanted kidney was the most consistent source of viral isolates. The graft of one patient who yielded virus premortem contained $10^{2.5}$ tissue culture infectious doses at the time of death. Inclusion bodies have never been observed in biopsies of the transplants or the renal parenchyma at autopsy. The immunosuppressive drug regimen presently employed to suppress homograft rejection appears to activate latent cytomegalovirus infections. In some cases, the infection may prove fatal, and in others it may complicate the clinical course and management.

Renal Secretion of Cystine in Cystinuria. JOHN C. CRAWHALL AND CHRISTOPHER J. THOMPSON, London, England (introduced by Jan Wolff *).

The renal clearance of plasma amino acids was measured in three patients with cystinuria. The first patient showed the expected pattern in which the clearance of cystine was approximately equal to the glomerular filtration rate. The other two patients had cystine clearance values 100% and 50% higher than their glomerular filtration rates respectively. In the first patient the lysine clearance was higher than the arginine clearance and was only on half of the glomerular filtration rate. In the second and third patients the arginine clearance was higher than the lysine clearance and was close to the glomerular filtration rate. These results suggest that the second and third patients differed significantly from the first not only in their clearance of cystine but also in that of arginine. The patients were started on penicillamine therapy, and the clearance of amino acids was again determined. It was found that although the level of plasma cystine had been reduced by about 50% in each case, the renal clearance of cystine remained unchanged. The results previously obtained for the basic amino acids were also unchanged. The renal clearance of cysteine-penicillamine disulfide closely followed the cystine clearance rates in all three patients and was similarly greater than the glomerular filtration rate in the last two patients. At present it is not possible to decide whether the excess of urinary cystine in the last two patients was derived from a high renal extraction of cysteine and cystine from the arterial blood or whether the cystine was secreted as a result of renal metabolic process. The data suggest that cystinuric patients with very high cystine clearances

could be genotypically different from those previously described.

A New Member of the Membrane ATPase Family. ANTHONY W. CZERWINSKI, HILLEL J. GITELMAN, AND LOUIS G. WELT,† Chapel Hill, N. C.

The magnesium-dependent, sodium-potassium activated, glycoside-inhibited adenosine triphosphatase of Skou has been found in diverse tissues; and because of characteristics in common with the active component of ion transport, many consider it to play a key role in this process. It is therefore of interest that an adenosine triphosphatase has been found in the rat erythrocyte membrane with similar and significantly dissimilar characteristics. This new enzyme system has the following properties: it is *1*) observed at low concentrations of adenosine triphosphate (e.g., 10^{-5} mole per L or less), *2*) magnesium-dependent, *3*) activated by sodium, and this activation is completely inhibited by glycoside (scillaren), and *4*) progressively inhibited, in lieu of being activated, with potassium at levels from 2×10^{-4} mole per L to 50×10^{-2} mole per L. One product of this enzyme reaction is adenosine diphosphate, which accounts for all the adenosine triphosphate that disappears. A second major product is inorganic phosphate, and within the limitations of the estimation at these low levels of phosphate, it equates with the disappearance of adenosine triphosphate on an equimolar basis. The implications of this new system are several. First, it raises a question concerning the relationship between this system and that of Skou regarding the transport of alkali metal ions, specifically sodium. Secondly, it raises the question as to whether this enzyme system may be a component in the active transport of other compounds that apparently require the presence of sodium and are inhibited by glycoside.

Suppression of Delayed Hypersensitivity *In Vitro* by Inhibition of Protein Synthesis. JOHN R. DAVID, New York, N. Y. (introduced by H. S. Lawrence†).

An *in vitro* system has been used to study the mechanisms of delayed hypersensitivity. Peritoneal cells obtained from guinea pigs exhibiting delayed hypersensitivity are inhibited from migrating out of capillary tubes by the specific sensitizing antigen (tuberculin, ovalbumin, or diphtheria toxoid). This inhibition is independent of serum antibody and is a direct reflection of cellular hypersensitivity. If as few as 2.5% of the cells within a population are specifically sensitive, the whole population can be inhibited by antigen. Such sensitive cells must be living to react with antigen and affect other cells. If these cells are desensitized by trypsin *in vitro*, they will recover their sensitivity after 24 hours of incubation in tissue culture. It was of interest to determine whether the inhibition of cell migration by antigen would occur if protein synthesis were blocked. Sensitive cells exposed to puromycin, 2×10^{-5} M, were no longer inhibited by specific antigen and migrated 88% of normal. Control sensitive cells and cells in the presence of puromycin, 1×10^{-6} M, were still inhibited by antigen and migrated 17% and 18% of normal. The concentration of puromycin that prevented the inhibition of cell migration was also sufficient to suppress incorporation of C^{14}-leucine into protein by peritoneal cells. Additional evidence that the action of puromycin on sensitive cells was due to its specific effect on protein synthesis was obtained with analogues of puromycin. L-Phenylalanyl and L-tyrosyl analogues, known to inhibit protein synthesis, prevented the inhibition of migration of sensitive cells by antigen. In contrast, the L-tryptophanyl analogue and 3′ aminonucleoside of puromycin, which do not interfere with protein synthesis, had no effect. It is concluded that active protein synthesis is necessary for the expression of delayed hypersensitivity *in vitro*.

Thyroid Iodide Kinetics and Evidence for an Iodide Leak. LESLIE J. DEGROOT,* Boston, Mass.

Iodine metabolism has been investigated by I^{131} and I^{127} analysis in 4 normal subjects and 20 patients with thyroid disease. In normal subjects thyroid clearance (C_G) averaged 10 ml per minute, thyroid iodine release rate (K_{GB}) was 1% per day, thyroid iodine content (Q_G) was 10.7 mg, absolute iodide uptake (AIU) measured by three techniques was 52 to 71 μg per day, and thyroid iodine secretion (H) estimated by three distinct methods was 107 to 138 μg per day. In 8 subjects with multinodular goiter there was a doubling of Q_G without a corresponding decrease in K_{GB}, resulting in a doubling of H. In 4 thyrotoxic subjects C_G and K_{GB} were markedly elevated. The increase in K_{GB} raised H to 485, although Q_G was one-half normal. Similar alterations in C_G, K_{GB}, Q_G, and H were present in 4 subjects with the butanol-insoluble iodine syndrome. In two subjects with Hashimoto's thyroiditis C_G was normal, K_{GB} increased, and Q_G was 1.5 mg. AIU and H were above normal although the patients were not hyperthyroid. Evidence was found for the release of iodine from the thyroid in nonhormonal form in both normal subjects and in those with disordered thyroid function. The rate of loss of [I^{131}] from serum during the period 12 to 96 hours after administration of tracer did not remain exponential but instead progressively slowed suggesting a return of I^{131} to plasma. Specific activity of urinary I was approximately equal to specific activity of PBI 7 to 14 days following tracer administration. More labeled iodide appeared in urine than predicted from degradation of PBI^{131}. Urinary isotope excretion declined in parallel with thyroid isotope content rather than plasma PBI^{131}. Thyroid iodine secretion was consistently higher than AIU. All of these factors suggest that release of iodide (as iodide or a form promptly metabolized to iodide) is a constant aspect of thyroid physiology. The extent of iodide release is greater in thyroid disease than in normal subjects. Spillage of endogenous thyroid iodide may contribute to goiter formation in the presence of adequate iodine intake.

Direct Evidence of Fetomaternal Passage of Leukocytes and Platelets in Man. R. G. DESAI, S. DRISCOLL, E. MCCUTCHEON, AND B. LITTLE, Boston, Mass. (introduced by Charles P. Emerson †).

Leukocytes and platelets have been demonstrated to pass from mother to fetus in man; hitherto, however, fetomaternal passage of these elements has not been demonstrated. The recent use of intrauterine transfusions in the management of erythroblastosis fetalis has afforded the opportunity to study the transfer of erythrocytes, leukocytes, and platelets from fetus to the mother. Fetuses of five women during 24 to 34 weeks of gestation were transfused with 100 to 150 ml of freshly packed group O Rh-negative blood labeled *in vitro* with Atabrine. One fetus received three, and two fetuses, two intrauterine transfusions; one fetus was transfused once. Twenty ml of maternal blood was collected 1, 2, 3, and 4 hours after intrauterine transfusion. In two cases, half-hour and 20-hour samples also were examined. Buffy coat smears were examined by means of fluorescent microscopy. Donor leukocytes and platelets were demonstrable in small numbers in all maternal samples. There appeared to be a peak incidence of fetomaternal transfer in the 2-hour sample. The results show that intact leukocytes and platelets can migrate from fetus to mother as early as 24 weeks of gestation. The high incidence of fetomaternal passage demonstrated in these cases may be due to pathological changes in the placentae associated with erythroblastosis fetalis. However, the exact mechanism of cell transfer remains to be elucidated.

The Hepatic Clearance of the Thrombosis-inducing Capacity of Serum. DANIEL DEYKIN, Boston, Mass. (introduced by A. S. Freedberg †).

Although it is well known that inhibitors of blood coagulation arise during clotting *in vitro,* mechanisms retarding the propagation of thrombosis *in vivo* have not been well characterized. The following experiments demonstrate the role of the liver in the inactivation of thrombogenic factors in the intact animal. Infusions of human serum into rabbits induce thrombosis in areas of vascular stasis. These thrombi are morphologically identical to red thrombi found in man. Activated Factors IX and XI in the infused serum have been shown to be essential for thrombus formation. Clearance of hypercoagulability engendered by these procoagulants was assayed by examining for thrombus formation in venous segments isolated immediately and at progressive intervals following serum infusion into rabbits. Decreased thrombosis was evident within 45 seconds and was striking by 180 seconds after infusion. When serum was infused into the portal vein, rather than into a peripheral vein, diminished thrombosis was evident within 15 seconds and was striking within 30 seconds following infusion. If the portal vein and hepatic artery were occluded following serum infusion into a peripheral vein, no decrease in thrombosis occurred within 10 minutes after infusion, and the rabbits died of widespread coagulation. In further studies, rabbit livers were perfused with human serum, and samples of the perfusate were assayed at intervals for Factors VII, IX, X, XI, and thrombosis-inducing capacity. Factors IX and XI and thrombosis-inducing capacity progressively decreased, whereas Factors VII and X were unaffected. In addition to its function in synthesizing blood coagulation factors, these experiments indicate that the liver plays a central role in the inactivation or removal of procoagulants whose presence in the circulation is associated with systemic hypercoagulability.

Bilirubin Encephalopathy: Experimental Models in Newborn and Adult Animals. IVAN DIAMOND AND RUDI SCHMID,* Chicago, Ill.

The occurrence of bilirubin encephalopathy as a complication of unconjugated hyperbilirubinemia is confined almost entirely to the neonatal period; this has been ascribed to an ill-defined immaturity of the blood brain barrier. It has recently been demonstrated that in the plasma, bilirubin is bound exclusively to albumin, and that this protein interaction is a major determinant of pigment distribution between the intra- and extravascular spaces. Moreover, human albumin has a much higher affinity for bilirubin than does albumin from rats or guinea pigs, and certain organic anions compete with the pigment for common binding sites. Based on these observations, experimental models were developed in animals that permitted reversible alterations of pigment distribution between the various body compartments. In newborn guinea pigs and in adult Gunn rats, constant levels of hyperbilirubinemia were achieved by infusion of C^{14}-bilirubin; the radioactive pigment was administered either bound to human albumin or dissolved in protein-free solution. Infusion of unbound C^{14}-bilirubin resulted in lower serum concentrations and much higher brain levels of radioactivity than when the pigment was given complexed with human albumin. Furthermore, C^{14}-bilirubin deposited in the brain by the administration of unbound pigment could be mobilized by subsequent infusion of human albumin. Induction of respiratory or metabolic acidosis increased the extravascular distribution of C^{14}-bilirubin. Injected salicylate, on the other hand, without changing the pH of the plasma, displaced bilirubin from albumin binding sites and similarly enhanced accumulation of the pigment in the brain. These findings indicate that in newborn guinea pigs and adult rats, the amount of pigment transferred into the brain is independent of the total concentration of serum bilirubin, but is determined rather by the size of the unbound pigment fraction. The studies, therefore, provide direct experimental support for the treatment of neonatal jaundice by human albumin infusion.

Intestinal Cholesterol Synthesis: Its Localization and Control. J. M. DIETSCHY AND M. D. SIPERSTEIN,* Dallas, Texas.

The location of intestinal sterol synthesis and the mechanisms of its regulation have been studied in the

rat. Every level of the gastrointestinal tract incorporated acetate-2-C^{14} into digitonin precipitable sterols; however, the synthetic rates were relatively low in the esophagus (25 mμmoles per g tissue), stomach (65 mμmoles per g), and jejunum (20 mμmoles per g), but increased markedly in the ileum (120 mμmoles per g). Similarly, synthesis was low in the cecum (30 mμmoles per g), but increased in the distal colon (100 mμmoles per g). When the small bowel wall was divided into villi, crypts, and muscle, cholesterogenic activity was found almost exclusively in the crypt preparation. In contrast to sterol synthesis by the liver, the specific synthesis of cholesterol (determined by TLC) at every level of the intestine was totally insensitive to prolonged cholesterol feeding and fasting. However, small intestinal cholesterogenesis was strikingly enhanced (10- to 15-fold) by 48 hours of biliary diversion. Furthermore, when bile was reinfused at any level of the small bowel in animals with biliary diversion, marked suppression of sterol synthesis occurred distal to the infusion point. The time course for this inhibition showed that no effect occurred with infusions of <6 hours, but at longer times, suppression of cholesterogenesis became evident reaching maximal values ($>90\%$) at 48 hours. The recent demonstrations that sterols made in the intestine may significantly contribute to the circulating cholesterol pool emphasize the physiologic importance of sterol synthesis by the intestine. The finding in these studies that the presence or absence of bile profoundly influences the rate of small bowel cholesterogenesis not only provides an explanation of the gradient of synthetic activity normally found between the jejunum and ileum, but may also offer an explanation for the hypercholesterolemia commonly associated with prolonged biliary obstruction.

Plasma Antidiuretic Hormone in Adrenal Insufficiency. JOSEPH F. DINGMAN, CARLOS GONZALEZ-AUVERT, ABDUL B. JALAL AHMED, AND AKIRA AKIMURA, Boston, Mass. (introduced by Frank N. Allan †).

On the basis of bioassays of whole plasma, Kleeman and associates contend that ADH secretion is normally suppressed in water-loaded hypoadrenal patients and that hydrocortisone corrects the impaired diuresis by a direct renal action. Our concept that hydrocortisone inhibits ADH secretion was investigated with a specific method for plasma arginine vasopressin (AVP), the human antidiuretic hormone. Trichloroacetic acid extracts of plasma were chromatographed on IRC-50 resin columns, AVP fractions were eluted with 50% acetic acid, and AVP was isolated by silicic-acid glass paper chromatography. Eluates of specific AVP zones were injected iv in alcohol-diuretic rats using 4 point assay against AVP standard, and AVP was confirmed by thioglycollate inactivation in all studies. Recovery of 4 μU AVP from inactive plasma averaged 90%. After overnight dehydration, plasma AVP averaged 5 μU per ml in 12 normal subjects and was elevated to 18 to 100 μU per ml in 5 patients with untreated adrenal insufficiency. After hydration, plasma AVP was abnormally sustained (11 to 60 μU per ml) in 4 studies showing impaired water diuresis and fell only to the normal range in 2 studies showing subnormal free water diuresis. Cortisone lowered plasma AVP to normal levels in 2 dehydrated patients. Plasma AVP was zero during normal water diuresis in 3 patients after cortisone therapy. High AVP plasmas simultaneously preserved and assayed by Kleeman's method showed no bioactivity. We conclude that *1*) hypersecretion of AVP does exist in hypoadrenalism; *2*) sustained AVP secretion despite hypo-osmolality accounts for the impaired water diuresis; *3*) glucocorticoids promote normal water diuresis by inhibiting AVP hypersecretion and normalizing osmoreceptor function; *4*) circulation AVP may be present during subnormal free water diuresis; *5*) rigorous purification of plasma AVP by column and paper chromatography affords the most precise technic now available for studying AVP secretion.

Phagocytic Defense against Acute Bacterial Infection in Experimental Diabetes Mellitus. ROBERT H. DRACHMAN, Baltimore, Md. (introduced by W. Barry Wood, Jr.†).

Rats made chronically diabetic with alloxan are more susceptible than control rats to acute bacterial pneumonia experimentally induced by intrabronchial inoculation of type 25 pneumococci. Although the diabetic animals are only hyperglycemic (i.e., do not exhibit ketoacidosis), they die sooner and in greater numbers as a result of the infection, and their pneumonic lesions contain many more (100 to 1,000 ×) viable organisms. When studied histologically, their lesions show a striking depression of phagocytosis. Similar relationships obtain in normal and chronically diabetic rats with experimental pneumococcal peritonitis. Furthermore, *in vivo* phagocytosis experiments performed by injecting type 25 pneumococci intraperitoneally 18 hours after a starch-aleuronat injection reveal a marked depression of phagocytosis in the diabetic animals. The results of comparative *in vitro* phagocytic tests, performed with the same strain of type 25 pneumococcus and with leukocytes and sera obtained from both normal and diabetic rats, indicate that the factor responsible for inhibiting phagocytosis is in the serum rather than in the cells. Indeed, the phagocytic capabilities of leukocytes harvested from starch-aleuronat–induced peritoneal exudates in normal, as well as in diabetic rats, are depressed not only in diabetic serum, but also in normal serum made hypertonic by hyperglycemic levels of added glucose, other hexoses, or pentoses. Phagocytosis by granulocytes in whole blood, on the other hand, is unaffected in hyperglycemic plasma, as is that of exudate leukocytes preincubated in plasma. Thus the impaired phagocytic defense of the nonketotic diabetic host appears to be due to the osmotic effect of the high glucose levels on the granulocytes that have left the blood stream and are operating in inflammatory exudates. The observation that abnormally high levels of glucose depress phagocytosis in acute exudates suggests that hyperglycemia should be rigorously controlled in

diabetic patients with impending, or established, bacterial infections.

Changes in Renal Blood Flow and Possibly the Intrarenal Distribution of Blood Accompanying the Natriuresis of Saline Loading. LAURENCE E. EARLEY AND ROBERT M. FRIEDLER, Boston, Mass. (introduced by Maxwell Finland †).

The possibility that renal hemodynamics may be involved in the diminished tubular reabsorption accompanying saline loading was investigated in anesthetized dogs receiving desoxycorticosterone and vasopressin. Sodium excretion ($U_{Na}V$), concentrating capacity ($T^c_{H_2O}$), and hemodynamics including *p*-aminohippurate extraction (E_{PAH}) were measured before, during, and after infusion of isotonic Ringer's solution, and during unilateral reductions of renal blood flow during loading. In 28 experiments increased $U_{Na}V$ averaged 978 μEq per minute per kidney during loading. In 5 experiments no increased filtered sodium (F_{Na}) accompanied the natriuresis. In 5 of 7 studies followed after loading F_{Na} remained maximal or increased further despite returns toward control of $U_{Na}V$. In all studies E_{PAH} decreased during loading (average control .79, falling to .63), and renal blood flow increased. These latter values returned toward control as $U_{Na}V$ decreased following loading, independent of increasing F_{Na}. During natriuresis, modest unilateral reductions of renal perfusion pressure (70 to 85 mm Hg) increased E_{PAH}, increased $T^c_{H_2O}$, and reduced $U_{Na}V$ without equivalent reductions in F_{Na}. Greater reductions in perfusion pressure (40 to 60 mm Hg) decreased F_{Na} below preloading values, whereas $U_{Na}V$ remained elevated, and E_{PAH} remained lower than control. PAH transport was unaffected by "saline" loading as assessed by Tm, altering plasma levels, and extraction of tracer amounts of radioactive Diodrast. Thus, decreased E_{PAH} during saline infusion may represent a relative increase in noncortical blood flow, which could include medullary flow. These data are consistent with the concept that redistribution of intrarenal blood flow may affect net tubular reabsorption of sodium. Shifts of GFR to juxtamedullary nephrons may exceed reabsorptive capacity, or decreased medullary hypertonicity secondary to increased blood flow could reduce water loss from the descending limb of Henle's loop leading to diminished sodium reabsorption as an increased flow of more dilute fluid passes transport sites of the ascending limb.

Quantitative Evaluation of Free Fatty Acid and Glyceride Fatty Acid Metabolism in Man. R. PHILIP EATON, DANIEL STEINBERG,* AND MONES BERMAN, Bethesda, Md.

Information on the extent to which free fatty acids (FFA) recycle through the plasma and the extent to which they contribute to plasma triglyceride (TG) formation is limited and difficult to obtain by direct methods. The digital computer, programmed for multicompartmental model analysis, was used in the present studies for kinetic analysis of FFA and TG metabolism in man. C^{14}-palmitate was infused iv at a constant rate for 60 minutes and then stopped; plasma samples were taken over a total of 3 hours, and specific radioactivity (SA) of FFA and TG was measured. Radioactivity was first detected in plasma TG 30 minutes after the start of the C^{14}-palmitate infusion, and then SA of TG rose rapidly; cholesterol esters and phospholipids contained very little radioactivity. The SA of TG continued to rise for several hours after the labeled FFA infusion was stopped and then fell slowly. The combined FFA and TG data were closely fitted with a model in which labeled plasma FFA is the sole precursor for labeled plasma TG, and in which a significant recycling pathway returns labeled FFA to the plasma compartment. A catenary system of intermediate pools between the precursor FFA and product TG is required to account for the observed time course of TG SA. In 6 young fasted normal volunteers the FFA recycling pathway accounted for $22 \pm 3\%$ of the total plasma FFA flux, which varied from 270 to 560 μEq per minute. In two normal subjects the half-life of endogenous TG was 2.8 and 4.9 hours, whereas in a patient with familial fat-induced hyperlipemia it was greater than 21 hours.

A Study of the Distribution and Binding of Insulin to the Subcellular Organelles of Striated Muscle. P. MICHAEL EDELMAN, JEAN C. EDELMAN, AND IRVING L. SCHWARTZ,† Cincinnati, Ohio, and Upton, N. Y.

A survey of the subcellular distribution of I^{131}-insulin in muscle was undertaken to obtain information bearing on the site or sites of the stimulatory effect of the hormone on protein synthesis and on glucose transport. Adult rat femoral muscle was incubated for 30 minutes in Krebs-Ringer bicarbonate buffer containing I^{131}-insulin, washed three times in saline, bathed in 10^{-3} M *N*-ethylmaleimide to bind available free SH groups, and then washed in dilute acid, 0.15 M NaCl and 8 M urea solutions until the radioactivity in the wash was at background levels. The muscle was then homogenized and fractionated by differential and sucrose density gradient centrifugation into mitochondrial, microsomal, nuclear, sarcolemmal, and soluble constituents, and the radioactivity and protein content of each component were determined. The fractions were characterized and their relative purity was ascertained by phase and electron microscopy, by enzyme assay (cytochrome oxidase, succinic dehydrogenase, and ATPase), and by measurement of RNA and DNA. The binding of hormone to muscle cell membrane was studied by incubating the isolated sarcolemmal fraction (obtained from labeled whole muscle) in media designed to disrupt electrovalent and disulfide bonds and then measuring its specific radioactivity. Most of the label was fairly evenly divided between the soluble and the sarcolemmal fraction of the muscle cell, binding to the latter fraction depending in part on covalent linkage involving the 6 to 11 bridge of the A chain. Less than 2% of the total radioactivity was found to be associated with the highly purified nuclear fraction; less than 0.5% of the total radioactivity was associated with

the microsomal and mitochondrial fractions. These findings support the concept that insulin acts at the cell membrane. In addition, the large amount of I^{131}-insulin associated with the soluble fraction invites attention to a possible hormonal effect at the level of the aminoacyl transfer sRNA systems.

Selective Intestinal Infection with Type 4 Adenovirus and Its Specific Protective Effect against Epidemic Disease. W. P. EDMONSTON, R. H. PURCELL, W. LUDWIG, J. W. P. LOVE, B. F. GUNDELFINGER, AND R. M. CHANOCK,* Bethesda, Md.

When volunteers were fed type 4 adenovirus enclosed in an enteric coated capsule, a symptom-free infection occurred that was limited to the lower intestinal tract. In this manner the area in which adenovirus pathology usually occurs, i.e., the nasopharynx and lower respiratory tract, was bypassed. Selective intestinal infection stimulated moderately high levels of neutralizing antibody. Furthermore, such enteric infections were not communicable to susceptible contacts who were exposed for prolonged intervals to volunteers shedding virus. Efficacy of the live adenovirus enteric capsule mode of immunization was then evaluated in a military recruit population. This group was chosen because extensive outbreaks of type 4 adenovirus illness occur every year during late January, February, and March. The type 4 virus used for immunization was grown in human diploid fibroblast (W1-38) tissue cultures and was shown to be free of adventitious microbial contaminants and of oncogenic activity in suckling hamsters. Two hundred seventy-nine military recruits were randomized by serial number. One hundred thirty-five men were fed 10^6 $TCID_{50}$ of encapsulated type 4 virus, and 144 men were fed placebo capsules. The men were examined and specimens for virus isolation taken 3 times a week. All the men vaccinated who lacked neutralizing antibody became infected and developed moderate to high levels of antibody. Enteric infection was not associated with detectable illness. Infection did not spread to antibody-free contacts despite their prolonged close contact with the men vaccinated. A specific protective effect of the vaccine was demonstrated when the vaccinated and placebo men were transferred to a camp at which type 4 adenovirus was epidemic. Thirty-two of 132 men in the placebo group were hospitalized with an adenovirus febrile respiratory tract disease, whereas none of the men vaccinated were admitted to the hospital for adenovirus illness. Thus, the vaccine appeared to be 100% effective in preventing febrile adenovirus disease.

The Macromolecular Complexes and Bilirubin Binding in Human Gallbladder Bile. BAROUKH M. EL KODSI, SIDNEY R. COOPERBAND, AND IAN A. D. BOUCHIER, Boston, Mass. (introduced by Isadore N. Rosenberg*).

The bilirubin binding to macromolecular material has been studied in human bile. Bile was aspirated from 30 normal and pathological gallbladders at surgery. Analyses of biles in the Spinco model E ultracentrifuge with Schlieren optics revealed one main peak and a number of smaller faster moving peaks. The main peak constituted 85 to 95% of the sedimenting macromolecules, and sedimented at 1.94 $S°_{20,w}$ in normal bile, and 1.88 $S°_{20,w}$ in pathological biles. The bilirubin pigment sedimented at the same rate as the main peak in both groups when studied with absorption optics at 450 mμ. The two groups did not differ in total solids, but the quantity of material comprising the main peak of pathological bile (6.8 ± 3.2 g per 100 ml) was significantly lower than that of normal bile (10.7 ± 4.7 g per 100 ml). The binding of bilirubin to macromolecular molecules was studied by dialyzing bile against buffered saline at room temperature, and the rate of bilirubin movement from the dialysis sac was determined. The data suggest that there are two fractions: one escaping at a rate similar to free crystalline bilirubin, and a second escaping at a slower rate, presumably the result of dissociation of pigment from the macromolecular material. The first fraction of bilirubin amounted to $12.4 \pm 3.9\%$ of the total bilirubin in normal biles and $24.0 \pm 9\%$ in pathologic biles. At the end of 6 hours, normal bile had lost a mean of 46% and pathologic bile 70% of their total bilirubin. The quantity of unbound bilirubin and the rate of dissociation of the bound bilirubin correlated with the concentration of material comprising the main peak. It is concluded that bilirubin in bile is bound to a macromolecular complex. Alteration in the quantity of main peak macromolecules is associated with alteration in ability to bind bilirubin.

The Effect of Respiratory Alkalosis on Blood Lactate and Pyruvate in Humans. FREDERIC L. ELDRIDGE* AND JOHN M. SALZER, Palo Alto, Calif.

Since the effect of hypocapnia and respiratory alkalosis on blood lactate and pyruvate has generally been studied during severe hypocapnia, the effect on these acids of graded hypocapnia and alkalosis was determined. Ten studies were performed on six normal human subjects. *1*) After resting determinations of arterial lactate, pyruvate, pH, P_{CO_2}, HCO_3^-, and ventilation, subjects hyperventilated successively to monitored P_{CO_2} levels of approximately 35, 30, 25, and 20 mm Hg for 10 minutes at each level. Measurements were repeated at each level. *2*) On another day, the same subjects repeated the experiment but breathed 100% O_2 throughout. *3*) Similar measurements were made on subjects breathing with a P_{CO_2} of 30 mm Hg, first for 1 hour on room air and then for 1 hour on 100% O_2. No accumulation of pyruvate occurred unless the P_{CO_2} was less than 28 mm Hg and the pH greater than 7.50. On the other hand, both lactate and the lactate/pyruvate (L/P) ratio rose progressively as the P_{CO_2} fell below 35 mm Hg. One hundred per cent O_2 breathing in the same subjects eliminated the rise in pyruvate in all subjects even though P_{CO_2} and

pH values were <20 mm Hg and> 7.60, and led to a smaller rise in lactate but did not affect the increase in L/P ratio. Hyperventilation for 1 hour at a P_{CO_2} of 30 mm Hg led to insignificant changes in pyruvate but did increase lactate and L/P ratio. These studies show that moderate respiratory alkalosis does not lead to an increase in blood pyruvate, that even severe alkalosis does not produce increased pyruvate during oxygen breathing, and that lactate increases are out of proportion to pyruvate increases at all levels of alkalosis studied. These findings suggest a re-examination of the concept that increased blood lactate is purely secondary to increased pyruvate in hyperventilation.

Relationships between Coronary Artery Lesions and Myocardial Ischemia in Man. WILLIAM C. ELLIOTT, ERNEST L. FALLEN, MICHAEL D. KLEIN, FRANCIS J. LANE, AND RICHARD GORLIN,* Boston, Mass.

Multiple site sampling in the coronary sinus for lactate production has been helpful in defining areas of myocardial ischemia. Thirty-seven subjects were studied by selective coronary cinearteriography and mapping of the coronary sinus for evidence of glycolysis exceeding oxidation, i.e., lactate production. All were stressed by isoproterenol infusion (2 to 4 μg per minute) during sampling of blood draining the left ventricle. Normal lactate extraction was observed at rest and during isoproterenol stress in 20 subjects, 14 of whom had no coronary lesions. Four others had major right coronary stenoses, whereas only two had significant left coronary lesions. Seventeen showed lactate production in at least one site. Unexpectedly, some lesions of proximal left anterior descending artery alone resulted in lactate production from the inferior rather than anterolateral surface of the left ventricle. The observation that areas of ischemia may be spatially distant from arterial stenosis was similar to Blumgart's description of "myocardial infarction at a distance." With severe disease of both coronaries, revascularization of the anterior myocardium, either by spontaneous collateral development or internal mammary implant, eliminated anterior but not inferior myocardial lactate production. Conversely, others with left anterior descending lesions had lactate production that could only be detected by sampling blood deep in the coronary sinus coming from the anterior left ventricle. Apparently, dilution of this venous blood accounted for net lactate extraction noted nearer the coronary sinus ostium. Five patients with extensive lesions of all arteries showed lactate production at all sites. Similarly, two patients with aortic stenosis, angina pectoris, left ventricular hypertrophy, and no coronary lesions had lactate production at all sites. Localization of ischemic areas is often difficult by arteriography alone. The functional significance of morphologic lesions may be determined by careful study of venous effluent. Hence, both methods are essential for diagnosis and selection for revascularization procedures.

The Component Phases of Human Biliary Evacuation. EDWIN ENGLERT, JR., Salt Lake City, Utah (introduced by Frank H. Tyler †).

With a source of radioactivity in the gallbladder, external monitoring provides continuous records of radioactivity in the biliary tract. During physiologic stimulation these can be used to analyze phases in the process of biliary emptying. Fifteen normal subjects were studied with I^{131}-iopanic acid and a fatty meal. Emptying was irregular, comprising 4 to 10 (mean, 7.5) phases. Rapidly emptying and nonemptying steps were commonest, both initially and throughout the period of evacuation. In ten subjects the phasic sequence began inefficiently but produced effective emptying later. Thirteen individuals manifested a single major emptying phase at this transition point, which ranged from the thirty-third to the one hundredth percentile (mean, fifty-sixth of the total emptying period. The biliary tract empties in an irregular multiphasic fashion reminiscent of other smooth muscle processes. Animal observations relate the steps to changes in gallbladder contraction and activity of the sphincteric mechanisms in the bile duct within the duodenal wall. In man as well, the data suggest that biliary evacuation depends on the moment-to-moment interplay of these opposing forces.

Prenatal Antibody Synthesis as Measured in Healthy Human Newborn. WALLACE V. EPSTEIN, San Francisco, Calif. (introduced by Ernest Jawetz †).

To date there has been no demonstration of immune globulins of fetal origin having antibody specificity in normal human newborn. In response to intrauterine infection the immune globulins γ_M appear to be preferentially produced by the human fetus. This protein, like γ_A, appears incapable of transplacental passage. Fifty-six pairs of maternal and cord sera from apparently healthy mothers and their children were examined for the capacity to agglutinate sheep erythrocytes coated with normal human γ_G globulin or Bence Jones (BJ) proteins. Hemagglutinating activity for γ_G and type K, BJ protein-coated cells, was found in all maternal sera but in only 5% of cord sera. These few positive cord sera had titers equal to or less than the maternal titers. In contrast, hemagglutinating activity for type L, BJ protein was found in 54 cord sera. In 70% of these cord sera the titers were higher than those of maternal sera or occurred in the absence of similar activity in the maternal sera. By sucrose gradient ultracentrifugation and gel filtration column chromatography, hemagglutinating activity for all of these cell coatings was found to be restricted to the macroglobulin fraction of both cord and maternal sera. Agglutinating activity of cord serum appeared oriented toward light polypeptide chain sites obstructed in intact γ-globulins and in some instances demonstrated subtype specificity as determined by inhibition of hemagglutination. Twentyfold concentration of pooled maternal and cord sera revealed 0.016 mg and 0.002 mg, respectively, of free L-chain protein per milli-

liter of the original sera. The normal human fetus therefore would appear to synthesize macroglobulin antibodies that differ in specificity and in concentration from those of the mother.

Physiologic Observations of Human Cross Circulation. J. W. ESCHBACH, JR., R. B. EPSTEIN, J. M. BURNELL, AND E. D. THOMAS,* Seattle, Wash.

W.D. with terminal chronic glomerulonephritis and J.H. with acute leukemia refractory to drugs were cross circulated 2 hours daily for 4½ months. W.D. supplied sufficient leukocytes and platelets to J.H. to carry her through 2 months of marrow aplasia produced by 300 r irradiation. A remission followed but she relapsed and died with *Pseudomonas* septicemia. Many physiological studies were undertaken: *1*) Leukokinetic data demonstrated adequate leukopoiesis in the azotemic boy as indicated by daily transfer of 50 to 100 billion leukocytes from W.D. to J.H. *2*) Irradiation halted platelet destruction in the leukemic allowing W.D.'s platelets to equilibrate at half-normal levels in both. *3*) Increased erythropoietin transferred from J.H. failed to raise significantly the reticulocyte count of the azotemic boy. *4*) Immunologic reactivity was assessed by exchange of skin grafts. Slow graft rejection indicated that tolerance was not induced, but second set rejections did not occur. *5*) J.H.'s renal function remained normal while maintaining W.D.'s serum creatinine at 6 to 8 mg per 100 ml. Although anuric, W.D. was adequately dialyzed with daily cross circulation except for potassium, which required resins for control. *6*) Excess parathyroid and aldosterone activity in W.D. were manifested in J.H. *7*) Although hypertensive, W.D. had normal angiotensin levels. Blood pressure was controlled by shifting fluid into J.H. thereby removing excess salt and water from W.D. J.H. remained normotensive. *8*) There were no adverse effects of cross circulation except for chills and fever in J.H. immediately following each cross circulation, which disappeared when she was in remission. W.D., on chronic dialysis for 5 months, has shown no evidence of leukemia.

Selective Deficiency of Immunoglobulin Polypeptide Chains. J. L. FAHEY,* W. F. BARTH, R. ASOFSKY, AND D. S. ROWE, Bethesda, Md.

Clinical studies of the control of immunoglobulin formation were undertaken in the context of current concepts of the composition, heterogeneity, and genetic control of the immunoglobulins. Individual molecules are composed of two types of polypeptide chains, i.e., the heavy (mol wt, 50,000) and light (mol wt, 22,000) chains. Two forms of light chains, (κ and λ: types I and II) and four forms of heavy chain (γ, α, μ, and δ) have been identified. The forms of heavy chain, respectively, determine whether molecules are IgG (γ_G, 7 S γ_2-globulin), IgA (γ_A-, γ_{1A}-, β_{2A}-globulins), IgM (γ_M-, 18 S γ_1-macroglobulins), or IgD (γ_D). The IgD is a new class of immunoglobulin identified recently in this laboratory. Marked deficiency of two classes of immunoglobulin, IgG and IgA, was found in a 15-year-old girl with recurrent infection since age 2. Antibody formation was markedly impaired. Quantitative immunoglobulin measurements revealed trace amounts of IgG and IgA, superabundance of IgD and IgM, and normal amounts of IgK and IgL. Biosynthetic, immunofluorescent cytology and immunoglobulin turnover studies indicated that γ and α polypeptide chains were synthesized in minimal amounts, μ and δ chains at rates 10 times greater than normal, and κ and λ chains in normal amounts. Although a defect in structural genes for γ and α polypeptide chains has not been ruled out, the findings can be accounted for by a defect in genes regulating the synthesis of γ and α polypeptide chains.

An Effect of Mannitol on Renal I^{131}-Hippuran Excretion Independent of Urine Flow Rate. MELVIN H. FARMELANT, WALTER G. DUKSTEIN, AND BELTON A. BURROWS,† Boston, Mass.

It has been previously reported that mannitol loads minimize differences in I^{131}-Hippuran tracings between a kidney with renal arterial constriction and its "normal" counterpart in patients with renovascular hypertension. This effect can be explained by reduction in urine flow differences between the kidneys or by changes in renal hemodynamics or tubular secretory function. Following a single injection of I^{131}-Hippuran in dogs, renal radioactivity tracings could be described by the integral of arterial blood radioactivity minus this integral after a delay corresponding to renal passage time (shown by bladder appearance of radioactivity). The decrease in renal radioactivity for 3 to 5 minutes after its peak approximates an exponential slope, the half-time ($t_{\frac{1}{2}}$) of which can be related to simultaneous C_{PAH}. The relationship between $t_{\frac{1}{2}}$ and passage times to urine flow rates following water or mannitol loads was studied in normal human subjects. Increasing water loads resulted in shorter $t_{\frac{1}{2}}$ and passage time until urine flow exceeded 3 ml per minute, above which these values showed little change. Mannitol loads were associated with shorter $t_{\frac{1}{2}}$ and passage times compared to water loads at similar urine flow rates. Similar differences between water and mannitol loads were observed in chest radioactivity tracings that reflected changes in renal clearance of I^{131}-Hippuran. The ratio of chest radioactivity $t_{\frac{1}{2}}$ to renal radioactivity $t_{\frac{1}{2}}$ was constant at urine flows above 3 ml per minute with both mannitol and water loads. Therefore, the reduction in $t_{\frac{1}{2}}$ produced by mannitol as compared with water loads at similar urine flow rates reflects increased I^{131}-Hippuran clearance. Thus, the effect of mannitol in renal arterial constriction may be in part due to increased urine flow to a critical level, but also may indicate more rapid movement of tubular fluid proximal to the collecting ducts, resulting in a reduced passage time and changes in renal blood flow.

Kinetics of Plasma Lipoprotein Triglyceride in Man and the Dog. J. W. FARQUHAR, R. C. GROSS, P. W. WRIGHT, AND G. M. REAVEN, Palo Alto, Calif. (introduced by H. Holman *).

Previously we reported methods for calculation of turnover rates (t_r) of S_f 20 to 400 lipoprotein triglyceride (VLD-TG). Models of VLD-TG kinetics for man and dog were validated, and t_r (per kilogram body weight) were calculated in 13 subjects and in 10 dogs for varying VLD-TG concentration (C_{TG}). In man, percentages of calories from fat and carbohydrate were varied to test for effect on t_r. In the dog, t_r vs. C_{TG} is linear; therefore kinetics are consistent with simple diffusion or with any mediated transport mechanism, such as an enzyme-dependent clearing process in a region of great excess of unoccupied clearing sites. In man t_r and C_{TG} are nonlinear, but are related as a saturable system: kinetics are consistent with the Michaelis-Menten formulation. No difference in t_r was detected at equivalent C_{TG} in humans on diets either high or low in fat. As further evidence of the marked difference in the systems between man and the dog, at half maximal t_r in man, the ratio of t_r/C_{TG} was only 30% that of the dog. The data indicate: *1)* Species vary widely, but within a species there is surprising homogeneity of VLD-TG clearing efficiency. As a corollary, variations in C_{TG} in both species are controlled by variations in hepatic VLD-TG secretion rates, and not by variations in removal site "efficiency." *2)* Humans are much more likely to become hyperlipemic (at an equivalent t_r) than are dogs; therefore wide fluctuations in C_{TG} may be expected as maximal t_r are approached. Finally, *3)* knowledge of kinetics rather than concentration alone should allow precise study of mechanisms controlling substrate synthesis, steady-state concentrations, and removal mechanisms. Also differentiation between roles of substrates and of removal sites in clearing may be possible.

Effect of Human Leukocyte Extracts on Mitoses of Human Fibroblasts *In Vitro*. PHILIP J. FIALKOW AND STANLEY M. GARTLER, Seattle, Wash. (introduced by Arno G. Motulsky *).

As one approach to testing the hypothesis that immunologic mechanisms may predispose to chromosomal abnormalities, the effect of an extract of human circulating mononuclear cells on mitoses in fibroblast cell culture was studied. Since cells containing more than 55 chromosomes are relatively easy to detect, hyperploidy was used as an index of mitotic error. Fibroblast mitoses were examined 3 to 5 days after the addition of circulating mononuclear cell extract (prepared by freezing and thawing). It was found that extract added to fibroblasts of a nonrelated individual consistently increased the percentage of hyperploid mitoses by as much as 400%. The response was linear with respect to dosage of extract. Extract from two patients with chromosomal abnormalities and thyroid autoantibodies produced the most marked responses. In most cases the frequency of endoreduplicated mitoses was also increased. The hyperploidy effect was abolished by boiling the extract for 2 minutes. As compared with circulating mononuclear cell extracts, fibroblast extracts produced relatively slight responses to most of the doses tested. When extracts prepared from circulating mononuclear cells were tested on fibroblasts from the same individuals, only two of eight extracts produced marked responses. One of these latter subjects was an XO/XX mosaic with thyroid antibodies, and the other had a history of extensive autogenic skin grafting and chronic skin disease. The data suggest that extracts of circulating mononuclear cells contain a factor(s) that can cause mitotic errors or fusion in fibroblasts *in vitro* or both. The nature of this factor(s) is unknown, but the effect appears to involve self-recognition.

Studies on the Mechanism of the TSH Stimulation of TPN in Thyroid Slices. JAMES B. FIELD,* SHELDON EPSTEIN, HIROSHI OKA, AND MEISEI OHTA, Pittsburgh, Pa.

Previously we demonstrated the specificity, sensitivity, and rapidity of the TSH stimulation of glucose-1-C^{14} oxidation by thyroid slices. TSH increased TPN, which probably accounted for this effect. The rise in TPN was associated with an equivalent fall in DPN, suggesting stimulation of the DPN kinase reaction, which converts ATP and DPN to TPN. The present studies further elucidate the action of TSH on TPN synthesis. Dog thyroid slices, preincubated for 2 hours with nicotinamide-C^{14} to label endogenous DPN, were then incubated for an additional hour with or without TSH. Triphosphopyridine and diphosphopyridine nucleotides were then extracted and isolated by column chromatography. TPN obtained from TSH-stimulated thyroid slices consistently contained more radioactivity than TPN from control slices, providing direct evidence for an effect of TSH on TPN synthesis. TSH also increased ATP levels in thyroid slices, and the absolute increment in millimicromoles per gram was greater than the amount necessary for the augmented TPN synthesis. Inhibitors of oxidative phosphorylation blocked TSH stimulation of glucose oxidation and TPN synthesis, whereas moderate reductions in thyroid DPN induced by Carzinophilin did not diminish these TSH effects. These results suggest that ATP may be more important than DPN in TPN formation mediated by DPN kinase. Thyroid slices incubated with TSH and then homogenized synthesized more TPN from added excess ATP and DPN and oxidized more 6-phosphogluconate-1-C^{14} than did control slices, consistent with activation of DPN kinase. Actinomycin D and puromycin, which blocked RNA and protein synthesis respectively, did not inhibit TSH stimulation of glucose oxidation, suggesting that new protein and RNA synthesis is unnecessary for this action. Although most of the TPN including the TSH-mediated increment was found in the supernatant fraction of the cell, the nuclear fraction contained most of the DPN kinase.

Human Alveolar Lining Lipids and Cells Obtained *In Vivo*. T. N. FINLEY, E. W. SWENSON, D. E. SMITH, AND T. E. MORGAN, Albuquerque, N. M., Gainesville, Fla., and Seattle, Wash. (introduced by Maurice B. Strauss †).

We have found *in vivo* endobronchial saline wash in dogs yields surface active alveolar phospholipids. In a similar attempt in humans, we obtained alveolar lipids and cells in four awake patients using small washes of saline. The saline was instilled and recovered via a cuffed bronchographic catheter placed in a bronchopulmonary segment under local anesthesia. The procedure was carried out without morbidity in patients with normal lungs, but it was difficult to recover the saline in patients with obstructive airway disease. In one of these patients segmental atelectasis developed and persisted for several days. The recovered wash from the normal patients was not surface active possibly due to the presence of the local anesthetic Cyclaine, which has an inhibitory effect. The wash contained two cell types not seen in bronchial washings: small, round, and polygonal cells with clear cytoplasm and larger cells containing granules. No red cells were seen. Both cell types did not stain for acid phosphatase or PAS-tests for phagocytic activity and mucin respectively. These cells may represent the alveolar membranous and granular pneumocytes of Macklin. The wash was centrifuged and lipids were extracted from the supernate and the sedimented cells. The major lipid component of the supernate was lecithin, 43%. It contained 59% esterified palmitate and 74% total saturated fatty acids. This was similar to dog and cow lung wash. The major lipid component of the cells was lecithin, 32%. Cell lecithin contained 47% esterified palmitate and had a total saturation of 60%. The remainder of the phospholipids was similar in the supernate and cells. Cholesterol was 35% and triglycerides 3% in the cells, and 14% and 20% in the supernate respectively. Phosphatidyl dimethylethanolamine, a possible presursor of dipalmitoyl lecithin in the dog, was 1 to 2 % in both sources.

On the Fixation of Plasma γ-Globulins to Skin in Man. PHILIP FIREMAN, MARY BOESMAN, AND DAVID GITLIN,* Pittsburgh, Pa.

It is generally accepted that antibodies associated with immediate hypersensitivity have the capacity to fix firmly to tissues and that such fixation is a factor in the ability of these antibodies to produce the immediate hypersensitivity response upon reacting with specific antigen. To test this concept, γ_{1A}- and 7 S γ_2-globulins, which have been shown to be responsible for immediate hypersensitivity reactions in man, were radioiodinated, and their disappearance rates from intradermal injection sites were compared with those of radioiodinated albumin, 3 S γ_1-globulin, and 19 S γ_1-globulin, proteins that do not appear to be involved in immediate hypersensitivity reactions. The iodination did not appear to affect the property of γ_{1A}-globulins to produce the immediate hypersensitivity response. It was found that γ_{1A}-globulins, 7 S γ_2-globulins, and albumin behaved alike in normal individuals and that the fractional rate of disappearance of these labeled proteins from the injection site remained constant after the initial 12 to 24 hours: γ_{1A}-globulins disappeared with an average half-life of 36 hours, 7 S γ_2-globulins with a half-life of 42 hours, and albumin with a half-life of 32 hours. In children who had no detectable plasma γ_{1A}-globulin, the fractional disappearance rates of γ_{1A}-globulins, 7 S γ_2-globulins, and albumin from intradermal injection sites were similar to those in the normal person. The half-life of 3 S γ_1-globulins at the intradermal site was approximately 5 hours. The disappearance of 19 S γ_1-globulins from the injection site could be resolved into two exponential components: 90% had a half-life of 40 hours, and the remainder had a half-life of approximately 4 days. These studies suggest that unique or specific fixation of γ_{1A} or 7 S γ_2-globulins to skin does not occur in man.

Histidine Decarboxylase Induction Following Portacaval Shunt: A Possible Mechanism for Gastric Acid Hypersecretion. JOSEF E. FISCHER AND SOLOMON H. SNYDER, Bethesda, Md. (introduced by Oliver Cope †).

Following the construction of an Eck fistula in animals and portacaval shunt in man, gastric secretion and the incidence of duodenal ulcer are increased. Various groups of investigators have ascribed these effects to a secretagogue, originating in the stomach or intestine, that escapes inactivation by bypassing the liver. Gastrin and histamine originating in the stomach and histamine released by the small intestine have been proposed as this secretagogue. Two months following portacaval shunt in the rat, gastric histamine content was markedly elevated. After oral administration of histamine-C^{14}, no greater tissue accumulation of the amine was observed in such rats. There was, however, a fourfold increase in the activity of the histamine-forming enzyme, histidine decarboxylase, in the stomach of portacaval shunt rats. Histamine methyltransferase activity in the stomach and diamine oxidase activity in the small intestine were not elevated. The elevation of histidine decarboxylase activity with a consequent increase in gastric histamine may be causally related to the gastric acid hypersecretion following portacaval shunt. It is suggested that induction of histidine decarboxylase may be a final common pathway in gastric acid hypersecretion in several pathological and experimental states.

Studies of ACTH Antibodies and Their Reactions with Inactive Analogues of ACTH. NORMAN FLEISCHER, JAMES R. GIVENS, KAORU ABE, WENDELL E. NICHOLSON, AND GRANT W. LIDDLE,* Nashville, Tenn.

Immunological methods for assaying plasma adrenocorticotropic hormone (ACTH) have given higher values than biological methods, raising the possibility that there might be substances that are antigenically similar to

ACTH but biologically inactive. Antibodies that would neutralize the biological activity of ACTH were developed by repeatedly injecting porcine or human ACTH with Freund's adjuvant into guinea pigs or rabbits. These neutralizing antibodies were gamma globulins (electrophoretically) and stable at 56° C for 30 minutes. ACTH-antibody complexes that formed at neutral pH were dissociated at acid pH. Antibodies were found that would neutralize biological activity of synthetic α^{1-39} ACTH and ACTH derived from human pituitaries, tumors associated with "the ectopic ACTH syndrome," plasma of Addisonian patients, and plasma of patients with Cushing's disease. ACTH antibodies neutralized the intrinsic melanocyte-stimulating activity of pure ACTH but not that of MSH. Of twelve antisera tested, one (an antihuman ACTH) neutralized short-chain synthetic α^{1-24} ACTH. One of six animals injected with α^{1-24} ACTH developed antibodies that would neutralize both α^{1-24} and α^{1-39} ACTH. Antigenicity, therefore, is not limited to the C-terminal portion of the ACTH molecule. Studies were performed to determine whether biologically inactive ACTH analogues would "consume" neutralizing antibodies, that is, impair their capacity to neutralize active ACTH. Antibodies to porcine ACTH were consumed by polypeptides corresponding to the amino acid sequences 25 to 39 and 11 to 24 of pure porcine ACTH. The sequences 1 to 10 and 7 to 13, however, failed to consume antibodies. Human ACTH inactivated with hydrogen peroxide consumed antibodies. After intravenous administration of ACTH to human subjects, there appeared in the plasma a material that consumed ACTH antibodies but lacked the biological activity of ACTH. We conclude that biologically inactive analogues of ACTH can react with ACTH antibodies; this could explain the higher estimates obtained in immunological assays as compared with biological assays of ACTH.

Studies in Fasting Ketosis. DANIEL W. FOSTER, Dallas, Texas (introduced by J. P. Sanford *).

It is generally held that ketosis in starvation is the result of overproduction of ketone bodies by the liver with no decrease in peripheral utilization of ketones. Two major theories of hepatic overproduction of ketone bodies exist: *1*) increased oxidation of fatty acids (FA) and *2*) decreased synthesis of FA. These parameters have been examined in rats during onset and recovery from fasting ketosis by correlating acetoacetate (AAA) levels in the blood with AAA synthesis, FA synthesis, and FA oxidation in liver slices. FA oxidation was unchanged despite 48 hours of starvation. On the other hand, by 12 hours, when blood ketones had increased fivefold, FA synthesis fell tenfold and AAA synthesis increased fivefold. Because decreased FA synthesis and AAA production occurred simultaneously with onset of fasting, similar studies were performed during the recovery phase of ketosis. After injection of glucose, blood ketones fell sharply within 10 minutes despite unaltered AAA or FA production in the liver. These *in vitro* studies indicate that the overproduction of ketone bodies by the liver is unrelated to either a block in FA synthesis or increased fatty acid oxidation and further suggest that recovery from fasting is initiated by increased peripheral utilization of ketones. Confirmatory evidence of impaired utilization of ketones was obtained by AAA tolerance tests *in vivo*, which showed a turnover time of 9.3 minutes for control and 23.8 minutes for ketotic rats. Finally, radioactive AAA turnover studies were performed before and after glucose injection. Blood ketone levels fell sharply after glucose with no increase in turnover time, clearly indicating reversal of ketosis without change in hepatic production of acetoacetate. The sequence of events in fasting can thus be considered as *1*) carbohydrate deprivation, *2*) primary stimulation of AAA synthesizing enzymes in the liver, and *3*) impaired peripheral uptake of AAA.

The Fd Fragment: A Third Structural Unit of G Immunoglobulins? E. C. FRANKLIN * AND B. FRANGIONE, New York, N. Y.

Abundant evidence indicates that IgG consists of two heavy and two light polypeptide chains. However, the occurrence of Fc fragments in "heavy chain disease" and some genetic studies in rabbits suggest that the Fc and Fd fragments, which together constitute the "heavy chain," may be separate structural units. Since Fd fragments have not been isolated, their composition must be analyzed indirectly either by comparing peptide maps of heavy chains and Fc fragments, or Fab fragments and light chains. Heavy chains and Fc fragments from 12 We(b) and 3 Vi(c) G myelomas and Fab fragments of 6 of the former and 2 of the latter were examined. Peptide maps of Fc fragments from proteins in each class were strikingly similar and differed only in an occasional peptide in addition to those related to Gm type. Normal heavy chains had 7 additional peptides; those from myeloma proteins had 5 to 14 of which 3 to 7 were present normally. The group of Fd fragment peptides was unique for each protein although many were present in several proteins. Most of these spots (5 to 10) were also present in the Fab fragments. Immunologic studies also demonstrated antigenic specificity in Fd fragments from 3 proteins. Similar variations were also present in 7 peptides in heavy chains from 15 macroglobulins. This variability in the Fd fragments of myeloma proteins and probably also antibodies, together with the constancy of the Fc fragments, suggests that the Fd fragment contributes to the heterogeneity among different G immunoglobulins and supports the concept that it may be a distinct structural unit, possibly under separate genetic control.

Estrogen Stimulation of Growth Hormone Release. ANDREW G. FRANTZ AND MITCHELL T. RABKIN, Boston, Mass. (introduced by Anne P. Forbes †).

Suppression of pituitary growth hormone production by large doses of estrogens has been suggested as a rationale for their empirical use in acromegaly and certain malignancies. To test this possibility a normal male was given

large doses (50 mg per day) of diethylstilbestrol for a month, and plasma growth hormone (HGH) response to insulin hypoglycemia was tested at approximately weekly intervals. The normal rise of HGH, without apparent suppression, occurred in all tests. Very high initial fasting HGH levels (7.4 to 24 mμg per ml), however, were noted at the start of each test during estrogen administration. Fifteen similar control determinations while off estrogens were <1 mμg per ml. Subsequently, four normal males were given diethylstilbestrol, 50 mg per day, for 7 to 10 days. Control HGH determinations were made beforehand and on the last days of treatment, in the fasting state on arrival at work ("ambulatory"). To evaluate the effect of exercise, basal specimens on awaking were also obtained on each test day. (Our normal range for males is <1 to 3, ambulatory, and <1 mμg per ml, basal.) Fifteen basal specimens were all low, <1 mμg per ml, before and after estrogens, except for one value of 4.2 mμg per ml. The ambulatory specimens showed low HGH levels, <1 mμg per ml, during the control periods, but marked elevation after estrogen administration (mean 14, range 4.2 to 30 mμg per ml, seven specimens). Glucose administration produced normal suppression of elevated values. Preliminary tests with smaller diethylstilbestrol doses (5 mg per day) have yielded similar results. Two women were followed throughout menstrual cycles with daily basal and ambulatory HGH determinations. Basal values were all low. Ambulatory levels, as has been noted with other women, were higher than in males, and a distinct postovulatory rise in HGH occurred. It is suggested that exogenous and endogenous estrogens act to enhance the normal stimulation of HGH release caused by physical activity.

Cystathionine Excretion. GEORGE W. FRIMPTER,* JAMES C. STRICKLER, AND HOWARD G. WORTHEN, New York, N. Y.

Cystathionine is unique among amino acids in that it is cleared to a high degree, has a reabsorptive Tm, and net tubular secretion can be demonstrated. Cystathionine excretion was studied in two unrelated patients with homozygous cystathioninuria, one patient with heterozygous cystathioninuria, in cystinuria, in normal subjects, and in dogs. Excretion of cystathionine is not increased in cystinuria; and the excretion of lysine, arginine, and ornithine is normal in cystathioninuria. In the dog, cystathionine clearance was high and reabsorption was rate limited. Amino acids of the lysine-ornithine (cystinuric) group markedly inhibited tubular reabsorption of cystathionine, suggesting that they share a transport mechanism. However, other amino acids, e.g., alanine, also inhibited reabsorption to some extent. In several instances, following administration of the inhibiting amino acid, cystathionine clearance exceeded that of inulin. This was observed in dogs and in one patient after intravenous administration of lysine. L-Cystathionine-H^3 was injected into a renal artery, and multiple small specimens of urine were collected from both ureters. Twenty minutes later injection of nonradioactive lysine into the renal artery was followed by a sharp secondary peak of radioactivity. Lysine given intravenously after the renal arterial injection of cystathionine-H^3 also demonstrated displacement from the injected kidney. Tissue analyses also demonstrated that lysine displaced cystathionine from the kidney. It was further shown that renal arterially administered lysine-C^{14} was displaced by the subsequent intravenous injection of arginine, suggesting that this may be a more general phenomenon.

Metabolic Implications of Renal Gluconeogenesis in Rate Control of Ammonia Synthesis. ROBERT E. FUISZ, A. DAVID GOODMAN, DONALD E. KAMM, GEORGE F. CAHILL, JR.,* AND ALEXANDER MARBLE,† Boston, Mass.

Previous investigations in this laboratory demonstrated increased gluconeogenesis (GNG) by kidney cortex slices from adrenalectomized rats given NH_4Cl and decreased GNG by slices from rats given $NaHCO_3$. The present study was designed to evaluate the direct effect of medium pH on renal GNG. Renal slices from normal rats were incubated in a glucose-free Krebs-Ringer buffer containing an appropriate precursor substrate and equilibrated with 5% CO_2:95% O_2. Bicarbonate concentrations were varied to produce acid (pH 7.1), normal (pH 7.4), and alkaline (pH 7.7) media. Glucose syntheses from glutamine, glutamate, α-ketoglutarate, oxaloacetate, and dihydroxyacetone were all significantly increased at pH 7.1 and depressed at pH 7.7. Similar acute alterations in GNG were observed by altering pH in perfused rabbit kidneys by changes in P_{CO_2}. In addition, the marked augmentation of renal GNG observed *in vitro* in slices from fasted or alloxan-diabetic rats could be suppressed by previous $NaHCO_3$ administration *in vivo*. Thus renal GNG appears to be more intimately related to alterations in acid-base balance than to hormonal or caloric metabolic control. The results suggest an immediate primary effect of pH on renal GNG independent of *in vivo* induction of enzyme activity. Also, the theory of primary control of NH_4^+ synthesis by glutaminase is inconsistent with the observation that acidosis acutely increases GNG from substrates far beyond the glutaminase enzyme system. The data support the primary effect of acidosis on accelerating the rate-limiting steps in GNG, and secondarily increasing NH_4^+ synthesis due to removal of product inhibition, such as glutamate. These data also suggest that in animals with elevated NH_4^+ production, renal glucose production may account for a major proportion of total glucose synthesis.

Excretion of Catecholamine Metabolites by Normal Children and Those with Familial Dysautonomia. STANLEY GITLOW, MILTON MENDLOWITZ,† ELIZABETH KRUK WILK, ROBERT WOLF, AND JOHN GLICK, New York, N. Y.

Neural crest tumors of childhood such as ganglioneuroma, pheochromocytoma, and neuroblastoma are usually

associated with abnormalities in catecholamine (CA) metabolism. Detection of CA-secreting tumors of childhood and proof of defective CA metabolism in dysautonomic children have been hampered by the use of relatively nonspecific techniques for the measurement of the high and variable excretion of CA metabolites by normal children. Excretion of vanillylmandelic acid (VMA), the metanephrines (NM), and homovanillic acid (HVA) by 106 normal subjects from 3 weeks to 18 years of age and by 40 dysautonomic children from 18 months to 21 years of age was measured by bidirectional paper chromatography (VMA and HVA) and a modification of the vanillin procedure of Pisano (NM). The paper chromatographic technique for measuring VMA and HVA is adequately sensitive and more specific than other assay procedures. The problem of incomplete urine collection was bypassed by expression of metabolite excretion as micrograms per milligram creatinine. Mean (and range) excretion of these metabolites varied with age, as follows: under 12 months = 7.0 (1.4 to 15) VMA and 1.48 (0.01 to 4.55) NM; 12 through 24 months = 4.6 (1.3 to 8) VMA and 1.37 (0.27 to 2.17) NM; 25 to 60 months = 4.3 (1.5 to 7.5) VMA, 0.91 (0.01 to 2.3) NM, and 7.3 (4 to 12) HVA; 5 to 10 years = 2.3 (0.5 to 5.5) VMA, 1.31 (0.43 to 2.7) NM, and 4.9 (0.5 to 8.5) HVA; 10 to 15 years = 1.9 (0.3 to 3.3) VMA, 0.86 (0.01 to 1.87) NM, and 2.1 (0.5 to 12) HVA; over 15 years = 1.3 (0.2 to 2.8) VMA, 0.21 (0.01 to 0.67) NM, and 1.0 (0.5 to 2.0) HVA. Hence, normal adult CA metabolite excretion was not achieved until after 15 years of age. Forty dysautonomic children excreted normal quantities of NM, but lower amounts of VMA and higher amounts of HVA than the normal controls at each age level. This suggests an abnormality in the distribution and perhaps storage of CA in familial dysautonomia.

Micropuncture Study of the Effects of Acute Reductions in Glomerular Filtration Rate on Sodium and Water Reabsorption in the Proximal Tubules. SHELDON GLABMAN, HAGOP S. AYNEDJIAN, AND NORMAN BANK, New York, N. Y. (introduced by A. B. Gutman †).

The factors regulating sodium reabsorption by the proximal tubules are incompletely understood. It has been demonstrated that when the filtered load of sodium is increased acutely in rats, the absolute amount of sodium reabsorbed by the proximal tubules rises proportionately. In spite of this demonstrated capacity for the reabsorption of increased quantities of sodium, some feature of proximal transport appears to prevent the complete reabsorption of sodium when the filtered load is reduced. The present micropuncture experiments were carried out to study the effect of acute reductions in GFR on sodium and water transfer by the proximal tubule. Glomerular filtration rate was reduced in rats by constriction of the abdominal aorta above the origin of the renal arteries. The fall in GFR per kidney ranged from 10 to 66% of control with a mean fall of 40%. Filtration rate was also measured in individual nephrons, and the average reduction of 30% was comparable to the reduction of over-all GFR. Thus, there was no evidence for loss of function in some nephrons while others remained intact. With the reductions in GFR, the TF/P inulin ratios (water reabsorption) remained essentially the same as under control conditions. Tubular fluid sodium concentrations during aortic constriction were also found to be the same as under control conditions (TF/P sodium ratios averaging 0.98 ± 0.3). These observations indicate that the absolute rate of reabsorption of sodium and water decreased in direct proportion to the fall in GFR. The results support the view that proximal sodium transport is regulated to a large degree by the supply of sodium to the sites of active transport. The adaptive reduction in salt transfer, in proportion to the decrease in supply, helps to explain the continued delivery of sodium to distal nephron sites and continued urine flow when the filtration rate is reduced.

Studies of the Renal Concentration Defect in Thyrotoxicosis. HAYDEN GLATTE, RALPH E. CUTLER, AND J. THOMAS DOWLING,* Seattle, Wash.

An impaired renal concentrating ability in thyrotoxicosis has been both suggested and denied. The problem was investigated by doing paired studies in eight patients and six normal subjects during both the thyrotoxic and eumetabolic states. Acute hyperthyroidism was induced in the normal subjects with L-triiodothyronine, 5 to 7 μg per kg daily, for an 8- to 10-day period. Eumetabolism was achieved in the thyrotoxic patients with radioactive iodine. Renal and cardiac hemodynamics were measured by standard techniques. Normal subjects during induced hyperthyroidism had an average increase of 20% in GFR and RPF, and the cardiac index increased 32%. No significant consistent change in renal hemodynamics between the two metabolic states was noted in the patients although most showed a significant reduction in cardiac index (29%) following treatment. Maximal urinary osmolality (U_{max}) was determined after 24 to 36 hours of dehydration plus exogenous vasopressin. Hypertonic mannitol was then administered and $T^c_{H_2O}$ measured at varying levels of C_{osm}. There was no significant difference in $T^c_{H_2O}$ in either group between the two metabolic states, but a significant decrease (mean 20%) in U_{max} was observed in all patients and in four normal subjects while hyperthyroid. This decrease in renal concentration was not due to hypercalcemia, bacteriuria, kaliopenia, decreased urinary urea, polydipsia, or increased solute excretion. These data confirm the impairment of renal concentrating ability in thyrotoxicosis. The decrease in U_{max} with normal $T^c_{H_2O}$ has also been seen in sickle cell disease in children and hepatic cirrhosis. The defect is probably due to a decrease in medullary hypertonicity sufficient to affect U_{max} without significantly impairing $T^c_{H_2O}$. In the case of thyrotoxicosis this could be due to an increased medullary blood flow.

The Effect of Cold Exposure on Plasma FFA Levels in Lean and Obese Subjects. JOSEPH A. GLENNON AND WOLFGANG J. BRECH, Madison, Wis. (introduced by Edgar S. Gordon †).

After an overnight fast of 10 to 12 hours 42 healthy normal weight controls (134 ± 3 SEM pounds; 21 male) and 26 obese (210 ± 10 pounds; 11 male) subjects in light clothing were seated in a room at 4 to 6° C for 30 minutes. Blood was drawn initially and after 30 minutes for determination of plasma FFA by the Dole method. The same procedure was carried out on 6 control subjects at room temperature. Subjects who shivered during cold exposure were excluded. Plasma FFA did not rise after 30 minutes at room temperature, but in the cold rose to 480 ± 29 to 611 ± 33 μEq per L in control and 539 ± 44 to 632 ± 40 μEq per L in obese subjects. Ten control and 6 obese subjects had no rise after cold exposure. In 12 controls (6 male) a polyethylene catheter was inserted in an antecubital vein, and a resting blood specimen was drawn after 30 minutes. Subsequently, blood was drawn at 5-minute intervals during 30 minutes of cold exposure. Plasma FFA level consistently fell during the first 5 minutes before rising and in 4 subjects never reached resting levels. On separate days 6 control (3 male subjects with a good response to cold (324 ± 69 μEq per L) received 200 mg of nicotinic acid or 5.0 mg of Propranolol. Both drugs eliminated the previous response. Exposure to cold for 30 minutes caused a rise in plasma FFA in most subjects. This response was biphasic with an early decrease and a subsequent rise in plasma FFA level. This rise appears due to catecholamine stimulation. There was no significant difference in response of lean and obese subjects to cold.

Correlation of Viral Invasion with Hepatic Dysfunction in Experimental Canine Hepatitis. DAVID J. GOCKE, RUDOLF PREISIG, THOMAS Q. MORRIS, JOSEPH G. SWEETING, AND STANLEY E. BRADLEY,† New York, N. Y.

Correlation of viral invasion with hepatic dysfunction has not been possible in human hepatitis because of lack of essential virologic techniques. Canine hepatitis, however, is a recognized adenoviral disease that provides an analogous system suitable for such correlative studies. Beagle puppies (5 to 7 kg), preimmunized against distemper and leptospirosis, were infected parenterally with 300 $TCID_{50}$ of virus. Daily hepatic biopsies, routine biochemical tests, serologic studies, and determinations of Bromsulphalein (BSP) storage capacity (S) and transport maximum (Tm) were carried out. In dogs without detectable antiviral antibody, the disease terminated in death after 5 to 7 days. Viremia was demonstrable by day 2 to 3, associated with fever (Δ2–4° F) and accumulation of inflammatory cells in hepatic sinusoids and virus in Kupffer cells (fluorescein-labeled and antiviral antibody). Subsequently, foci of hepatocellular necrosis containing virus developed, and elevations of SGOT appeared. BSP S and Tm were decreased from controls of 13.3 ± SD 0.7 to 8.8 ± SD 2.7 for S, and 2.91 ± SD 0.26 to 1.96 ± SD 0.30 for Tm, whereas the Tm/S ratio remained normal. This depression was proportional to cellular destruction by histologic examination. Death occurred 48 to 72 hours after appearance of viremia. At autopsy extensive hepatic necrosis was present. By contrast, in the occasional dog with low levels of antibody, a more prolonged course was observed. Small necrotic foci containing virus were found in the liver after 3 to 4 days. Minimal functional derangement was noted at this time, and the lesions appeared to regress, but after 2 to 3 weeks, extensive hepatic necrosis developed together with inflammatory infiltrates in portal areas and about central veins. Virus was no longer demonstrable in the liver. S and Tm decreased markedly. These findings make it possible to relate functional abnormalities to viral invasion and extent of hepatic damage, and demonstrate the importance of immunity in modulating the disease. The mechanism of the delayed hepatic necrosis in dogs with low antibody titers requires further study.

Specific Antigenic Similarity between Malignant Adult and Normal Fetal Tissues of the Human Digestive System. PHIL GOLD AND SAMUEL O. FREEDMAN, Montreal, Canada (introduced by Douglas G. Cameron †).

To demonstrate the existence of tumor-specific antigens in adenocarcinomata of the human colon, corresponding tumor-specific antisera were prepared by two techniques: *a*) antiserum produced by rabbits immunized with an extract of pooled human colonic cancers was absorbed with an excess of pooled normal colonic tissue components and with pooled human plasma, and *b*) rabbits made immunologically tolerant to pooled normal colonic tissue in neonatal life were immunized as adults with an extract of pooled colonic cancers. Antibodies directed against the same qualitatively tumor-specific antigens were demonstrated in both types of antisera by the techniques of double diffusion in agar gel, passive cutaneous anaphylaxis, immunoelectrophoresis, hemagglutination, and immunofluorescence. The tumor-specific antigens were also detected in individual specimens of human colonic cancers. To avoid confusion between individual-specific antigens and tumor-specific antigens, normal and tumor tissues were obtained from the same donors at operation. Antigenic components identical to those found in colonic cancers were demonstrated by the technique of precipitin-inhibition in specimens of primary cancers of the human rectum, duodenum, stomach, esophagus, and pancreas. These antigenic components were absent from all other adult malignant or normal tissues tested. However, human fetal gut, liver, and pancreas obtained between 2 and 6 months of gestation were shown to contain the same antigenic components found in cancers of the digestive organs. These findings suggest that the demonstrated tumor-specific antigens probably arise during malignant transformation as a consequence of a reversion of the entodermally derived epithelial cells of the human digestive system to a more primitive dedifferentiated state.

The existence of these system-specific "carcinoembryonic" antigenic components has not been demonstrated previously. Their function in normal fetal or malignant adult epithelium remains speculative.

Effects of Intrarenal Infusions of Bradykinin and Acetylcholine on Renal Blood Flow in Man. L. I. GOLDBERG,* C. T. DOLLERY, AND B. L. PENTECOST, London, England, and Atlanta, Ga.

Acetylcholine and bradykinin were infused into a renal artery of patients during diagnostic procedures, and effects on ipsilateral renal venous blood flow (RBF) were determined by a constant infusion, indicator-dilution technique. The vasodilators were compared in two hypertensive patients. In one, infusion of acetylcholine, 500 μg per minute, increased RBF from 210 to 469 ml per minute; bradykinin, 2.8 μg per minute, increased flow to 455 ml per minute. In the other, acetylcholine, 130 μg per minute, increased RBF from 334 to 786 ml per minute; bradykinin, 1.5 μg per minute, increased flow to 647 ml per minute. Dose-response relationships were studied in four patients. In a malignant hypertensive, acetylcholine, 200 μg per minute, increased flow from 278 to 489 ml per minute; similar increments, however, occurred with infusion rates of 25 and 100 μg per minute. RBF of a less severe hypertensive increased from 700 to 1,249 ml per minute with acetylcholine, 25 μg per minute, but 250 μg per minute decreased RBF below control values. Graded increases in flow were observed in two patients with bradykinin using infusion rates of 0.14, 0.42, and 1.4 μg per minute. In one of these patients, an oliguric nephrotic, RBF increased from 299 to 580 ml per minute and in the other, a hypertensive, from 587 to 1,235 ml per minute with the 1.4 μg per minute infusion rate. Unaccountably, bradykinin was ineffective in a patient with hypertension caused by unilateral renal disease, whereas acetylcholine increased RBF from 296 to 528 ml per minute. Flow returned to control value within 10 to 20 minutes of ceasing the drug infusion. Systemic actions were not observed. These results indicate that intrarenal infusions of acetylcholine and bradykinin can increase RBF of patients with hypertension or renal disease and that the constant infusion, indicator-dilution technique can be usefully employed in such patients.

Urea Accumulation in Renal Medulla of the Dog; Evidence for Uphill Movement. MARTIN GOLDBERG AND MANUEL A. RAMIREZ, Philadelphia, Pa. (introduced by J. Russell Elkinton †).

If all urea transport in the canine renal medulla is passive, then urea movement is always secondary to water movement, and final urine urea concentration (U_{Urea}) is never lower than renal papillary tissue urea concentration (P_{Urea}). We have devised a system to study renal accumulation of urea in the absence of a medullary electrolyte gradient, utilizing ethacrynic acid (ECA). Experiments were performed on hydropenic dogs receiving Pitressin; pelvic urine was collected from each kidney. During stable flow (V), both kidneys were removed and rapidly frozen. Slices from cortex, outer medulla, outer papilla, and papillary tip were analyzed for urea, H_2O, Na^+, K^+, and NH_4^+. Series 1: maximal priming and sustaining doses of ECA were given, and after a stable V and urinary isotonicity were achieved, 8% urea was infused to increase U_{Urea} to various levels. In some studies, V was lowered by bleeding. Series 2: ECA was given without urea. Series 3: 10% mannitol diuresis was instituted first, instead of ECA, and at high V, 8% urea was substituted for mannitol. Protocols 1 and 3 produce initial medullary urea depletion followed by controlled repletion. Data were compared to nondiuretic controls. In mannitol-urea experiments, urine:papilla electrolyte gradient was similar to controls (—76 mmoles per L), whereas $U_{Urea} - P_{Urea}$ (ΔU) fell to +6 mmoles per L (control ΔU + +185 mmoles per L). In all 10 studies with ECA, at levels of U_{Urea} varying from 35 to 205 mmoles per L, and at levels of V from 0.5 to 17 ml per minute: *1*) intramedullary [Na] + [K] was isotonic to plasma in all slices. *2*) A definite intramedullary urea gradient was still present. *3*) P_{Urea} was always greater than U_{Urea} ($\overline{\Delta U} = -20$ mmoles per L, range −5 to −43 mmoles per L, $p < 0.001$). Thus urea transport and accumulation in the canine renal medulla can occur without hypertonic sodium transport, and urea can leave the collecting duct against a concentration gradient. These data are incompatible with simple passive urea movement.

Variations in Dietary Protein and Sodium and the Solute Composition of the Urine in Man. CARL GOLDSMITH, CARLOS BARCENA, MICHAEL JOHNSON, AND GEORGE E. SCHREINER,* Washington, D. C.

The inverse relationship between urinary sodium concentration (U_{Na}) and urinary urea concentration (U_{Urea}) during hydropenia in man has been attributed to a fixed maximal urinary osmolality (U_{osm}) requiring competition by solutes for excretion. To extend this observation, six normal male volunteers were fed isocaloric diets in which the sodium and protein contents were independently varied as high, moderate, and low. After 4 days on each of the dietary combinations, urine collections were made during maximal hydropenia and then during maximal water diuresis. During hydropenia, maximal U_{osm} increased with increasing dietary protein. U_{Urea} at any given protein intake was inversely related to sodium intake, and conversely U_{Na} at any given sodium intake was inversely related to protein intake. The relationship of U_{Urea} to nonurea solute concentration was likewise inverse. These studies indicate that U_{Urea} and U_{Na} are functions of both protein and sodium intake. This relationship is not altered by water diuresis and is therefore not a phenomenon of hydropenia. This finding disputes the primacy of an osmotic ceiling. Alterations in dietary sodium and protein change the filtered load of urea and hence medullary urea concentration. This in turn could, by altering water reabsorption from the descending limb of the loop of

Henle, change sodium reabsorption by the gradient sensitive pump in the ascending limb. The result would be a simultaneous decrease in U_{Na} and increase in U_{osm} during high protein feeding and the opposite during protein restriction.

Treatment of Hypercalcemia with Small Phosphate Supplements. RALPH S. GOLDSMITH AND SIDNEY H. INGBAR,* Boston, Mass.

Current treatment of hypercalcemia is not entirely satisfactory because of variable effectiveness, transiency, and inherent toxicity. Albright and co-workers demonstrated that oral phosphate supplements alleviated the hypercalcemia of patients with hyperparathyroidism. This led us to evaluate the effect of phosphate in 12 patients with hypercalcemia (12 to 22 mg per 100 ml) due either to malignancy, with or without metastases to bone, or to myeloma. Symptoms of hypercalcemic crisis were often present. When possible, phosphate was administered orally, 1.2 to 3.1 g P (as Na_2HPO_4) daily. In some patients, coma or persistent vomiting necessitated intravenous infusion (3.1 g P as Na_2HPO_4-KH_2PO_4, pH 7.4, given over 6 hours, once daily). Although several patients were refractory to other therapy, including adrenocortical steroids, phosphate consistently induced a sustained reduction of serum calcium to normal or near normal, but not to subnormal, values. This effect was rapidly evident and was associated with abolition of hypercalciuria. Reversal of hypercalcemia was accompanied by relief of symptoms of calcium intoxication, correction of electrocardiographic abnormalities, and in several instances, lowering of elevated values of serum creatinine and BUN. In two patients, initial hyperphosphatemia associated with azotemia was abolished; in the others, hyperphosphatemia was not induced. Several patients showed sustained chemical and clinical improvement following one or two intravenous infusions, so that retreatment was necessary at only weekly intervals. Adverse clinical reactions to phosphate were not seen. Similar clinical and chemical responses to phosphate were obtained in five patients with hyperparathyroidism. At necropsy, one phosphate-treated patient with malignancy and long-standing hypercalcemia displayed renal calcification. In two others, nephrocalcinosis was not seen. Thus, oral or intravenous administration of relatively small amounts of phosphate appears to be highly effective in the acute treatment of hypercalcemia and its direct sequelae in patients with malignancy, myeloma, or hyperparathyroidism.

Prevention of Alcohol Hypoglycemia by Insulin Pretreatment. M. S. GOLDSTEIN AND J. ZINDER, Chicago, Ill. (introduced by L. N. Katz †).

Ethyl alcohol has been shown to produce hypoglycemia in the fasting but not in the fed state. The preventive effect of feeding has been attributed to the glucose derived from the foodstuff. However, it can be postulated that the event of eating per se, with insulin release serving as the signal, may prevent a shift in the metabolic economy from protein-carbohydrate dominance with very active regulation of blood sugar to the predominant fat metabolism of fasting. Dogs were fasted for 5 days and then infused with 15% ethanol in isotonic saline at 2 ml per minute. As reported by others, blood sugar levels fell to 20 or 30 mg per 100 ml over a 2- to 3-hour period with consequent hypoglycemic death. Nonfasted animals showed no such hypoglycemia. Animals fasted for the same 5-day period, but receiving, subcutaneously, $\frac{1}{20}$ U per kg of crystalline zinc insulin once a day instead of food, behaved as fed animals in accepting the infusion of ethyl alcohol without hypoglycemia. The fasted animals lost 2 to 5% of body weight over the 5-day period. By contrast, the fasted animals receiving daily injections of insulin lost 7 to 20% of body weight during the same period. This marked increase in weight loss is consistent with the view that the daily administration of insulin maintains the animals in a state of high carbohydrate turnover with protein used for gluconeogenesis as the primary fuel rather than fat. The lesser caloric value of protein and the higher associated content of water and electrolytes would lead to a larger weight loss over the same period. It is concluded that insulin, in nonhypoglycemic amounts, serves as a metabolic signal in preventing the shift from carbohydrate and protein to fat as the major fuel in fasting, thereby resisting the hypoglycemic effects of alcohol infusion.

Disorders of Calcium Metabolism in Uremia. HARVEY C. GONICK, JACQUES J. HERTOGHE, AND MILTON E. RUBINI, Los Angeles, Calif. (introduced by Morton I. Grossman *).

Previous studies have suggested a diminished intestinal absorption of calcium in uremia. We have further explored this problem utilizing a combination of conventional balance techniques and intravenous Ca^{47} as described by Aubert and Milhaud. Six patients with chronic renal disease, serum creatinines ranging from 2.0 mg per 100 ml to 16.2 mg per 100 ml, were studied. The diagnoses were gouty nephropathy (1), chronic glomerulonephritis (3), and polycystic kidney disease (2). The two patients with the most severe renal impairment also had extensive metastatic calcification. Serum calcium levels were normal in these two patients but reduced in two others with moderately severe azotemia. All patients showed normal intestinal absorption but increased endogenous secretion of calcium, accounting for the high fecal values previously described. The patients with metastatic calcification demonstrated a normal urinary excretion of calcium, increased exchangeable calcium pool size, and elevated values for bone accretion and resorption. In contrast, the remaining patients showed a markedly diminished urinary excretion of calcium, low or normal pool size, and low or normal accretion and resorption rates. The abnormalities of calcium metabolism in uremia may therefore be characterized as follows: *1*) low urinary excretion, *2*) normal intestinal absorption, and

3) increased endogenous secretion of calcium. We would also speculate that when parathyroid activity becomes markedly enhanced as a result of hyperphosphatemia or hypocalcemia or both, calcium is mobilized from bone (as evidenced by high resorption rates), and blood and urinary levels return to normal. The resultant high Ca $\times$ PO_4 product then may lead to metastatic calcification.

The Electrical Nature of Coupled and Uncoupled Transport of Sodium and Chloride in Reptilian Bladder. C. F. GONZALEZ, D. E. GENTILE, AND W. A. BRODSKY,† Louisville, Ky.

The electrical behavior of the turtle bladder mimics that of two oppositely oriented EMF (E_{Na^+} and E_{Cl^-}) coupled electrically in parallel through ion-selective paths in the membrane. Such behavior was elicited under conditions of short-circuiting of bladders bathed by sodium-free and sodium-rich mucosal fluids. When the mucosal surface was bathed by choline chloride, 110 mM, and serosal surface, by choline Ringer's, serosal fluid was 20 to 60 mv negative to mucosal, and short-circuiting current (Isc), was —20 to —50 μa. Replacement of mucosal choline Cl by equimolar $NaCH_3SO_4$ caused reversal of orientation of the potential (P.D.) and Isc, which reached values of 90 to 100 mv and +250 μa, respectively. Replacement of mucosal $NaCH_3SO_4$ by equimolar NaCl decreased P.D. to 70 to 75 mv and Isc to 200 μa. The decrease of P.D. and Isc occasioned by replacing $NaCH_3SO_4$ by NaCl was reversible and was quantitatively equal to the magnitude of P.D. and Isc with mucosal choline Cl. If we assume that Isc with mucosal choline Cl is carried by Cl^- alone and that Isc in $NaCH_3SO_4$ is carried by Na^+ alone, then Isc (NaCl) = (NaCH$_3$SO$_4$) — Isc (choline Cl), and Isc (NaCl) = I (Na^+) — I (Cl^-), provided that there is constancy of the membrane elements through which external electric current must pass. Additivity of ion-selective electrical currents was found in seven consecutive experiments. Additivity of currents remained intact, whereas mucosal pH was varied from 3 to 9 in one case. This suggests that the transport force for Cl^- is independent of and superimposable upon that for Na^+ ion, as would be the case in a simple electrical network.

Absorption and Metabolism of Radioactive β-Carotene and Vitamin A in Man. DEWITT S. GOODMAN,* ROLF BLOMSTRAND, BENGT WERNER, HELEN S. HUANG, AND TATSUJI SHIRATORI, New York, N. Y., and Stockholm, Sweden.

Tritium or C^{14}-labeled β-carotene, vitamin A alcohol, and vitamin A acetate were fed to patients in whom polyethylene cannulae had been implanted in the thoracic duct in the neck. Serial samples of lymph were collected, and the lipid was extracted and chromatographed on columns and on thin-layer plates of alumina. Absorption of labeled derivatives mainly occurred between 3 and 10 hours. During this time washed chylomicrons contained three-fourths of the absorbed radioactivity. Labeled vitamin A esters predominated in all lymph samples and represented 90% of the absorbed radioactivity after the feeding of vitamin A and 70% after β-carotene. The fatty acid composition of the vitamin A esters in lymph was remarkably constant, regardless of the fatty acid composition of the diet, regardless of whether chylomicron or nonchylomicron lipid was analyzed, and regardless of whether the vitamin A esters were derived from dietary vitamin A or from β-carotene. Vitamin A palmitate predominated in all samples, and saturated esters (vitamin A palmitate + stearate) consistently comprised 75 to 85% of the labeled esters. Small amounts of vitamin A oleate and linoleate were also found in all samples. Unchanged labeled β-carotene comprised only one-fourth the absorbed radioactivity, after the ingestion of H^3-β-carotene. The biosynthesis of vitamin A, from β-carotene, in man therefore mainly takes place in the intestine, during the absorption of dietary β-carotene.

Studies on the Presence of Renal Pressor Material in Various Hypertensive Diseases. WARREN E. GOORNO AND NORMAN M. KAPLAN, Dallas, Texas (introduced by Carleton B. Chapman †).

This study was designed to examine the validity of the angiotensin infusion test as an index of endogenous levels of pressor material in various forms of hypertensive disease and to elucidate the mechanism of hypertension in acute and chronic renal parenchymal disease. Renal venous blood from 45 hypertensive patients was independently assayed for pressor material by the method of Grollman, and, in some patients, aldosterone excretion and plasma volume were measured. Patients with essential hypertension had a 20 mm Hg rise in diastolic blood pressure with less than 5.0 mμg per kg per minute of angiotensin and did not have significant levels of pressor material in the renal venous blood. Patients with renovascular hypertension, malignant hypertension, and some with accelerated hypertension required 6.0 mμg per kg per minute or more of angiotensin and had significant levels of pressor material in the renal venous blood from one or both sides. All 35 patients with chronic renal parenchymal disease and hypertension were sensitive to less than 5.5 mμg per kg per minute of angiotensin, and pressor assays were negative in all 12 so tested. Aldosterone excretion and plasma volume were not elevated in such patients. All 4 patients with acute glomerulonephritis were sensitive to less than 5.5 mμg per kg per minute of angiotensin, and the pressor assay was positive in only one. Of 12 patients with renal vascular lesions who were sensitive to less than 5.5 mμg per kg per minute of angiotensin, pressor assays were negative in all 8 tested, and surgical repair was unsuccessful in all 7 subjected to operation. The data suggest that *1*) the angiotensin infusion test is a valid index of circulating pressor material, *2*) hypertension seen in acute and chronic renal parenchymal disease is usually not related to the release of pressor material from the kidney, and *3*) the functional significance of renal vascular lesions can be ascertained in order to select those amenable to surgical repair.

The Long-Term Stability of Triglyceride Molecules in Adipose Tissue. ENOCH GORDIS, New York, N. Y. (introduced by Vincent P. Dole†).

During transport of triglycerides from intestine to blood and from blood to adipose tissue, hydrolysis and re-esterification take place. Yet it may be asked whether triglycerides stored in adipose tissue are in a dynamic state. Do hydrolysis and re-esterification continue to occur in the depots, or do triglyceride molecules, once deposited, remain intact? This question was studied by feeding rats different oils in sequence and determining whether fatty acids from the different oils were incorporated into the same triglyceride molecules. Rats were fed a diet containing coconut oil as the sole fat for the first 3 weeks, safflower oil as the sole fat for the next 3 weeks, and triolein as the sole fat for the final 9 weeks. Lauric and myristic acids occur only in coconut oil, and linoleic acid only in safflower oil. None of these acids are synthesized to any significant extent by adipose tissue. The extent of mixing in triglyceride stores during the final weeks was determined from the composition of two groups of triglycerides isolated from adipose fat by chromatography on $AgNO_3$-impregnated silicic acid. *1*) Monosaturated-dilinoleins: This group of glycerides occurs in safflower oil, but not in coconut oil. Any incorporation of lauric and myristic acids into this group must represent mixing of triglycerides in adipose tissue. Since monoenoic acids are absent from this glyceride group, its composition is unaffected by triolein feedings. After the coconut and safflower oils had been fed, only a small proportion of lauric and myristic acids was present in this group compared to the whole fat. During the next 9 weeks, little penetration of short-chain acids into this group occurred. *2*) Oleodilinoleins: The proportions of the positional isomers, 1-oleodilinolein and 2-oleodilinolein, deposited during the safflower oil feedings, changed only slightly during the final 9 weeks. These findings suggest that most of the triglyceride molecules, after deposit in adipose tissue, remain intact until mobilized for release.

A Moving Source Phenomenon: The Result of Slow Passage of Label across the Red Cell Membrane. CARL A. GORESKY, Montreal, Canada (introduced by Francis P. Chinard†).

The present experiments are designed to examine the form of the indicator dilution curve resulting when a substance that passes slowly through the red cell membrane is injected into a well-defined system, the hepatic circulation of the dog. Thiourea was selected as a suitable test substance. Labeled red cells (which are confined to the vascular space), labeled water (which enters and leaves red cells and liver cells rapidly), and thiourea (which enters and leaves red cells slowly and liver cells rapidly) were incubated together, so that the thiourea equilibrated within the red cells. The hematocrit of the mixture was adjusted to that of the blood in the animal. The mixture was rapidly injected into the portal vein, and serial samples of hepatic venous blood were collected. Effluent label was expressed in terms of fractional recovery per milliliter of blood. Red cells reached an early and high peak and then decayed rapidly; water reached a low later peak and decayed slowly; thiourea emerged in a biphasic pattern, a first sharp peak occurring synchronously with the red cell peak, and a second spread out peak occurring later in time than the water peak. Raising the hematocrit increased the proportion of thiourea emerging in the early peak; increasing the time of transit diminished the proportion in the early peak. The thiourea curves may be interpreted as resulting from two interacting phenomena: a red cell source, traveling with the mean velocity of flow in the hepatic sinusoid, progressively shedding label, and a delayed plasma wave which, in turn, loses label into red cells containing no thiourea. The plasma thiourea wave propagates more slowly than the water wave because the red cells are not freely accessible to the thiourea. These studies imply that red cell trapping significantly affects the extravascular distribution of label in an organ.

Effect of Vasopressin on Water Permeability of Isolated Perfused Collecting Tubules. JARED GRANTHAM, JACK ORLOFF,* AND MAURICE BURG,* Bethesda, Md.

To investigate further the mechanism of action of vasopressin, its effect on water absorption by collecting tubules *in vitro* has been tested. Unbranched portions of collecting tubules .7 to 2 mm long were dissected from cortex and outer medulla of rabbit kidneys. The isolated tubules were perfused at a constant rate between 5.4 and 30.8×10^{-9} L $minute^{-1}$ with a hypotonic solution (70 mOsm kg^{-1}, outside bath 290 mOsm kg^{-1}) including tracer albumin-I^{131}. Fluid leaving the distal end of the perfused tubule was collected in a suitable pipette, and the volume and albumin-I^{131} concentrations of successive timed collections were measured. The difference between the rate of perfusion and collection was used to calculate the rate of water absorption. Significant leak of perfusion fluid was ruled out by measurement of albumin-I^{131} recovery. Initially, fractional absorption of water was .11 to .52, depending on the perfusion rate and length of the tubule. Water absorption in the control periods averaged 4.7×10^{-3} cm^3 per cm^2 surface area per minute. Addition of Pitressin (0.025 to 100 mU per ml) to the outside bath caused water absorption to approximately double (mean increase 109% ± 27% SEM, 6 experiments). Water absorption returned to previous control values following washing with Pitressin-free solutions when the lowest concentrations of Pitressin (.025 to 2.5 mU per ml) had been used. These results directly confirm the generally accepted theory that vasopressin acts on the mammalian collecting tubule to increase water flow along an osmotic gradient. It is suggested that *in vitro* microperfusion of isolated tubule segments provides an improved means for direct study of the function of specific portions of the nephron.

The Pathogenesis of Colloid Goiter. MONTE A. GREER,* HUGO STUDER, AND ANN K. STOTT, Portland, Ore.

Although hyperplastic goiters can regularly be produced by a variety of techniques that result in chronic TSH stimulation of the thyroid, experimental production of colloid goiter has been reported only in the hamster. Since pathogenetic mechanisms for colloid goiter are currently ill-defined and highly speculative, this problem was studied in a large series of rats. We have found that statistically significant thyroid hypertrophy can be produced within 1 week of feeding a low iodine diet (LID). These goiters, however, are of the usual hyperplastic variety. Refeeding a high iodine diet (HID) supplies adequate substrate for thyroid hormone production so that TSH secretion is decreased through negative feedback. The resultant decline in thyroid weight is slower than either the reaccumulation of colloid in the follicles or the change of the thyroid epithelium from columnar to low cuboidal or squamous. Between 1 and 2 weeks of HID refeeding is required to produce characteristic changes of flat follicular epithelium and marked colloid reaccumulation. If LID is fed for 2 months or more before reinstitution of HID for 2 weeks, colloid-filled thyroids two to three times larger than normal can regularly be produced. Colloid goiter can be produced by HID whether the initial thyroid hypertrophy is produced by LID or by chronic propylthiouracil (PTU) feeding, but no change in morphology or size of the hyperplastic glands occurs if PTU is continued with the HID refeeding. Colloid goiter can also be produced by hypophysectomy of animals with hyperplastic goiters even if LID or PTU is continued. It is concluded that colloid goiter is produced by a decrease in TSH stimulation of an enlarged, hyperplastic thyroid. An increased quantity of available iodine is not essential for its development.

The Pyrogenic Refractory State during Continuous Intravenous Infusions of Bacterial Endotoxin. SHELDON E. GREISMAN * AND WILLIAM E. WOODWARD, Baltimore, Md.

Pyrogenic tolerance following repeated daily single intravenous injections of bacterial endotoxins has been studied intensively. In contrast, the pyrogenic refractory state that develops rapidly during the course of a continuous intravenous infusion of endotoxin has been virtually neglected. The importance of defining pyrogenic refractoriness stems from the likelihood of sustained endotoxinemia during gram-negative bacterial septicemia. Five healthy volunteers, seven with tularemia, one with typhoid fever, and over 150 normal rabbits received constant intravenous infusions (ranging from 18×10^{-6} to 18×10^{-2} μg per minute) of *Escherichia coli* or *Salmonella typhosa* endotoxin for 8 to 14 hours. Fever increased progressively during the initial 3 to 5 hours, then returned progressively to base line despite the continuing infusion. This pyrogenic refractory state possessed the following characteristics: *a*) reticuloendothelial blockade with Thorotrast neither prevented nor reversed its course, *b*) passive transfer was unsuccessful with refractory-phase plasma, *c*) infusion of normal plasma or fresh whole blood failed to restore responsiveness, *d*) circulating antibody titers to endotoxin remained unaltered, *e*) peripheral leukocytosis appeared, *f*) infusion of febrile-phase plasma re-evoked an immediate, monophasic fever, *g*) endotoxinemia could be demonstrated by pyrogen bioassay, *h*) tenfold increases in endotoxin infusion rates re-evoked fever, *i*) dermal inflammatory responses to endotoxin were suppressed in man while tuberculin reactivity remained unimpaired, and *j*) pyrogenic reactivity to endotoxin reappeared within 24 hours in man; refractoriness persisted in rabbits. It is concluded that the pyrogenic refractory state reflects an inability to continue to mobilize endogeneous pyrogen during sustained endotoxinemia. Such observations, together with previous studies, are consistent with two distinct immunologic mechanisms of resistance to endotoxin pyrogenicity: *1*) desensitization at the cellular level, and *2*) elaboration of circulating antibodies that assist reticuloendothelial clearance and destruction of endotoxin. Whereas both such mechanisms may contribute to pyrogenic tolerance, the characteristics of the pyrogenic refractory state suggest the participation only of the former.

Effects of pH and Theophylline on the Uptake, Elution, and Antidiuretic Action of Adenosine-3',5'-Monophosphate. PAUL F. GULYASSY AND ISIDORE S. EDELMAN,* San Francisco, Calif.

The proposal of Orloff and Handler that the antidiuretic action of the neurohypophyseal hormones is mediated by adenosine-3',5'-monophosphate (cyclic AMP) is supported by a convincing body of evidence. Little is known, however, of the mechanism of nucleotide-induced changes in resistance to osmotic flow of water. As a first step in the study of this problem, we studied the effects of two variables (i.e., pH and theophylline) on the uptake, elution, and antidiuretic action of cyclic AMP in the isolated toad bladder. The uptake of cyclic AMP-H^3 failed to show saturation kinetics. For the first 30 minutes, there was a curvilinear rise in cyclic AMP-H^3 uptake, followed by a continued linear rise in tissue radioactivity for the next 2.5 hours. At 180 minutes, the volume of distribution was 135% of tissue water at a pH of the serosal medium of 7.0 and 159% at a pH of 8.4. In contrast maximal antidiuretic activity of cyclic AMP was obtained at pH 7.0 and 70% of maximal activity at pH 8.4. Elution of cyclic AMP-H^3 (or labeled metabolites) from prelabeled hemibladders revealed a trapped component representing 40% of the total uptake, and the quantity of trapped metabolite was independent of pH over the range 7.0 to 8.4. Theophylline, an inhibitor of cyclic AMP degradation via nucleotide phosphodiesterase, inhibited uptake of cyclic AMP-H^3 by 25%, 36%, and 34% at 30, 60, and 120 minutes, respectively, but did not reduce the fraction of trapped metabolite in elution studies. Additional studies also showed that the pH optimum for the antidiuretic action of theophylline was 8.0.

Alterations in Activity of Plasma Antihemophilic Factor (Factor VIII) Induced by Brain Stimulation. C. G. GUNN AND JAMES W. HAMPTON, Oklahoma City, Okla. (introduced by Stewart Wolf †).

Many years ago Cannon showed that stimulation of splanchnic nerves in the cat would accelerate blood clotting. More recently Lechler and Penick reported reduced levels of Factors VIII and IX in hibernating ground squirrels. Both studies suggest a central regulating mechanism for blood clotting factors. The present study explores the effects of brain stimulation on the plasma levels of Factor VIII. Each of 12 dogs had from 1 to 5 permanent electrodes implanted in its brain under stereotaxic guidance. Every 3 weeks each dog was subjected either to 10-minute periods of electrical stimulation or to equivalent sham periods without stimulation. Factor VIII assays were made before electrical or sham stimulation, immediately after, and up to 60 minutes. The assay for Factor VIII used a modification of the thromboplastin generation test for mixtures of dog plasma with human hemophilic plasma. The assays were stable except immediately following the periods of electrical stimulation, when significant, transitory alterations in Factor VIII activity occurred in either direction depending upon the site stimulated. The alterations in Factor VIII were not related to changes in hematocrit, fibrinolytic activity, or prothrombin times. Brain areas in which stimulation induced an increase in Factor VIII included the mesencephalic reticular formation and the dorsomedial nucleus of the thalamus. Brain areas in which stimulation induced a decrease in Factor VIII included the field of Forel and certain hypothalamic areas. Stimulation of the hippocampus, the preoptic area, the spinothalamic tract, and superior colliculus caused no Factor VIII changes. Similarly, no changes were encountered in the 40 sham procedures. It is concluded that central neural regulatory mechanisms exist that may increase or decrease plasma Factor VIII activity in the dog.

The Mechanism of Action of Androgens on Erythropoiesis. CLIFFORD W. GURNEY * AND WALTER FRIED, Chicago, Ill.

Stimulation of erythropoiesis by testosterone in some anemias of man encouraged us to investigate the mechanism of this androgen effect. In mice whose rate of erythrocyte formation has been suppressed by transfusion, erythropoiesis increases 6- to 20-fold following multiple testosterone injections, the magnitude of the response being inversely related to the intensity of the plethora. Forty-eight hours after the second of two daily subcutaneous injections of 2.5 mg testosterone propionate, plasma of normal female mice contains an elevated titer of erythropoietic stimulating factor (ESF). However, no ESF is detected in the plasma of identically treated polycythemic mice. Following a short period of hypoxia, the increase of ESF is modest in control animals, and six to eight times greater in mice receiving testosterone. The rise in ESF following one injection of testosterone is abolished if, 7 hours before plasma collection, bilateral nephrectomy is performed. Ureter-ligated, testosterone-injected, control animals show ESF titers comparable to those of unoperated mice. Erythropoiesis is suppressed to a lesser degree in male mice by polycythemia than it is in females. Plethoric males respond to testosterone less vigorously than do females, and ESF titers in plasma of males treated with testosterone are lower than those of identically treated females. We interpret these experiments as indicating that the erythropoietic effect of androgen is the consequence of action of this hormone or one of its products upon the kidney, with the resultant elaboration of erythropoietin. For any given erythropoietic stimulus, a larger than normal amount of erythropoietin is produced in animals receiving testosterone. The degree to which androgens influence erythropoietic rates in female and male animals is demonstrated by quantitative differences in the response of the sexes coincident to plethora and also by the quantitative differences to exogenous testosterone.

The Role of Monoglyceride in Triglyceride Hydrolysis within the Fat Cell. HERBERT A. HAESSLER, Boston, Mass. (introduced by John D. Crawford *).

Lipase preparations from fat tissue exhibit low levels of hydrolytic activity against pure triglyceride substrates. Since triglyceride comprises 99% of depot lipid stores and fat is released only as free fatty acids, there is an apparent discrepancy between measurable triglyceride lipase and the facility with which intact fat cells release fatty acids. To further investigate adipose tissue lipolysis, tissue was homogenized and centrifuged at low speed to separate aqueous and lipid fractions. The fat fraction was then ultracentrifuged producing a cake with three layers. Lipolytic activity was found in the lower two, but was virtually absent from the upper oily layer using the fat as its own substrate. When each fraction was studied by thin-layer chromatography, all contained approximately equal amounts of triglyceride, but only the upper layer lacked phospholipid and probable traces of monoglyceride. It was thus possible to separate intracellular lipolytic activity from triglyceride and associate it with other lipid elements. Hydrolysis of pure trilinolein by the aqueous fraction was barely measurable although this extract contained 28% of the total lipolytic activity when heat-inactivated endogenous fat served as substrate. When monoolein was added to the system, the release of linoleic acid increased about 100-fold. Activity was unaffected by addition of coenzyme A or ATP. With tissue that had been preincubated for 1 hour, the system was stimulated by a 10-minute terminal exposure to epinephrine before homogenization. These findings suggest that for release the bulk of intracellular triglyceride is not hydrolyzed as such, but is first converted to diglyceride by an epinephrine-sensitive enzyme that transfers a fatty acid from triglyceride to monoglyceride. Diglycerides are rapidly split. The association of lipolytic activity with phospholipid suggests that this transferring enzyme

together with traces of monoglyceride is located in a subcellular particle.

Relation of Altered Hemostasis to Idiopathic Aseptic Necrosis of the Femoral Head. HENRY E. HAMILTON, MICHAEL BONFIGLIO, RAYMOND F. SHEETS, AND WILLIAM E. CONNOR,* Iowa City, Iowa.

Aseptic (avascular) necrosis of the femoral head is termed "idiopathic" when trauma has been excluded, since no other causal relations have been observed. The chance observation of excessive hemorrhage during an arthrotomy for this disorder prompted a search for associated disturbances in blood coagulation. Study of nine consecutive cases of nontraumatic necrosis of the femoral head yielded the following associated conditions: one had thrombocythemia and inconsequential bruising that developed concurrently with onset of hip pain; one had drug-induced marrow hypoplasia with thrombocytopenia; one had hyperlipidemia and reduced prothrombin consumption; two brothers had chemical findings of gout but without tophi or arthritis, one having demonstrable hypercoagulability and excessive hemorrhage during operation; two others had previous symptoms of gout and evidence of hypercoagulability; one had prolonged clotting; one had Raynaud's phenomenon, accelerated erythrocyte sedimentation, and rouleaux formation. Gout has been associated with enhanced platelet adhesiveness and increased thromboplastic activity. Reports in the literature have associated aseptic necrosis of the femoral head with sickle cell disease, congenital hemolytic anemia, caisson disease, prolonged use of adrenal cortical steroids, lupus erythematosus, and collagen disease. These disorders and those observed by us may be correlated by a hypothesis that any one of a constellation of events disturbing hemostasis by initiating thrombosis, hemorrhage, or sludging of the blood in the femoral head with its peculiarly limited blood supply can cause aseptic necrosis.

Receptor Mechanisms for the Metabolic and Circulatory Actions of Epinephrine in Man. WILLARD S. HARRIS, CLYDE D. SCHOENFELD, RICHARD H. BROOKS, AND ARNOLD M. WEISSLER,* Columbus, Ohio.

The many diverse actions of catecholamines appear to be mediated through two distinct types of adrenergic receptors, named alpha and beta. Selective beta receptor blockade by intravenous propranolol (10 mg) has made it possible to determine the specific receptors involved in the metabolic and circulatory actions of epinephrine in man. In six normal, fasting volunteers, epinephrine (5 μg per minute for 30 minutes) significantly increased oxygen consumption +30 ml per minute per m^2 (+25%) and arterial levels of free fatty acids +998 μEq per L (+218%), glucose +44 mg per 100 ml (+56%), lactate +1.06 mmoles per L (+131%), and pyruvate +0.15 mmoles per L (+83%). Beta blockade completely eliminated all these responses except for the rise of glucose (+30 mg per 100 ml or +37%). Thus, epinephrine increases oxygen consumption, lipolysis, and lactate and pyruvate production by activating adrenergic beta receptors, but mobilizes glucose, in large part, through other mechanisms. In ten normal volunteers epinephrine (5 μg per minute) significantly increased heart rate, cardiac output (indicator dilution), and stroke volume, without changing mean arerial pressure (MAP). In a clear reversal of these actions, epinephrine, after beta blockade, significantly decreased heart rate (−11 beats per minute), cardiac output (−0.62 L per minute per m^2), and stroke volume and increased MAP (+14 mm Hg). The emergence of this new pattern of hemodynamic responses to epinephrine is a consequence of its potent, but usually unapparent, alpha (vasoconstrictive) action, which was unmasked only by blocking its predominant beta activity. Except for vasoconstriction and the major component of the elevation of blood glucose, the metabolic and circulatory actions of epinephrine in man appear to be mediated through adrenergic beta receptors.

Aldosterone Secretion in Congenital Adrenal Hyperplasia. ROBERT I. HENKIN, GEORGE T. BRYAN, AND FREDERIC C. BARTTER,† Bethesda, Md.

Aldosterone secretion rates (ASR) were studied in seven patients with virilizing, salt-losing, nonhypertensive (type I) and seven patients with virilizing, nonsalt-losing, nonhypertensive (type II) congenital adrenal hyperplasia (CAH). In patients with type I CAH in sodium balance, mean ASR was 10 ± 4.4 (SEM) μg per 24 hours, significantly below normal, compared to seven "control" patients of similar age (mean ASR, 104 ± 23.6 μg per 24 hours). Sodium depletion, which produced a significant rise in these "control" patients (mean ASR, 324 ± 59.5 μg per 24 hours), produced no significant rise in mean ASR in type I CAH (mean ASR, 21 ± 10.1 μg per 24 hours). In patients with type II CAH in sodium balance, mean ASR was 587 ± 174 μg per 24 hours, significantly above normal. Sodium depletion produced a further rise in ASR (mean ASR, $1{,}382 \pm 364$ μg per 24 hours), significantly greater than the normal rise, as did 2 days of ACTH stimulation (mean ASR, $1{,}018 \pm 216$ μg per 24 hours). Thus, these two types of CAH, which can be easily differentiated on the basis of their ASR, require different explanations for the underlying mechanism. In type I CAH a block of steroid 21-hydroxylation limits the formation of cortisol from 17-hydroxy progesterone and of aldosterone from progesterone; the latter may explain their salt loss and their low ASR. As expected, sodium restriction produces no significant increase in ASR. In type II CAH, there is a block of 21-hydroxylation that limits formation of cortisol from 17-hydroxy progesterone. However, their elevated ASR, which is responsive to both sodium depletion and ACTH stimulation, suggests that the 21-hydroxylating enzymes required to produce aldosterone from progesterone are not impaired in type II CAH. In these patients, the block in 21-hydroxylation presumably leads to accumulation of 17-hydroxy progesterone, and secondarily, of progesterone. This, by in-

creasing precursor substrate concentrations, could induce abnormally high secretion of aldosterone.

Radioimmunoassay of Insulin: A Rapid, Simple Method Using "Instant Dialysis" with Coated Charcoal. VICTOR HERBERT,* KAM-SENG LAU, CHESTER W. GOTTLIEB, RONALD A. ARKY, AND SHELDON J. BLEICHER, New York, N. Y., and Boston, Mass.

A radioimmunoassay for plasma insulin was developed by Berson and Yalow employing radioisotope dilution and a fixed quantity of insulin-binding antibody. The method requires 4 days of incubation at 4° C, which appears to permit stabilization of antigen-antibody complexes, and then paper hydrodynamic flow or chromatoelectrophoresis or both to separate free from antibody-bound insulin. The modification proposed here replaces electrophoresis by the much simpler "instant dialysis" using coated charcoal and sharply reduces incubation time, since it appears to recognize insulin-antibody complexes, which the charcoal studies suggest begin to form almost instantly. Two ml of 2.5 g per 100 ml Norite A pharmaceutical grade charcoal coated with 0.25 g per 100 ml dextran 80 adsorbs free insulin from albumin solutions within 10 seconds, but rejects insulin-antibody complexes, which remain in the supernatant. Counting of the radioactivity in the supernatant after precipitation of the charcoal by centrifugation yields ratios of bound-to-free I^{131}-insulin, plotted against unlabeled insulin concentrations, which are essentially identical to those obtained by standard methods when the same antibody and I^{131}-insulin concentrations are employed. An interesting sidelight of the present study was the finding that insulin-antibody complexes appear to be kept out of charcoal more completely by human than by bovine serum albumin. The methodologic simplifications here reported, reducing the assay time from 4 days to less than 3 hours, should be equally applicable to measurement of plasma levels of other hormones such as growth hormone, parathyroid hormone, and ACTH. Instant dialysis with coated charcoal may also be used to assay for antibodies to hormones and has already been used to assay for circulating antibody to insulin in diabetic patients.

The Pathophysiology of "Cholesterol Anemia" in Rabbits. ROBERT HESTORFF, PETER WAYS, AND SUSAN PALMER, Seattle, Wash. (introduced by Clement A. Finch †).

Cholesterol-fed rabbits (CFR) develop very high plasma cholesterol levels and hemolytic anemia. This study documents a concomitant increase in total cholesterol (primarily true cholesterol, some dihydrocholesterol) per erythrocyte in most CFR [normals, 1.10 (1.06 to 1.17×10^{-10} mg per RBC); CFR, 1.81 (1.12 to 4.12×10^{-10} mg per RBC)] without increases in erythrocyte phospholipid or triglyceride. Also, the proportion of CFR erythrocyte cholesterol, which is esterified, increases in proportion to the total RBC cholesterol (normal, 1 to 2%; CFR, 4 to 56%). Younger mean cell age in the CFR does not account for the higher RBC cholesterol, as reticulocyte-enriched (50 to 60%) populations separated from the blood of normal bled rabbits have a maximal cholesterol increase of 20% per cell. In some CFR, by contrast, sterol per erythrocyte was twice normal with fewer than 20% reticulocytes. To ascertain whether the additional red cell sterol was intrinsic or adsorbed, hemoglobin free ghosts were prepared. These contained 95 to 98% of the sterol found in intact CFR erythrocytes. In addition, sedimentation of the CFR erythrocytes through D_2O saline (density, 1.16) did not change their sterol content. In stained films or wet preparations of CFR whole blood, red cells were distorted and cytoplasmic vacuoles observed. The latter, seen within reticulocytes, mature cells, and ghosts, appeared lipophilic with oil red O, or osmic acid stains. In addition to shortened *in vivo* survival, the *in vitro* autohemolysis of CFR erythrocytes in defibrinated blood (37°, 48 hours) is 4 to 5 times normal and is uncorrected by glucose or normal plasma. Thus, erythrocytes from CFR have striking elevations of cholesterol ester either in the membrane or adsorbed to its inner surface. This chemical abnormality exists concomitantly with abnormal morphology and impairment of membrane integrity both *in vivo* and *in vitro*.

Tolerance to the Induction of Interferons by Endotoxin and Virus. MONTO HO* AND YUJI KONO, Pittsburgh, Pa.

It has been recently demonstrated that an intravenous inoculation of bacterial endotoxin will induce the transient appearance of an interferon-like inhibitor in the experimental animal similar to that induced by viruses. This represents a new parameter of the biological activity of endotoxin. In the course of studying this phenomenon, we tested the hypothesis that an animal may become tolerant or resistant to the interferon-inducing effect of endotoxin, just as it becomes tolerant to other effects of endotoxin. It was found that 12 hours after the inoculation of 0.01 to 10 μg of *Escherichia coli* endotoxin extracted according to the Boivin method, a dose (10 μg) ordinarily sufficient to induce the interferon-like inhibitor in the rabbit failed to do so. Tolerance persisted for 2 to 3 days, but the animal reverted to its sensitive state in 5 days. Intravenous Thorotrast was largely ineffective in reversing tolerance. However, serum collected 24 hours after one dose of endotoxin, when incubated *in vitro* with 10 μg endotoxin at 37° C for 1 hour, abolished the inhibitor-inducing capacity of the endotoxin, whereas normal rabbit serum had no such effect. One dose of either endotoxin or Sindbis virus (10^7 p.f.u.) was also effective in rendering the animal, within 24 hours, resistant to the interferon-inducing effect of Sindbis virus. However, Sindbis virus (10^9 p.f.u.) did not render the animal tolerant to the inhibitor-inducing effect of endotoxin. It is postulated that the tolerant state induced by either endotoxin or virus is partially due to the rapid appearance of a circulating humoral factor that inactivates the interferon-inducing capacity of endotoxin or virus. Such

a state may help explain the sporadic rather than the regular appearance of interferon-like inhibitors in patients with severe infections from gram-negative bacteria.

The Replication Pattern and Time of Experimentally Induced Breast Tumor Cells. JOSEPH HOFFMAN AND JOSEPH POST,† New York, N. Y.

Traditionally the growth advantage of tumor cells has been ascribed to their relatively rapid and chaotic proliferation. With the introduction of pulse labeling with radioactive DNA precursors and the scoring of mitotic labeling, it has become possible to study these aspects of tumor cell multiplication *in vivo*. In an earlier communication on rat carcinogen-induced hepatoma, it was found that the tumor cells replicated in a relatively orderly and rhythmic polycyclical manner. In addition the respective times for the replication cycle and its component intervals were relatively slow, when compared to those of normal replicating liver cells. The present report is concerned with the same time parameters of the carcinogen-induced breast tumor, which follows a single feeding of 7-12 dibenzanthracene in female Sprague-Dawley rats. The results show that these cells have a regular, polycyclical rhythm of replication. The time intervals are as follows: generation time, 45 hours; DNA synthesis time, 10 hours; post-DNA synthesis time plus mitotic time, 1.5 hours; and postmitotic time, 34.5 hours. It is obvious that these tumor cells replicate more slowly than do normal cells, such as those in liver, intestine, and bone marrow. In addition, the DNA synthesis time of this homogeneous and predominantly diploid tumor cell population is similar to that of normal diploid cells. These findings are of significance in the understanding of tumor growth and chemotherapy.

The Uptake, Distribution, and Disappearance of Intravenously Administered *d*-Aldosterone-1,2-H^3 in the Heart, Arteries, and Kidney. W. HOLLANDER,* D. M. KRAMSCH, A. V. CHOBANIAN, AND J. C. MELBY,* Boston, Mass.

The metabolism of aldosterone in heart, kidney, and arteries was studied because of its possible role in hypertensive cardiovascular disease. The uptake of H^3-*d*-aldosterone by dog tissues was determined from 5 to 120 minutes following intravenous injection of the isotope. The distribution of H^3-aldosterone was studied by radioautography after the injected aldosterone had reached a maximal level in the tissues. Subcellular fractionation of the tissues was carried out by differential ultracentrifugation. H^3-aldosterone was extracted from the tissues with chloroform and isolated chromatographically. The uptake of H^3-aldosterone was maximal in kidney, heart, and aorta within 15 minutes following its injection. The ratio of peak radioactivity in kidney, heart, and aorta was 6:3:1. At and after the time of peak radioactivity, aldosterone activity in the tissues was 1 to 3 times higher than the radioactivity in the blood. The disappearance of radioactivity in the tissues appeared to parallel the decline in blood radioactivity, which had a $t_{\frac{1}{2}}$ of 42 minutes. Subcellular fractionation of kidney, aorta, and heart tissues revealed over 60% of H^3-aldosterone in the supernatant fraction, about 20% in the nuclear debris, and less than 10% in the mitochondrial and microsomal fractions. Radioautographs of the heart muscle and kidney indicated a selective accumulation of H^3-aldosterone *1*) along the cell walls of cardiac muscle fibers adjacent to capillaries, *2*) along the cell walls of the convoluted tubules, Henle's loops, and Bowman's capsule, and *3*) along the inner wall of glomerular arterioles and capillaries. No concentration of radioactivity appeared in the collecting ducts. Pretreatment with spirolactone "aldosterone antagonists" did not alter the aldosterone uptake by the tissues or the radioautographic and tissue fractionation findings. The concentration and distribution of H^3-aldosterone in kidney, heart, and arteries suggest that the hormone is acting at these sites.

The *In Vivo* Synthesis, Interconversion, and Glyceride Distribution of Fatty Acids in Rat Adipose Tissue. C. H. HOLLENBERG,* Montreal, Canada.

To explore factors regulating the composition and glyceride distribution of adipose tissue fatty acids, epididymal fat pads of male rats were incubated attached to their vascular supply in a medium containing U-C^{14}-glucose and either removed or replaced for later study. Immediately after incubation 67% of lipid radioactivity was recovered as triglyceride, the remainder as diglyceride. Within 24 hours all tissue radioactivity was as triglyceride. Immediately, and 1 day after incubation, nearly 80% of the total fatty acid radioactivity was as saturated acids, 20% as monounsaturated acids, and these proportions remained almost unchanged over the next 2 weeks. Recirculation of label was trivial. During this interval while fat pad lipid content increased 60%, total fatty acid composition remained unaltered at 40% saturated, 40% monounsaturated, and 20% diunsaturated acid. These data indicate that the maintenance of the predominantly unsaturated character of the tissue during the 2-week interval was not due to extensive interconversion of acids synthesized *in situ* and thus imply that plasma triglyceride is a major precursor of rat adipose tissue lipid. The extent of mixing of newly synthesized acids with fatty acids derived from tissue stores or plasma was explored by determining the distribution of radioactive acids among various species of adipose tissue triglyceride. Although the specific activity of saturated acid in trisaturated triglyceride was higher than in other triglyceride types, radioactive saturated acid was also present in triglycerides containing dienoic acids, not synthesized in the tissue. Hence, although the data suggest that the newly synthesized acids are not delivered into a single large fatty acid pool, they also indicate that at either the fatty acid or lower glyceride stage of the esterification process some mixing must occur between these acids and those derived from tissue stores or plasma lipid.

Two Types of Liver Phosphorylase Deficiency: Excessive Deactivation with Brain Disease and Elevated Urinary Catecholamines (Case I), and Isolated Reduction of Activity (Case II). GEORGE HUG, WILLIAM SCHUBERT, JOHN GARANCIS, AND GAIL LEHNHOFF, Cincinnati, Ohio (introduced by Edward L. Pratt †).

Case I, a white girl, age 6, with progressive brain deterioration, has been studied for 4½ years with repeated needle biopsies of the moderately enlarged liver. Glycogen level was elevated (8 to 17%). Glycogen structure on stepwise enzymatic degradation was normal. Hepatic phosphorylase activity was decreased (5.8 to 17 μMP_i per minute per g of tissue). With glucagon iv (1.5 mg per minute for 3½ minutes) or epinephrine iv (1.8 μg per kg per minute for 5 minutes) phosphorylase could be activated (glucagon, 12.9 to 26.6, and epinephrine, 16.1 to 28.5 μMP_i per minute per g) to values within the normal range of phosphorylase activity (48 control measurements: range, 19.5 to 46.9 μMP_i per minute per g; average, 27.4). Urinary epinephrine and norepinephrine were consistently elevated 2 to 6 times normal. Case II, a white girl, age 4½, who is well except for marked hepatomegaly, showed increased liver glycogen (8.8 to 11.8%) and decreased phosphorylase activity (0.7 to 9.6 μMP_i per minute per g). Epinephrine iv consistently activated phosphorylase but never above 10 μMP_i per minute per g. In both, hepatic electron microscopy revealed markedly elevated glycogen. In Case II, more glycogen was of the monoparticulate form. To elucidate the mechanism in Case I, epinephrine was infused continuously in dogs for 12 hours. An initial increase in phosphorylase activity declined to preinfusion values or below. In Case I, maximal depression of phosphorylase activity occurred after administration of reserpine. Simultaneously, urinary epinephrine rose 20 times and norepinephrine 8 times normal. These results suggest that in Case I excessive deactivation of phosphorylase occurs as a consequence of increased levels of catecholamines. The primary defect might be in the metabolism of catecholamines leading to their elevation and causing, unrelated to the hepatic effect, neurological disease by a mechanism yet unexplained. In Case II, the defect is a reduction in phosphorylase without neurological consequences.

A Piston for the Sodium Pump. EDWARD S. HYMAN, New Orleans, La. (introduced by Quentin Deming *).

Whatever the mechanism may be that moves sodium across a living membrane against a concentration gradient, energy is required. This energy must be linked to sodium. A study of sodium by the methods of physical chemistry limits the possible energy links and singles out one that must occur. Movement of sodium is governed by its activity and not by its concentration, as movement of CO_2 is governed by P_{CO_2} and not CO_2 content. Theoretically, at a given concentration sodium activity is a function of the dielectric constant of the medium, the distribution of negative charges in solution, and of physical constants. Too massive a change in the medium is found necessary to significantly alter sodium activity. However, in a study of 50 sodium salts by potentiometry, the fall in activity per unit concentration exceeds the prediction based on ionic strength alone. That this excessive fall is due to ion association is confirmed by self-diffusion of Na^{22}, conductance, and transference. At the same normality sodium activity is lower with a divalent anion than with a univalent one (ionic strength is greater and some is bound). Both factors increase with valence. Any metabolic reaction that splits a multivalent anion into two or more anions of lesser valence will raise sodium activity. The most prominent instance is hydrolysis of ATP. By measurement sodium activity is higher in the hydrolytic products (22% at 0.1 N and pH 7, utilizing 114 cal per mole). Sodium will flow out of an area of increased activity to re-establish equilibrium. Should the enzyme orient the less polar end of ATP to serve as gate, flow would become directional. The Donnan potential would be changed, generating voltaic changes in the membrane.

Hemodynamic Studies in Adult Dogs with Coarctation of the Aorta Artificially Produced *In Utero*. BENJAMIN T. JACKSON AND RICHARD H. EGDAHL,* Boston, Mass.

Studies of aortic coarctation in the human have been limited in scope, and studies in experimental animals have been carried out largely following acute production of the lesion in mature or nearly mature animals. To study this disorder experimentally, while approximating human coarctation as closely as possible, partial aortic constriction (total occlusion was found to be fatal) was produced in dog fetuses *in utero*. Fetal operations for production of lesions were accomplished by placing a running stitch through the uterus and the skin of the fetus so that an elliptical area was circumscribed. An incision was made within the ellipse directly into the thorax of the fetus thus averting both loss of amniotic fluid and removal of the fetus from the uterus. A ligature was placed about the aorta distal to the ductus, fetal and uterine incisions were closed, and the fetus was separated from the uterus. Six such animals, with littermate controls, were studied at age 12 to 18 months with regard to the development of collateral circulation. Blood flow was measured with an electromagnetic flowmeter in the brachiocephalic and subclavian arteries and in the aorta proximal to the coarctation and at the diaphragm; blood pressure was determined above and below the coarctation. Mean blood pressure was elevated proximal and normal or elevated distal to the coarctation, and femoral pulse pressure was markedly narrowed. Mean blood flow in the distal thoracic aorta was similar in the coarctation dogs and controls. Total acute occlusion of the coarctation reduced to near zero the pulse pressure and pulsatile flow distally, but a reduced nonpulsatile flow persisted. In controls, occlusion of the aorta at the same level completely eliminated blood flow distally.

The rapid infusion of 5% dextrose in water increased the cardiac output and blood flow in the distal thoracic aorta both in controls and in coarctation dogs. Cardiac hypertrophy of a significant degree was characteristically present in coarctation animals. We conclude: *1*) Collateral circulation in the dog fetus is insufficient for survival with acute total aortic constriction. *2*) Although a large blood flow continues through coarctations of two to three mm diameter, pathophysiologic changes, including development of collateral circulation and cardiac hypertrophy, result. *3*) Severe coarctation in dogs does not prevent increased blood flow distally with increased cardiac output. *4*) The methodology utilized in this study may provide the means for fetal production of a wide range of lesions in experimental animals. *5*) The normal birth and development of animals operated upon *in utero* suggest the possibility that certain congenital anomalies might be surgically correctable in the human fetus *in utero*.

Concomitant Increase of Membrane Phosphatide Metabolism and Sodium Transport in Hereditary Spherocytosis (HS). HARRY S. JACOB AND MANFRED L. KARNOVSKY, Boston, Mass. (introduced by James H. Jandl *).

To preserve viability, HS red cells must actively transport sodium at nearly twice normal rates. Consequently, they afford a unique opportunity to relate the metabolism of membrane constituents to transport processes. Although the phospholipid composition of HS red cells is normal, labeling of phosphatides with inorganic phosphate (P^{32}_i) in washed red cells from five splenectomized HS donors (normal reticulocyte count) was double that in normal cells. Through the use of thin-layer chromatography and deacylation followed by paper chromatography, the increase was found primarily and consistently in phosphatidyl serine (PS) and (tentatively identified) phosphatidyl inositol (PI). Phosphatidic acid (PA), previously implicated by others in cation transport, did not consistently show increased labeling. Suspension of HS red cells in sodium-free (choline-potassium) media did not alter P^{32}_i incorporation into total phosphatides, but diminished the activities of PS and PI by 20%. Increasing the permeability of normal red cells by osmotic swelling produced 30% increments in PS and PI activity, despite unchanged or diminished over-all phosphatide and PA labeling. Ouabain, previously shown to depress glycolysis and elevate sodium content in HS but not normal red cells, resulted in increased P^{32} activity in PS, PI, and PA of HS cells only. We conclude that metabolism of these phosphatides with net negative charge is crucial to, and stimulated by, sodium transport in red cell membranes, but a prime role is indicated for PS and PI rather than PA. Analogous behavior of these same entities in such diverse systems as phagocytizing leukocytes and secreting acinar glands suggests their fundamental importance in membrane transport generally.

Interferon Study in Volunteers Infected with Asian Influenza. RODOLFO L. JAO, E. F. WHEELOCK, AND GEORGE GEE JACKSON,* Chicago, Ill., and Cleveland, Ohio.

Interferon, a protein with viral inhibitory activities, was demonstrated in the nasopharyngeal secretions and sera of patients infected artificially with Asian influenza. Daily nasopharyngeal washings were collected 24 hours before and 10 days after intranasal spray of 30 $TCID_{50}$ virus suspension of influenza A_2 (Elisberg strain) to 30 carefully screened adult male volunteers. Sera were collected a day before and every other day for 10 days and on the fourth and sixth weeks after virus challenge. Virus recovery from the nasal secretions was done in primary rhesus monkey kidney cells. Continuous cell line of human embryonic lung was employed in the assay of interferon using Sindbis as the interfering virus. Twelve (40%) of the 30 subjects challenged with influenza virus became ill and none of the 9 subjects given buffer control. A drug, being tested for its antiviral activity, was given to 15 virus-challenged and the 9 buffer-challenged subjects. The drug did not affect the rate of illness or interferon production. A significant amount of interferon was found in both the nasal washings and sera of 7 patients, all of whom had clinical illness and recovery of influenza virus from their nasal secretions. Five subjects had interferon in the serum only and one in the nasal washings only. Interferon was not demonstrated in all virus-positive subjects. It was found in only one subject who did not shed virus. Recovery of the virus preceded the onset of symptoms and the appearance of interferon in the nasal secretions or the blood or both. The cellular elaboration of interferon in response to viral infection previously shown in animal models is thus confirmed in a human induced viral infection.

Effects of Insulin on Triglyceride Metabolism in Man. DON P. JONES AND RONALD A. ARKY, Boston, Mass. (introduced by Charles S. Davidson †).

In four patients with diabetic ketoacidosis, initial plasma triglyceride (TG) concentrations of 382 to 450 mg per 100 ml returned to normal during insulin treatment over 24 hours. This suggested an effect of insulin on TG metabolism, which was studied by three techniques. In 6 normal subjects, rapid intravenous injections of insulin (0.12 U per kg) produced symptoms of hypoglycemia, and plasma glucose and free fatty acid (FFA) concentrations decreased 45 to 61% and 27 to 41% from control, respectively, followed by mild "rebounds" of FFA (to 173%). During the following 8 hours, after which a meal was given, and the next morning, no consistent TG changes occurred. Sustained infusions of low insulin doses (0.01 to 0.02 U per kg per hour for 5 to 8 hours) in seven subjects caused no symptoms of hypoglycemia. FFA initially decreased (to 38% control) but returned to control values in all patients after 1 to 2 hours in spite of sustained mild hypoglycemia (57 to 75% control) during insulin; FFA then increased 310%—much

more than after acute insulin—whereas glucose returned only to control values. Nevertheless, TG decreased in three patients during the test and in all seven by the next morning (65 to 86% control). During control studies without insulin, TG concentration did not change. During constant infusions (to 5 hours) of C^{14}-labeled palmitic acid in four patients, both acute and sustained insulin caused decreased rates of incorporation of C^{14} into plasma TG despite simultaneous increases in FFA specific activity. Thus, insulin affects TG metabolism in the normal as well as the insulin-deficient state. This effect is not just secondary to decreased FFA turnover, since plasma TG decreased after infusions of low insulin doses in spite of large increases in FFA. Depression of C^{14} incorporation into TG, seen acutely, suggests that insulin also decreases triglyceride production in the liver.

The Effect of Natural Estrogens on Hydroxyproline Excretion in Man. FRED H. KATZ AND ATTALLAH KAPPAS,* Chicago, Ill.

The heterocyclic imino acid hydroxyproline is a major component of collagen, and the amount excreted in urine reflects the metabolic turnover of connective tissue. Several natural hormones increase hydroxyprolinuria in man; excessive hydroxyproline excretion is also observed in certain connective tissue diseases and in bone disorders such as metastatic cancer and Paget's disease. This study demonstrates that a pronounced decrease in urinary hydroxyproline follows administration of the natural hormone estradiol and its principal metabolite estriol. Patients were placed on fixed low-gelatin diets and after appropriate control periods received intramuscular injections of estradiol or estriol in amounts approximating total daily estrogen production in late pregnancy. During steroid treatment, total hydroxyproline excretion diminished substantially, reaching levels as low as 40% of control values. Plasma hydroxyproline concentrations did not change significantly. Estrogen suppression of hydroxyproline excretion was demonstrated in men as well as women; it was also observed in prostate carcinoma metastatic to bone. The activity of estriol does not reflect conversion to its precursor hormone estradiol, since the transformation estradiol $\rightarrow$ estriol is not reversible *in vivo;* further, α-amino acid excretion decreased significantly during estriol treatment demonstrating anabolic activity for this hormonal metabolite. Thyroid and parathyroid hormones increase hydroxyproline excretion probably through catabolic effects on connective tissue; human growth hormone, although anabolic in action, also increases hydroxyproline excretion presumably by increasing the pool size of hydroxyproline peptides. In this study, estrogens antagonized the hydroxyprolinuric effect of all three hormones. The mechanism by which estradiol and estriol affect hydroxyproline metabolism is unknown; since estrogens, like growth hormone, are anabolic, their action could involve increased incorporation of hydroxyproline peptides into collagen, without necessarily increasing the pool size of these peptides.

Pulmonary Megakaryocytes: Their Origin and Significance. RICHARD M. KAUFMAN, ROMANO AIRO, SIMEON POLLACK, AND WILLIAM H. CROSBY,† Washington, D. C.

Megakaryocytes have long been known to inhabit the capillaries of the lung. Their origin, however, has been disputed, and their physiological significance has remained obscure. Two studies, in dogs and humans, inquire into these problems. The pulmonary blood flow of a group of dogs was redirected so that the right lung was perfused almost exclusively by venous blood and the left lung solely by arterial blood. The dogs were killed 1, 2, and 4 weeks after surgery and sections of their lungs examined. Megakaryocytes were confined almost entirely to the right lung, strongly suggesting that these cells do not originate in the lung but migrate there in venous blood. Further, many pulmonary megakaryocytes contain cytoplasm, implying that a significant number of platelets are released in the lung. The human study was undertaken to evaluate the incidence and morphology of circulating megakaryocytes and to attempt to quantify pulmonary platelet release. Blood from the right heart was obtained from 23 persons during cardiac catheterization, and megakaryocytes were isolated by a technique of saponin hemolysis and leukocyte concentration. Megakaryocytes were present in the blood of every person studied (mean, 2.4 cells per ml; range, .7 to 5.9 cells per ml). One-third of these cells appeared to contain a full complement of cytoplasm. We conclude that megakaryocytes normally enter the blood from the bone morrow. On the basis of megakaryocyte recovery one may compute that *1)* 24 to 68% of the mature megakaryocytes migrate to the lung, and *2)* 9 to 26% of the body's platelets are released in the pulmonary capillaries.

Metabolism of Mithramycin, an Antitumor Antibiotic. B. J. KENNEDY, JOHN W. YARBRO, MAGNHILD WOLLHEIM, AND MERLE LOKEN, Minneapolis, Minn. (introduced by Ivan D. Frantz, Jr.†).

Mithramycin, an antibiotic, produced objective regression of metastatic embryonal cell carcinoma of the testis in 7 of 12 patients. Other histologic types of testicular cancers have not demonstrated similar effects. Two of 6 patients with glioblastoma multiforme tumors of the brain also responded to therapy with mithramycin. The administration of mithramycin to 37 patients was associated with severe toxicity consisting of nausea, vomiting, fever, flushing, epistaxis, hematemesis, central nervous system disturbances, and muscular irritability. Among the biochemical alterations were a marked elevation of serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, lactate dehydrogenase, ornithine carbamoyl transferase, and alkaline phosphatase, without increases of cephalin flocculation or Bromsulphalein retention. There was an increase in prothrombin time and the two-stage prothrombin test, decrease in platelets but not leukocytes, decrease in serum calcium and phosphorus, proteinuria,

elevation of blood urea nitrogen, and occasionally renal failure. These toxic symptoms were associated with a mortality of 30%. To evaluate the metabolism of this agent, tritium-labeled mithramycin was administered intravenously and intraperitoneally to C3H mice with sacrifice of the animals at intervals following the injection. The concentration of mithramycin was determined in the lever, kidney, spleen, brain, testis, muscle, blood, and excreta. Significant differences in the localization of mithramycin in these tissues occurred. In counts per minute per milligram of tissue compared to muscle there was a fourfold increase in concentration in the kidney and a threefold increase in the liver. More than 75% of the measured excretion occurred within 8 hours. The areas of greatest concentration coincided proportionally with the systems demonstrating the most intense systemic reactions. Studies by the investigators have revealed that mithramycin inhibited the synthesis of RNA to a marked degree with little or no effect on DNA synthesis.

The Differential Nature of the Defect in Hepatic Lipogenesis in Acute Versus Chronic Insulin Deprivation. DAVID M. KIPNIS * AND RONALD KALKHOFF, St. Louis, Mo.

The effects of acute and chronic insulin deficiency on hepatic lipogenesis were studied by determining the conversion of acetate-1-C^{14}, acetyl-1-C^{14} CoA, and malonyl-1,3-C^{14} CoA to fatty acids by liver homogenates prepared from rats injected with either anti-insulin serum (AIS) or alloxan monohydrate. Ninety minutes after injection of AIS, acetate and acetyl CoA conversion to fatty acids was depressed ~70%, whereas malonyl CoA incorporation was unaffected, indicating a block at acetyl CoA carboxylase. The same pattern of impaired lipogenesis was produced in normal liver homogenates by addition of 1 to 5 μEq of palmitate (salt or fatty acid-albumin complex) or 125 mμmoles of palmityl CoA per ml incubation mixture. Reduction of the level of palmityl CoA and other long chain acyl CoA derivatives by addition of carnitine (5 μmoles per ml) reversed the inhibition of lipogenesis seen in these conditions. In the 72-hour alloxan diabetic rat, acetate, acetyl CoA, and malonyl CoA conversion to fatty acids was depressed 70 to 90%. Insulin (2 U per 100 g body weight, subcutaneously) restored lipogenesis to normal within 6 to 8 hours. Actinomycin (20 μg to 100 g body weight, intraperitoneally, 30 minutes before insulin) completely blocked the ability of the hormone to restore lipogenesis, but did not alter its hypoglycemic or plasma NEFA lowering effect. These data indicate that *1*) impaired lipogenesis following acute insulin deficiency is a result of feed-back inhibition of acetyl CoA carboxylase activity due to increased hepatic content of long chain acyl CoA derivatives, *2*) prolonged insulin deficiency results in an absolute decrease in both acetyl CoA carboxylase and malonyl synthetase content, in all probability secondary to impaired protein synthesis and decreased substrate flow through the lipogenic pathway, and *3*) the effects of insulin on glucose utilization and lipolysis (e.g., lowering of plasma NEFA) are not mediated through DNA-RNA dependent protein synthesis.

Uptake of Palmitic Acid by Forearm Muscle Measured Simultaneously with Palmitic Output from Forearm Adipose Tissue. G. A. KLASSEN, D. RABINOWITZ, A. KARMEN, AND K. L. ZIERLER,† Baltimore, Md.

RQ of man's forearm at rest in the basal state is 0.7, suggesting lipid oxidation. It is suspected that the major lipids oxidized are free fatty acids (FFA), but FFA uptake by forearm muscle has not been measurable by the Fick principle because forearm adipose tissue releases FFA into the venous effluent. Experiments were designed that would permit quantitation of FFA uptake by muscle and simultaneous FFA release from adipose tissue if certain conditions could be met, chiefly that there be no intercommunication between the deep vein (dv), draining mostly muscle but some adipose tissue, and a superficial vein (sv), draining mostly adipose tissue. The experiments rely on constant brachial arterial infusion of tracer labeled palmitic acid (PA), with measurement of blood flow and of a-sv and a-dv of tracer and stable PA. Absence of sv-dv communication was demonstrated in each experiment by retrograde venous double-injection of markers. We found that adipose tissue extracts no labeled PA from blood (hence has no PA uptake) and releases 0.25 μmoles PA per 100 g forearm per minute; muscle extracts 43% of arterial PA and takes up PA at a rate of 0.4 μmoles per 100 g forearm per minute. If PA were oxidized fully, it accounts for 23% of observed forearm O_2 uptake. PA is 29% of arterial FFA. If other FFA are extracted similarly, 80% of forearm O_2 uptake is due to FFA oxidation. In preliminary experiments, oleic acid, which is 41% of arterial FFA, was extracted by muscle to about the same extent as PA, supporting the validity of extrapolating PA results to total FFA. These experiments support the hypothesis that resting muscle of man consumes mainly FFA. It is also important to emphasize that in contrast to excised fat, adipose tissue *in situ* does not take up PA.

The Interrelationship of Protein Synthesis and Hormone Action. HEINZ KOHLER AND MAURICE M. PECHET,† Boston, Mass.

The interrelationship of protein synthesis and parathyroid hormone action has been investigated by studying the effects of actinomycin D and of puromycin in a special preparation utilizing unanesthetized rats with indwelling gastric, venous, and bladder catheters. A 2.5% dextrose, 1% NaCl, 0.09% KCl intragastric infusion and a 5% dextrose intravenous infusion were maintained. Urine samples were collected at 30-minute intervals for 4 days and analyzed for Ca, P, Na, K, Cl, and creatinine. The infusion of parathyroid extract (PTE) induced marked

increased excretion of P and K and decreased excretion of Ca. The infusion of actinomycin or of puromycin with PTE does not negate the PTE effects on the excretion of P, K, or Ca. For example, during PTE infusion at the rate of 5 U per hour, the average urinary deviations from control per 30-minute period were P, —502.4 μg; K, —18.1 μEq; Ca, +26.5 μg. With actinomycin 5 μg per hour and PTE 5 U per hour the values were P, —356.8 μg; K, —19.9 μEq; Ca, +25.7 μg. With PTE 10 U per hour the values were P, —460.8 μg; K, —25.2 μEq; Ca, +192.6 μg. With puromycin 1.8 mg per hour and PTE 10 U per hour, the values were P, —334.4 μg; K, —18.8 μEq; Ca, +195.6 μg. The effects of the infusion of actinomycin and puromycin in normal rats are dose dependent and biphasic in nature consisting of *1*) early decrease in P and increase in Ca excretions, *2*) late increase in P and decrease in Ca excretions. Our studies in contrast to published reports indicate that actinomycin and puromycin do not inhibit the action of administered parathyroid hormone, but cause a transient inhibition of the synthesis or release, or both, of endogenous parathyroid hormone.

False Neurochemical Transmitters and Their Relation to the Hypotensive Action of Monoamine Oxidase Inhibitors. IRWIN J. KOPIN, JOSEF E. FISCHER, JOSE M. MUSACCHIO, AND ROBERT J. WESTLAKE, Bethesda, Md. (introduced by Seymour S. Kety †).

After administration of monoamine oxidase inhibitors, norepinephrine levels in tissue are elevated, but sympathetic responsiveness is diminished. The increased exercise tolerance in anginal patients and the hypotensive effects seen after chronic administration of these drugs have been shown to be related to urinary amine excretion. Tyramine and *m*-tyramine are among the amines excreted; these amines can be β-hydroxylated in sympathetic nervous tissue. After administration of monoamine oxidase inhibitors, octopamine, the β-hydroxylated derivative of tyramine, accumulates in the tissues. This accumulation is markedly inhibited in organs of animals that have been chronically sympathetically denervated, indicating that the amine is probably in the sympathetic nerves. When examined *in vitro* by sucrose density centrifugation, octopamine is retained in the same particulate fractions that contain norepinephrine. When tyramine-H^3 is administered to cats, octopamine-H^3 formed in the heart and spleen can be released by sympathetic nerve stimulation. Endogenous octopamine formed after chronic treatment with monoamine oxidase inhibitors is also released by sympathetic nerve stimulation of these preparations. Octopamine is excreted in increased amounts in the urine of patients treated with these drugs and accumulates in the tissues. It is proposed that replacement of a portion of the norepinephrine at the sympathetic nerve endings by inactive false transmitters reduces the effectiveness of sympathetic nerve activity and results in hypotension and apparent adrenergic blockade.

Stabilizing Effects of Oxygen and Carbon Dioxide in Periodic Breathing. RAMON L. LANGE,* THEOFILOS J. TSAGARIS, JAMES T. BOTTICELLI, AND JAMES D. HORGAN, Milwaukee, Wis.

Periodic or Cheyne-Stokes respiration (CSR) may be abolished by administering CO_2 (a respiratory stimulant favoring cerebral blood flow) or O_2 (a respiratory and cerebral blood flow depressant), indicating that other influences are operative. Blood gases and circulatory and ventilatory function were measured before, during, and after CO_2 and O_2 administration. Twenty exposures to P_{ICO_2} (10 to 24 mm Hg) and 6 exposures to 100% O_2 were made in nine patients with CSR. All had heart disease and two had neurological disorders. While breathing room air mean values were cardiac index, 2.1; lung to artery circulation time, 45 seconds; cycle period, 80 seconds; VE, 8.6. Cyclic variations in P_{ACO_2} (24.2 to 34.6) about 28.7 mean were related to Pa_{CO_2} and Pa_{O_2} by a 180° phase shift. An unstable negative feedback system was suggested. O_2 and CO_2 produced minimal hemodynamic changes. CO_2 increased VE to 12.9 and induced cyclic ventilation without apnea. Mean P_{ACO_2} rose (31.1) with reduced excursion (29.4 to 32.7 mm Hg). Apnea rapidly returned with room air. O_2 produced damping of oscillation in three patients with mean VE of 7.3 and P_{ACO_2} of 31 on room air. Three patients with continued apnea had VE of 11.7 and P_{ACO_2} of 27 on room air. O_2 induced little change in VE and P_{AO_2}. Pa_{O_2} always exceeded 200 mm Hg. O_2 stabilization failure seemed related to insignificant hypoxic drive with high VE and low P_{ACO_2}. Respiratory control system simulation employed the observed prolonged circulation times but used normal chemoreceptor characteristics and cerebrospinal fluid effects. Computer simulated ventilation was periodic with room air. With CO_2, oscillation of reduced amplitude continued without apnea. O_2 induced progressive damping of oscillation. CO_2 stabilizes CSR because impaired CO_2 excretion reduces the over-all system gain despite increased VE. O_2 abolishes apnea when chemoreceptor function is normal. Persistent CSR implies metabolic or neural afferent synergism with CO_2 drive.

Stimulation and Suppression Testing of Aldosterone Secretion in Man. DAVID P. LAULER, GEORGE W. PAUK, ANTONIO I. VAGNUCCI, AND GEORGE W. THORN,* Boston, Mass.

To gain further insight into the genesis and perpetuation of edema disorders, evaluation of the aldosterone system in man has been standardized. Aldosterone, and often cortisol, secretory rates have been performed before, during, and after stimulation and suppression of aldosterone secretion. All studies were conducted in patients on standard metabolic balance protocol and with strict definition of posture and activity. Studies were conducted in normal subjects and patients with primary aldosteronism or secondary aldosteronism (congestive heart failure, cirrhosis with ascites, nephrosis). Stimulation testing consisted of salt restriction (5 days), blood letting

(500 to 1,000 ml), ACTH infusion (80 U daily for 48 hours), acute and sustained diuretic-induced volume depletion (ethacrynic acid every 4 hours), and subpressor infusions of angiotensin II. Suppression testing consisted of either salt loading (200 mEq sodium daily for 5 days) or albumin infusion. On a sodium intake of 90 mEq daily, aldosterone secretion ranged between 50 and 250 μg per day in normal subjects and on suppression by dietary salt loading, secretory values always were less than 50 μg per day. Patients with either primary or secondary aldosteronism failed to suppress their elevated secretory rates during comparable sodium loading; however, the former rapidly approached sodium balance and did not manifest edema, whereas the latter group went into positive sodium balance and rapidly accumulated edema. A patient with primary aldosteronism did increase aldosterone secretion (589 to 986 μg per day) on salt restriction, whereas patients with rapidly forming edema fail to do so, yet these patients did increase their secondary rates with diuretic-induced volume depletion. ACTH infusion in these patients caused only a modest rise in aldosterone secretion (20 to 40 μg per day).

Fixation of Complement Components to Autoantibody Eluted from Human RBC. J. P. Leddy, R. F. Bakemeier, and John H. Vaughan,* Rochester, N. Y.

The autologous proteins sensitizing human RBC and causing a positive direct antiglobulin (Coombs) reaction in acquired hemolytic disease fall into three serological patterns: *1*) complement (C′) alone, *2*) γ_G-globulin alone, *3*) γ_G-globulin and C′. The third pattern of RBC autosensitization could, in theory, result *a*) from the independent but concomitant operation of the mechanisms responsible for the first and second patterns, or *b*) from the capacity of some γ_G-globulin autoantibodies to fix C′ to the RBC that they are sensitizing. Concentrated eluates from three washed RBC samples showing autosensitization with both γ_G and C′ (pattern 3) induced *in vitro* sensitization of normal RBC to both anti-γ_G and anti-C′ sera. Incubations were at 37° C, without added C′. This finding suggested that C′ components that had originally been bound to these patients' RBC *in vivo* had been eluted with the γ_G autoantibody and carried piggyback by the autoantibody onto the normal RBC *in vitro*. When eluates from three other patients showing RBC autosensitization with γ_G and C′ were tested in this system, only γ_G-globulin was detectably transferred to normal RBC. This difference may be attributable to smaller amounts of C′ bound to these patients' RBC *in vivo*, but other differences may exist. RBC eluates from three patients exhibiting only γ_G-globulin autosensitization repeatedly failed to show the C′ piggyback phenomenon. The above anti-C′ serum possessed specificity for both the β_{1E}-globulin (C′4) and β_{1C}-globulin (C′3a) components of C′, as judged by independent hemagglutination inhibition studies with highly purified β_{1E}- and β_{1C}-globulins provided by H. Müller-Eberhard. The data indicate that some γ_G-globulin autoantibodies are responsible for *in vivo* C′ fixation to RBC and that at least some of the components in the C′ sequence are firmly fixed to the sensitizing antibody.

Mesenchymal Cell Proliferation in Persistent Viral Hepatitis. Carroll M. Leevy, Mansour Jabbari, and Willem tenHove, Jersey City, N. J. (introduced by Harold Jeghers †).

An elevated gamma globulin and improvement with adrenal steroid therapy are cited as evidence that persistent viral hepatitis is an autoimmune disease. The relationship of these parameters to replication of mesenchymal cells, which primarily contain gamma$_2$ globulin, was investigated, using H^3T and radioautographic techniques. Sprague-Dawley rats received 0.1 to 0.3 ml of CCl_4 three times weekly. Increased serum gamma$_2$ globulin (GG), liver cell necrosis, fragmentation of endoplasmic reticulum, prominent lysosomes, and active fibrosis were present after 2 to 4 months. A 50- to 100-fold increase in incorporation of H^3T into DNA by mesenchymal cells was noted. Identical findings occurred in patients with active viral hepatitis of 6 or more months duration. The influence of adrenal steroids on *in vitro* incorporation of H^3T into DNA was determined in this group. Four patients (group I) with marked hypergammaglobulinemia had 25- to 50-fold increase in labeled mesenchymal cells (normal 2 to 5 per 10,000 liver cells). Nine (group II) with moderate increase in GG had 2- to 5-fold increase in labeled mesenchymal cells. Four (group III) with normal or slightly increased GG had no labeling. Adrenal steroids suppressed DNA synthesis without influencing liver cell damage. Dramatic clinical improvement with reduction in GG was noted in group I; no significant alteration in clinical state or GG occurred in groups II or III. A group I patient had spontaneous decrease of H^3T uptake and GG; two group III patients had spontaneous increase in H^3T uptake and GG. Similar relationships were noted among mesenchymal cell proliferation, hypergammaglobulinemia, and response to adrenal steroids in alcoholic and drug-induced hepatitis. These data indicate mesenchymal cell proliferation and hypergammaglobulinemia are nonspecific reactions to liver injury, and are consistent with the postulate that persistent hepatitis represents a continuing viral infection.

Enterovirus Hemagglutination (HA): Specific Inhibition by Sugars. A. Martin Lerner and Elizabeth Jane Bailey, Detroit, Mich. (introduced by E. E. Muirhead †).

Recent experiments from this laboratory have shown that rheovirus types 1, 2, and 3 HA are specifically inhibited by *n*-acetyl-D glucosamine, but not by over 20 other sugars. HA of adult human red cells by the enteroviruses ECHO-3, 7, 11, 12, and 19 and of human cord cells by Coxsackie viruses, B-1 and B-5, was subsequently studied. Enzymatic digestions with β-glucosidase removed the specific receptors for HA on the virus capsids of all of these viruses indicating that these enterovirus

capsomeres are glycoproteins like those of rheoviruses. Specific sugars were found to inhibit HA with ECHO viruses 3, 7, 11, and 19 and Coxsackie B viruses 1 and 5. None of the 18 sugars tested inhibited HA with ECHO-12. These inhibitions of HA could be used to rapidly identify each of the viruses. Inhibition reactions with D-arabinose (1), D-ribose (2), and L-fucose (3) distinguish several of the ECHO viruses tested. ECHO-7 HA is inhibited by (3) only; ECHO-11 and ECHO-19 by (1) and (2), but not (3); ECHO-3 by (1), (2), and (3); and ECHO-12 by neither (1), (2), nor (3). ECHO-19 is distinguished from ECHO-11 in that HA of the former is inhibited by D-glucose, but the latter is not. Coxsackie B-1 HA is inhibited by D-glucosamine, whereas Coxsackie B-5 is not. The sugars block the HA reaction by attaching to the virus capsid but not to the erythrocyte.

Preparation of α- and β-MSH for Clinical Use. AARON B. LERNER, SAUL LANDE, AND G. VIRGINIA UPTON, New Haven, Conn. (introduced by Philip K. Bondy †).

In the commercial preparation of ACTH, oxycellulose is added to an extract of pooled pituitary glands from hogs. ACTH is absorbed on the oxycellulose, and the mother liquor is discarded. However, when new oxycellulose was added to the mother liquor, a fraction was obtained that possessed approximately 16% β-MSH, 6% α-MSH, and 5% ACTH. Further fractionation on a carboxymethylcellulose column developed with a stepwise concentration gradient of sodium succinate-acetic acid buffers gave 9 fractions. One contained primarily α-MSH, another β-MSH, and a third new β-MSH. Three of the 9 fractions were purified further by separate runs through a long Sephadex G-25 column. Alpha- and β-MSH were obtained in good yield as homogeneous peptides. The fraction containing the new β-MSH was composed of 12 peptides as shown by Sephadex chromatography. Some of these as well as those obtained from the Sephadex purification of the α-MSH fraction contained less than 13 amino acids. By these procedures α- and β-MSH and also ACTH can be prepared in sufficient quantity for clinical trial. The sequences as well as the biologic activity of the other small peptides are being investigated.

A Collagen-like Plasma Protein in Hodgkin's Disease and Experimental Fever in Man. E. C. LEROY, S. M. WOLFF, P. P. CARBONE, AND A. SJOERDSMA,* Bethesda, Md.

A hydroxyproline-containing protein has been identified in human plasma and a specific method developed for its assay. Characterization by ammonium sulfate precipitation and Sephadex G-200 gel filtration indicates that it is a large molecule; its resistance to digestion by proteolytic enzymes suggests further that it may be a form of collagen. In 54 normal adults the mean level of this protein, expressed as protein-bound hydroxyproline (PBH), was 8.0 μg per ml ± 0.1 (SE). Measurement of PBH in 150 patients with a variety of diseases revealed elevations in neoplastic diseases, particularly Hodgkin's disease, and in a variety of acute inflammatory disorders. Thirteen patients with active Hodgkin's disease had the highest PBH levels observed (mean 20 μg per ml ± 1.1). Seven patients studied serially during therapy showed a return to normal as the disease regressed. Six other patients in remission for 6 months following therapy had normal levels. Since six of the patients with Hodgkin's disease were febrile, the effect of experimental fever on PBH levels of normal volunteers was studied. Seven subjects were given three 20-mg intramuscular injections of etiocholanolone during a 6-day period; fever, local inflammation, and elevated PBH levels uniformly ensued (mean 13 ± 1.0, mean increase 96%, $p < 0.01$). In contrast, no alterations of PBH levels were noted following fever produced by three to five intravenous injections of a bacterial endotoxin (*S. typhosa*). Thus fever per se was not associated with alterations in PBH. Despite the presence of elevated PBH, urinary hydroxyproline levels remained normal. The spontaneous and experimentally induced elevations of PBH that have been observed should facilitate further characterization of this protein and evaluation of its role as a new parameter of collagen metabolism.

Immune Responses to Penicillin in Man and Penicillin Allergy. BERNARD B. LEVINE AND MICHAEL J. FELLNER, New York, N. Y. (introduced by Philip Y. Paterson *).

Previously, we demonstrated that in man benzylpenicillin induces immune responses mainly specific for the benzylpenicilloyl haptenic determinant. Presented here are the immune responses made by different patient populations in relationship to occurrence of allergic penicillin reactions. Serum antibodies were quantitatively assayed by highly sensitive passive hemagglutination methods, which detect mainly benzylpenicilloyl (BPO)-specific antibodies of both 7 S and 19 S molecular classes. Skin-sensitizing antibodies (SSA) were assayed by direct skin testing with BPO-polylysine conjugates and with crystalline benzylpenicillin. This allowed detection of SSA against BPO as well as the minor haptenic determinant specificities, respectively. Of 76 randomly selected patients, 97% showed BPO-specific immune responses. These consisted mainly of low titers of 19 S antibody; 13% showed 7 S antibody. Only 3% had SSA. Of 54 patients completing courses of penicillin without allergic reactions, 100% showed BPO-specific responses, again mainly 19 S, but here 39% showed 7 S antibody, and 9% had SSA. None of these patients showed SSA specific for minor determinants. In contrast, each of 7 patients with recent immediate systemic reactions showed SSA specific for minor determinants (1 showed also BPO-specific SSA). Of 17 different patients with late reactions, 14 showed SSA (5 for BPO, 7 for minor determinants, 2 for both). Nine of these 17 patients showed high titers of BPO-specific 7 S antibody. These results demonstrate that although im-

mune responses to penicillin are widespread among nonallergic patients, allergic reactors make immune responses that differ qualitatively and quantitatively from nonreactors. In terms of mechanisms of penicillin allergy, immediate systemic reactions appear to be mediated by SSA mainly of minor determinants specificity; late allergic reactions appear to be mediated by a variety of immunopathological mechanisms.

Cardiovascular and Metabolic Effects of Adenosine 3',5'-Monophosphate in Man. ROBERT A. LEVINE, Denver, Colo. (introduced by S. G. Blount, Jr.†).

In vitro experiments suggest that the glycogenolytic and myocardial responses to catecholamines are mediated by adenosine 3',5'-monophosphate (3',5'-AMP). Previous studies by the author in unanesthetized dogs have demonstrated positive inotropic and chronotropic effects of 3',5'-AMP that occur before changes in blood glucose or plasma free fatty acids (FFA). Single intravenous or intracardiac doses of 3',5'-AMP (8 to 12 mg per kg) administered to 15 human subjects, including 8 during cardiac catheterization, were attended within seconds by an increase in heart rate. The maximal increase in cardiac rate averaged 40% above control levels ($p < .005$), with a range of 20 to 150% of the initial rate, and was accompanied by a mean increase in cardiac output of 44% ($p < .025$) 5 minutes after 3',5'-AMP injection. Cardioacceleration persisted for 15 minutes. There were no significant changes in blood pressure. Five other normal subjects received a constant 3',5'-AMP infusion at the rate of 0.5 mg per kg per minute for 1 to 2 hours, which was associated with persistent tachycardia and an average blood pressure elevation of 22% during the infusion. Plasma cortisol, measured on the day preceding and the day of infusion, rose from an initial mean value of 11.7 ± 3.5 μg per 100 ml to 24.6 ± 9.0 μg per 100 ml ($p < .025$) at the end of the infusion. In the acute injection experiments, cortisol significantly increased in 6 of 14 subjects ($p < .025$). Although hyperglycemia was observed in all 20 subjects, FFA response was variable. Maximal mean increase in blood glucose was 34 mg per 100 ml above control values ($p < .025$) 10 minutes following a single dose of the nucleotide. There were no significant changes in plasma osmolarity. It would appear that 3',5'-AMP penetrates cells in significant concentration to regulate intracellular metabolic events, as reflected by glycogenolysis and steroidogenesis. The cardiovascular actions of 3',5'-AMP demonstrated *in vivo* support *in vitro* studies indicating that 3',5'-AMP may represent the biochemical basis for catecholamine-induced effects on the heart.

Myocardial Tensions in the Human Left Ventricle during Systole. GILBERT E. LEVINSON AND MARTIN J. FRANK, Jersey City, N. J. (introduced by Harper K. Hellems*).

Previous investigators have postulated that an optimal relationship of end-diastolic and stroke volumes to pressures will permit the heart to diminish myocardial wall tension during ejection, despite rising intracavitary pressure. The extent to which this hemodynamically advantageous mechanism operates in the human left ventricle (LV) was investigated, by means of left heart catheterization, in 5 normal subjects, 16 patients with increased LV loads (aortic stenosis, aortic regurgitation, systemic hypertension, and coarctation of the aorta), and 12 patients without increased LV loads (pure mitral stenosis, atrial septal defects, and chronic bronchopulmonary disease). End-isometric, mid-systolic, and end-systolic myocardial tensions were measured as $4P\pi R^2$, where P is pressure and R is the corresponding radius calculated, assuming a spherical geometry, from volumes measured by indicator washout. In the normal LV, tension fell during ejection: mean mid-systolic did not exceed mean end-isometric tension (14.7×10^6 dynes), and end-systolic was 14% less than end-isometric tension ($p < 0.025$), confirming the original postulate. In contrast, with increased LV loads, mid-systolic and end-systolic tensions exceeded end-isometric tension by 13 to 77% ($p < 0.025$) in all lesions except compensated aortic regurgitation. In the latter, because the LV dilated no more than was necessary to accommodate the augmented stroke volume, end-systolic tension was, as in the normals, lower than end-isometric tension. With increased RV loads, despite subnormal LV end-isometric tensions secondary to reduced LV end-diastolic volumes, a qualitatively identical loss of the normal hemodynamic advantage was observed: LV tensions rose by 12% during ejection ($p < 0.005$). It is concluded that: *1*) the normal LV exhibits a unique hemodynamic advantage: tension falls, as pressure rises, during ejection; *2*) this advantage is lost with dilatation or systolic overloading, but is retained in the diastolic overloading of compensated aortic regurgitation; and *3*) by changing LV end-diastolic and stroke volumes, lesions that overload the right ventricle alter the function of the left ventricle.

A Functional Role for Plasma Alpha$_1$ Lipoprotein. ROBERT I. LEVY, ROBERT S. LEES, AND DONALD S. FREDRICKSON,* Bethesda, Md.

A physiological role for the plasma alpha$_1$ (high density) lipoproteins has never been defined. In this study it is demonstrated that, in combination with beta lipoprotein, alpha$_1$ lipoprotein forms the very low density lipoproteins that take part in the transport of endogenous triglyceride. Furthermore, the presence of alpha$_1$ lipoprotein accounts for the pre-beta or alpha$_2$ electrophoretic mobility of these very low density lipoprotein complexes. It can be shown experimentally by dietary induction of hyperlipemia in normal subjects and patients that *1*) as concentrations of pre-beta lipoprotein rise, there is an immediate reciprocal fall in alpha$_1$ lipoprotein (high density lipoprotein) and a rapid reversal of this as hyperlipemia declines; *2*) the changes in alpha$_1$ lipoprotein are dependent on changes in plasma triglyceride, but independent of plasma concentrations of cholesterol, phospholipid, and beta lipoprotein; *3*) although isolated pre-beta lipo-

protein is apparently homogeneous, it is decomposed by removal of triglyceride through lipolysis or by mild delipidation with ether to two other lipoproteins. These have been positively identified as alpha$_1$ lipoprotein and beta lipoprotein; *4*) in contrast to normal subjects, when patients lacking circulating beta lipoprotein (a-beta lipoproteinemia) are fed high carbohydrate diets, they have no rise in plasma triglycerides and make no pre-beta lipoprotein; *5*) on similar high carbohydrate diets patients having only small amounts of alpha$_1$ lipoprotein (Tangier disease) have a greater than normal increase in plasma triglycerides but make no pre-beta lipoprotein; the triglycerides are carried by a single lipoprotein band having beta mobility. It is therefore suggested that alpha$_1$ lipoprotein facilitates the transport of endogenous triglycerides. It is also evident from these studies that the arbitrary separation of the plasma lipoproteins into high and low density lipoproteins no longer can be considered to define two structurally or functionally independent fat transport systems.

What Causes Uneven Ventilation? BENJAMIN M. LEWIS,* JOHN B. DALTON, AND LEONIDAS ROJAS, Detroit, Mich.

Ventilation is uneven not only per unit of alveolar volume, but also in the sequence in which alveoli empty and fill. If a small amount of neon is interpolated early into a slow maximal inspiration starting from residual volume, the concentration of neon in the subsequent expiration continuously rises. If the same amount is interpolated late in inspiration, concentration falls with expiration. This is found in normal subjects and patients with various types of pulmonary disease. The same study done during repetitive deep breathing at 20 to 30 cycles per minute gives the same results except in emphysema, where early interpolation results in a less steeply falling expiratory concentration than late interpolation. The regions that empty last, then, fill early in inspiration. Two (or more) regions of the lung differing in resistance would produce the opposite result. This would also hold true if the resistance to airflow were due to poorly supported bronchioles whose lumen varied with the pressure difference between the lumen and the surrounding parenchyma ("check valve" obstruction). A more probable explanation lies in the *S* shape of the volume-pressure (compliance) curve of the lungs. If the better ventilated region were on the lower limb of the *S* at residual volume, its filling early in inspiration and its emptying late in expiration would be retarded relative to the poorly ventilated region, which was on the middle portion of the curve at this volume. The latter region would fill readily early in inspiration, fill slowly as it reaches the top limb of the *S*, contribute little to early expiration, but increasingly to late expiration. Since this region is nearer the top of the curve at the start of inspiration, it can expand less during inspiration, thus explaining the lower ventilation of this region per unit of volume.

Transplanted Hematopoietic Stem Cells: Their First Ten Days. JERRY P. LEWIS, ROBERT D. LANGE, AND FRANK E. TROBAUGH, JR., Chicago, Ill., and Knoxville, Tenn. (introduced by Richard B. Capps †).

Successful hematopoietic transplantation is effected by grafted stem cells, those cells that differentiate into mature hemic elements and renew their own numbers. In mice, hematopoietic repopulation occurs in the spleen as visible colonies composed usually of only one cell type. These colonies permit detailed studies of the early events in hematopoietic repopulation. Hematopoietic repopulation was studied in CAF$_1$ female mice injected with measured doses of isologous marrow cells following 750 r irradiation. The cellular composition of each colony was determined from serial microscopic sections. Stem cell renewal was studied by assaying the stem cell content of spleens at daily intervals. The stem cell content of a spleen was estimated from the number of colonies formed on the spleen of an irradiated assay host injected with the minced test spleen. Growth rate of erythroid colonies was estimated from changes in volume. The volume of a colony was determined by summing the volumes of serial sections, estimated from planimetry of photomicrographs. Host factors affecting differentiation were studied by injecting marrow into hypertransfused mice, some of which received exogenous erythropoietin. Erythropoietin levels were determined by a standard assay utilizing incorporation of iron^{59} into erythrocytes of hypertransfused mice. The following observations are reported: *1*) After transplantation, stem cells first differentiate. *2*) Stem cell renewal begins after the fourth post-transplant day. *3*) Plasma erythropoietin does not increase until day 8 after irradiation. *4*) No erythroid colonies develop in hypertransfused animals unless exogenous erythropoietin is administered. *5*) Through the fourth post-transplant day the mean doubling time of erythroid colonies is 9.3 hours; from day 5 through day 10, 14 hours. These studies suggest that hematopoietic stem cells are pluripotential; they are directed at the time of seeding to differentiate along one cell line, and this direction may be controlled by erythropoietin. Renewal of their own population follows differentiation.

Ethanol-induced Fatty Liver on Fat-free and Fat-containing Diets: Role of Dietary, Adipose, and Endogenously Synthesized Fatty Acids. CHARLES S. LIEBER * AND NORTON SPRITZ, New York, N. Y.

Fatty liver developed in 18 rats fed ethanol (36% of calories) for 24 days with isocaloric adequate diets, either fat free (including 2 mg per cal linoleate) or containing fat (43% cal). Total hepatic lipids were significantly lower without dietary fat (90 vs. 140 mg per g). Similarly, in a human volunteer, 18 days of ethanol (46% cal) produced less steatosis, morphologically and chemically, with fat-free than with fat-containing diets; in percutaneous liver biopsies, total lipids were 63, 44, 156, triglycerides 32, 18, 108 mg per g, on isocaloric ethanol fat-free, control, and ethanol fat-

containing diets, respectively. Although one large dose of ethanol (7.5 g per kg) produced hepatic accumulation of adipose fatty acids (FA) in rats, fatty livers after 18 to 24 days of ethanol and fat-free diets (4 rats, 2 humans) had triglyceride FA strikingly different from depots, with 50% more palmitate (synthesized endogenously) but 3-5X less linoleate. To differentiate depot from dietary FA in the studies with fat-containing diets, corn, coconut, or linseed oils were fed to 12 rats and 1 volunteer until adipose FA contained 20 to 35% linoleate, laurate + myristate, or linolenate, respectively. Then, simultaneously another oil was substituted and ethanol begun; after 11 to 18 days, fatty liver triglycerides consisted largely of FA fed with ethanol, whereas FA given before ethanol were 6X lower than in depots. The role of dietary FA was confirmed by significantly higher liver lipid radioactivity 2 to 6 hours after oral palmitate-H^3 or intravenous chylomicrons-C^{14} and ethanol, vs. isocaloric carbohydrate controls; lipid absorption, hepatic chylomicron uptake, and labeling of circulating lipids were unaffected, but $C^{14}O_2$ production in liver slices incubated with ethanol and palmitate-C^{14} or chylomicrons-C^{14} was decreased. Thus, although one large dose produces hepatic accumulation of adipose FA, fatty liver following prolonged ethanol intake contains endogenously synthesized, and, when available, dietary FA, whose oxidation is decreased by ethanol. Elimination of dietary FA reduces alcoholic steatosis significantly.

Replication of Epithelial Cells in Atrophic Gastric Mucosa in Pernicious Anemia. MARTIN LIPKIN, BERTRAND BELL, PAUL SHERLOCK, AND YOUNG SHIK KIM, New York, N. Y. (introduced by Thomas P. Almy †).

After injection of thymidine-methyl-H^3 (H^3 TdR), cell replication was studied in atrophic gastric mucosa, jejunum, and esophagus of a patient with treated pernicious anemia. The findings were compared with those observed in stomach and small intestine of patients who did not have pernicious anemia. In both atrophic and normal gastric mucosa, replication of epithelial cells proceeded at the same rate, and new cells filled 0.5 cell position per hour as they migrated to the luminal surface of the mucosa. Since fewer epithelial cells were present in the atrophic mucosa, 0.9 cells per 100 cells per hour were produced, compared to 0.5 cells per 100 cells per hour in normal gastric mucosa. Thus, epithelial cells in the atrophic mucosa in treated pernicious anemia rapidly proliferated and were extruded early (normoproliferative cytopenia). In the jejunum of the patient with pernicious anemia, 0.6 cell position per hour was filled with new epithelial cells, yielding 0.6 cell per 100 cells per hour. The mean rate of DNA synthesis, measured as the number of grains appearing over metaphases in epithelial cells of atrophic stomach, normal stomach, and jejunum, revealed a constant synthesis rate during a 6-hour mid-portion of the *S* phase. In normal stomach and jejunum there was an increase in the synthesis rate during the first 2 to 3 hours of the *S* phase and a decrease during the last 2 to 3 hours. The distributions of grains over individual cells before mitosis were broader than Poisson distributions with twofold deviations. In jejunum, grain densities were reduced over cells migrating to the luminal surface, indicating cell division before migration. In atrophic stomach mucosa, grain densities over many cells were not reduced during migration, indicating increased complement of DNA and increased duration of the G_2 premitotic phase of the proliferative cycle. This property is also present in normal gastric mucosa with variation among individuals.

Studies on the Hybridization of Mammalian Cells in Culture. JOHN W. LITTLEFIELD,* Boston, Mass.

Further studies have been completed on the hybridization of mouse fibroblasts (L cells) in culture. As one partner, a clonal line was used that lacks guanylic acid-inosinic acid pyrophosphorylase and is resistant to 3 μg of 8-azaguanine per ml, and as the other, a line lacking thymidine kinase and resistant to 30 μg of 5-bromodeoxyuridine per ml. Both lines were sensitive to 4×10^{-7} M aminopterin, in contrast to wild-type cells. Spontaneous revertants did not occur in either line. After growth of the two lines together for 4 days, hybrid cells (frequency about 10^{-6}) were selected by virtue of their resistance to aminopterin. As in the previous studies, the hybrid nature of these cells was suggested not only by the presence of both enzymes, conferring resistance to aminopterin and return of sensitivity to 8-azaguanine and 5-bromodeoxyuridine, but also by the high number of chromosomes. The amounts of the two enzymes per cell were consistent with gene dosage. The frequency of hybridization was the same in monolayer and suspension cultures and has not been markedly increased by various agents. During growth for several months the two hybrid lines followed appear to have lost a few chromosomes; and variants (resistant to 8-azaguanine, 5-bromodeoxyuridine, or both) are present, some of which have lost many chromosomes. So far it has not been possible to accelerate this process of chromosome loss. The frequencies of drug-resistant variants in the hybrid lines are much the same as in the original wild-type population, as if in the latter also variation was due mainly to loss of chromosomes.

Placental Diffusion Studies Using Carbon Monoxide in Sheep. LAWRENCE D. LONGO, GORDON G. POWER, AND ROBERT E. FORSTER II,* Philadelphia, Pa.

Carbon monoxide was used to study placental diffusion because of the relative independence of the Pco gradient from the state of the fetal and uterine blood flow. Serial blood samples from the uterine and umbilical artery and vein of five anesthetized, near-term pregnant ewes and their lambs *in utero,* before and after the introduction of CO into the ewe via a closed rebreathing circuit, were analyzed for carboxyhemoglobin saturation (COHb%), P_{O_2}, P_{CO_2}, pH, oxyhemoglobin satu-

ration ($O_2Hb\%$), and CO_2 content. COHb% in the mother ($COHb_m\%$) before the introduction of CO averaged 1.0% (SD ± 0.43) and in the fetus ($COHb_f\%$) averaged 3.4% (SD ± 0.73). The ratio $COHb_f\%/COHb_m\%$ ranged from 2.8 to 3.6 (mean 3.3, SD ± 0.37). Administration of CO to the ewe resulted in elevation of $COHb_m\%$ to 5 to 10% within 5 minutes and a gradual increase in $COHb_f\%$, reaching the maternal value within 1½ to 2 hours and equilibrating at an average 2:1 ratio of $COHb_f\%$ to $COHb_m\%$ in approximately 6 hours. The Pco gradient was calculated from COHb% and $P_{O_2}/O_2Hb\%$ using the Haldane relationship. The milliliters of CO diffusing per minute was calculated from the rate of change of $COHb_f\%$ and the fetal blood volume. Placental diffusing capacity (D_{PCO}) ranged from 0.29 to 0.59 ml per minute per mm Hg kilogram of fetal weight (mean 0.50, SD ± 0.14). The Haldane relationship was also used to calculate the mean P_{O_2} gradient during the control period when the Pco gradient was negligible. From this, and values of fetal oxygen consumption in the literature, estimates of D_{PO_2} of 0.083 to 0.18 (mean 0.12, SD ± 0.04) were obtained. We conclude that: *1)* Measurements of D_{PCO} offer advantages in studying the limiting processes of placental exchange. *2)* The existence of 2 to 4 times as much COHb% in the fetus as in the mother under steady-state conditions may have profound implications in the development of the fetus *in utero* in mothers with elevated COHb%. *3)* The discrepancy between D_{PCO} and D_{PO_2} may be explained by errors in the estimates of fetal V_{O_2}, the presence of vascular shunts, or uneven distribution of blood flow within the placenta.

Renal Blood Volume and Intrarenal Distribution of Blood Flow in Normal and Hypertensive Man. JEROME LOWENSTEIN, PHILIP R. STEINMETZ, RICHARD M. EFFROS, DOMINGO M. GOMEZ, HERBERT CHASIS,† AND DAVID S. BALDWIN,* New York, N. Y.

Renal ischemia in essential hypertension has been adduced from previously observed reduction in the ratio of renal blood flow to functional tubular mass (Tm_{PAH}). Whether this reduction in blood flow is uniform or focal within the renal vascular bed is not revealed in the measurement of over-all renal blood flow by clearance techniques. We have developed a method of determining functional blood volume and intrarenal distribution of specific blood flow (blood flow per unit blood volume) in the kidney of man. After retrograde catheterization of the right renal artery and vein, indicator-dilution curves using indocyanine green were recorded across the renal vascular bed. Curves representing the continuous distribution of blood flow through theoretic units of equal volume (specific blood flow, ζ) were obtained by transformations according to formulae derived by Gomez. Renal plasma flow (C_{PAH}) and extraction of *p*-aminohippurate (E_{PAH}) were measured concurrently. Functional renal blood volume was calculated from mean specific blood flow ($\bar{\zeta}$) and renal blood flow [$C_{PAH}/E_{PAH} \times$ (1-hematocrit)]. Mean specific blood flow was significantly reduced in eight patients with essential hypertension, 0.190 $second^{-1}$, as compared to eight normotensive subjects, 0.230 $second^{-1}$ ($p < 0.05$). Renal blood volumes, however, were equal in the two groups, 37.5 ± 5.8 ml (1.73 m^2 body surface area) in hypertensives and 36.7 ± 10.9 ml in normotensives. The frequency distribution curve of ζ was unimodal, ranging from 0.32 $\bar{\zeta}$ to 2.60 $\bar{\zeta}$ in the normotensives, and did not differ significantly in the hypertensives. Functional renal blood volume provides an estimate of nephron units in terms of vascular channels rather than in terms of tubular mass. With the method employed in this study, the finding of reduction in mean specific blood flow ($\bar{\zeta}$) provides new evidence for the presence of renal ischemia in essential hypertension. Further, unaltered distribution of ζ demonstrates that this ischemia is uniform throughout the renal vascular bed.

Micropuncture Studies on Individual Nephrons in the Rat with Chronic Pyelonephritis. HERBERT LUBOWITZ, MABEL PURKERSON, AND NEAL S. BRICKER,* St. Louis, Mo.

Micropuncture techniques were used to examine the patterns of salt and water reabsorption in individual surface nephrons of chronically diseased (pyelonephritic) kidneys of rats. Studies were performed under two conditions: *1)* With the contralateral control kidney present, salt and water reabsorption could be compared in nephrons from the control and diseased kidneys in the same animals in a nonuremic environment. *2)* With the diseased kidney as the only source of functioning nephrons, salt and water reabsorption was evaluated in the diseased kidney in a uremic environment. Experimental pyelonephritis was induced in rats. After a stable chronic lesion had been established, tubular fluid was collected using conventional micropuncture techniques during mannitol and Pitressin infusion. Tubular fluid (TF) and plasma (P) inulin and osmolality were measured, and values for TF/P ratios were expressed as a function of tubular length. In the nonuremic environment salt and water reabsorption was found to proceed in a closely comparable manner in surface nephrons of diseased and contralateral control kidneys. All proximal tubular samples were isosmotic with plasma, and no greater scatter was observed in diseased than control organs. In the uremic rats, TF/P inulin values also increased progressively along the course of the proximal tubule. However, the per cent of filtered water reabsorbed at any level of the proximal tubule was less than in nonuremic animals. Proximal tubular fluid again was isosmotic with plasma. These observations show no evidence of an intrinsic tubular defect in salt and water reabsorption in the residual nephrons of the chronically diseased kidneys. Neither was there evidence of abnormal nephron heterogeneity. The decrease in the percentage of salt and water reabsorbed by the diseased kidney in uremia may result from adaptive changes involving an increase in GFR per nephron.