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Abstracts

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171. Reversible Ion Permeability Changes in Membranes of Metabolically Depleted Erythrocytes. PAUL L. LACELLE* AND ROBERT I. WEED, Rochester, N. Y.

ATP depletion induces the disc-to-sphere transformation of human erythrocytes accompanied by decreases in cell deformability and filterability, factors thought important in determining red cell life-span. Cells sphered by depletion of ATP have been examined to evaluate possible concomitant modification of passive ion permeability. Passive permeability was examined in fresh normal erythrocytes and after depletion of energy stores by 24–48 hr incubation in serum at 37°C. The depleted cells accumulate Ca^{++}, show sphering, and have a predictable decrease in sulfate ($^{35}SO_4$) efflux and in potassium (^{42}K) influx in the presence of ouabain at 37°C and pH 7.4. The K^+ influx decreased from 3.78×10^{-4} mole/liter cells per hr to 1.68×10^{-4} mole/liter cells per hr and was returned to 3.32×10^{-4} mole/liter cells per hr after incubation of depleted cells with adenosine to regenerate cellular ATP. $SO_4^=$ efflux decreased from 1.18×10^{-6} mole/liter cells per sec to 0.68×10^{-6} mole/liter cells per sec and returned to 1.12×10^{-6} mole/liter cells per sec after incubation with adenosine. When depletion occurs in Ca^{++}-free buffer, only minimal changes of shape and permeability are evident. However, addition of 1 mM Ca^{++} induced both the sphering and the decreased permeability. These data are interpreted as indicating that in addition to producing cell shape changes and decreased deformability, metabolic depletion in the presence of Ca^{++} leads to reversible decreases in cation and anion permeability. It is suggested that all these changes are consistent with reversible Ca^{++}- and ATP-dependent alterations in cell membrane thickness.

172. Cytomegalovirus Infection Associated with a Postperfusion Mononucleosis-like Illness. DAVID J. LANG,* Boston, Mass. (introduced by John D. Crawford**).

Between April and November 1967 seven cases of the postperfusion syndrome were recognized in patients who had recently undergone cardiopulmonary bypass procedures. As in previously reported cases, the clinical features of these illnesses resembled those of infectious mononucleosis. Heterophile agglutination reactions were negative. Evidence, primarily based upon serologic data, has been presented by other investigators suggesting that cytomegalovirus infection may be associated with these illnesses. It has been proposed that virus may be transmitted with transfusions of fresh blood. An alternative explanation postulates the reactivation of latent cytomegalovirus. In the present cases, cytomegalovirus was recovered from all seven patients. Virus was isolated from urine, throat swabs, and washed peripheral leukocytes. Significant changes in antibody titer were demonstrated as well. In the one instance where it was available, preoperative serum contained no cytomegalovirus antibody. In this case it was possible to study all donors of the fresh blood which had been received by the patient. Cytomegalovirus was recovered from one donor. Cytomegaloviremia was demonstrated only after inoculation of a sensitive feeder layer of tissue culture with leukocyte-rich plasma or intact washed leukocytes. Prolonged incubation of inoculated cultures was required for the demonstration of a cytopathic effect. Virus was not isolated from cell-free plasma, washed erythrocytes, or disrupted leukocytes. The data presented strongly support the concept that cytomegalovirus infection is responsible for some febrile postperfusion illnesses and that this virus may be transmitted with fresh blood. It is suggested that a prolonged asymptomatic cytomegaloviremia may exist in apparently healthy individuals. Intracellular cytomegalovirus apparently loses infectivity when refrigerated. Thus the risk of cytomegalovirus transmission by whole blood is reduced after storage.

173. Respiratory and Postural Factors in Venous Return Deduced from Flow Patterns in Tricuspid Stenosis. RAMON L. LANGE, MICHAEL H. KEELAN, JR.,* JAMES T. BOTTICELLI,* AND HOWARD W. SHORT,* Milwaukee, Wis.

Pulmonary congestion and edema result from augmentation of right ventricular (RV) stroke volume when left ventricular (LV) function is reduced. Orthopnea and paroxysmal nocturnal dyspnea imply postural factors. Active inspiratory efforts increase RV output by (1) increasing the capacity of the pulmonary vascular bed and the RV diastolic filling (intrathoracic factors); and (2) in the supine human, increasing flow from the abdominal veins by increased abdominal pressure (extrathoracic factors). The normal inspiratory fall in RA pressure reflects limited effectiveness of the latter mechanism. With increased venous pressure and volume as in congestive heart failure, the extrathoracic component may become significant. Assessment of this element of venous return is possible in patients with tricuspid stenosis since, commonly with inspiration, RA pressure is maintained or rises despite increased diastolic flow from RA to RV. In these circumstances, the augmented venous return from extrathoracic sites equals or exceeds the accelerated outflow from RA to RV. Since transvalvular flow may be described by orifice meter formula, the magnitude of extrathoracic elements may be estimated. In eight patients with tricuspid stenosis, supine with quiet respiration, the mean RA pressure (mm Hg) was: inspiration (I), 11.2; expiration (E), 10.9. The diastolic RA-RV pressure gradient was I = 7.5, E = 3.0; diastolic flow, I = 128%, E = 72% of mean. With moderate elevation of RA pressure and related increase in abdominal venous volume, quiet inspiration provides 50% augmentation of right heart filling from abdominal volume. Centripetal flow results from the rectifying effect of venous, tricuspid, and pulmonary valves. In sitting or erect positions, hydrostatic forces reduce or prevent extrathoracic augmentation—a factor related to orthopnea. Prevention of pulmonary congestion requires that venous pressure and volume be maintained below levels that allow significant influences of postural and extrathoracic

augmentation of venous return. In patients without heart disease, extrathoracic augmentation may contribute to pulmonary edema with head injury or overtransfusion.

174. Hyperinsulinemia during Alpha Adrenergic Blockade in Obesity. A. M. LAWRENCE* AND H. NEVIS,* Chicago, Ill. (introduced by R. L. Landau**).

Alpha adrenergic blockade in nondiabetic, obese subjects was associated with a greater than 100% rise in immunoreactive insulin (IRI) in the absence of a β-cell stimulus such as a glucose load. No change in plasma glucose (PG) occurred despite this rise in IRI. Normal volunteers were characterized by lack of any change during α-adrenergic blockade alone, but by worsening of glucose tolerance and heightened IRI when glucose was administered with an α-blocker simulating more nearly the PG and IRI responses of obese individuals receiving an oral glucose load alone. Indeed, PG and IRI responses of obese subjects to oral glucose were essentially similar with and without concurrent infusion of an α-blocker. Obesity is characterized by hyperinsulinemia demonstratable in the basal state, in response to insulinosecretory stimuli and during exercise. The role, if any, of the autonomic nervous system in this altered metabolic pattern is uncertain, although some degree of control due to α- and β-adrenergic receptor activity may be inferred from data demonstrating the inhibitory effect of epinephrine on insulin release. Since this effect is manifest during β-adrenergic blockade and is nullified during α-adrenergic blockade, it is reasoned that α-receptor activity inhibits, β-receptor activity augments insulin release to provocative stimuli. The results of this study lend support to the suggestion that hyperinsulinemia seen in obesity may be related to an imbalance of adrenergic receptor responses of the β-cell, and if associated with exaggerated secretory responsiveness could be responsible for premature release of immunologically similar but biologically different insulin moieties which could account for absence of hypoglycemia despite hyperinsulinism evoked during α-adrenergic blockade in obese subjects. Alternatively, intact α-recptor activity may be important to the peripheral effectiveness of circulating insulin.

175. Human Granulocyte Collagenase. GERALD S. LAZARUS,* JOHN R. DANIELS,* ROBERT S. BROWN,* HAROLD M. FULLMER,* AND HOWARD A. BLADEN,* Bethesda, Md., and New Haven, Conn. (introduced by James A. Shannon**).

Degradation of collagen fibrils may occur in inflammation concomitantly with leukocyte infiltration. This report describes a specific collagenase which is extractable from the granule fraction of human granulocytic leukocytes. The immediate availability of granulocyte collagenase differentiates it from other reported animal collagenases in which culture of tissue is necessary for enzyme detection. When aqueous extracts of 9×10^7 normal or chronic myelogenous leukemia granulocytes were incubated with ^{14}C-labeled reconstituted collagen fibrils, 21% of the substrate fibrils were solubilized (pH 7.4, 37°C, 18 hr). On fractionation of the granulocytes nearly all collagenase activity was found to be associated with the granule fraction. Chromatography of the crude granulocyte extract on a diethylaminoethyl cellulose column yielded a stable collagenase which was free of detectable caseinolytic activity. Incubation of the partially purified enzyme with a solution of native collagen resulted in a 65% fall in specific viscosity upon completion of the reaction (pH 8.5, 25°C, 20 hr). The band pattern of the reaction products seen on acrylamide gel electrophoresis indicated a single site of cleavage for each chain of the collagen molecule. Electron micrographs of segment long spacing aggregates prepared from these digests demonstrated loss of the carboxy-terminal one-quarter of the molecule. In contrast, incubation of the crude granulocyte preparation with collagen in solution at 25°C resulted in numerous products separable by acrylamide gel electrophoresis. This suggests that granulocytes are capable of extensive digestion of collagen through the combined action of a specific collagenase and other proteases.

176. The Effects of Infusion of β-Lipoprotein-Rich Plasma on Fat Transport in Abetalipoproteinemia. R. S. LEES* AND E. H. AHRENS, JR.,** New York, N. Y.

Chylomicrons and pre-β-lipoproteins are vehicles for transport of glycerides from gut and liver into plasma. In abetalipoproteinemia all three of these transport proteins are absent. To test whether lack of circulating β-lipoprotein or of its protein moiety is responsible for the inability to form the glyceride-bearing lipoproteins, a patient with abetalipoproteinemia was repeatedly transfused with β-lipoprotein–rich plasma. Evidence for the production of chylomicrons and pre-β-lipoprotein was sought when the transfusions were completed. While on a diet containing 88 g fat per day, an 11 yr old boy with abetalipoproteinemia was given intravenously over 4 days a total of 2150 ml of citrated plasma from a patient with familial hyperbetalipoproteinemia (type II). Levels of β-lipoprotein in the patient's plasma were measured immunochemically, and chylomicrons and pre-β-lipoproteins by preparative ultracentrifugation, followed by paper and immuno electrophoresis. Fat absorption was estimated by measurement of plasma radioactivity after oral administration of ^{14}C-linoleate. Four plasma transfusions raised β-lipoprotein cholesterol from zero to 285 mg/100 ml plasma and total plasma cholesterol from 35 to 344 mg. The half-life of the infused β-lipoprotein was 2.2 days; therefore, its concentration in plasma was normal or higher than normal for 5 days. Nevertheless, plasma glycerides never rose above 18 mg/100 ml plasma; no chylomicrons or pre-β-lipoproteins were detected at any time; and no more radioactivity appeared in plasma after ^{14}C-linoleate feeding at the peak of β-lipoproteinemia than during a control test before the transfusions. Two earlier findings from this laboratory—(1) that cholesterol synthesis is normal in abetalipopro-

teinemia and (2) that an apoprotein of β-lipoprotein is present in plasma—suggest that the primary defect in abetalipoproteinemia is an inability to assemble complete lipoproteins from their lipid and protein components. The failure of plasma infusion to cause glyceride mobilization in abetalipoproteinemia supports this hypothesis.

177. The Immune Response of the Kidney in Experimental Pyelonephritis. JAMES D. LEHMANN,* JAMES W. SMITH,* T. E. MILLER,* JACK A. BARNETT,* AND JAY P. SANFORD, Dallas, Texas.

Most bacteria causing pyelonephritis stimulate the production of circulating antibodies, but controversy exists concerning the role of such antibodies in protection against reinfection. Antibody synthesis within kidneys might better reflect host resistance than circulating antibody levels. Experiments using an in vitro method of assessing protein synthesis were designed to determine whether pyelonephritic kidneys are capable of local antibody production. Unilateral pyelonephritis was produced in rabbits by transient occlusion of one ureter followed by intravenous injection of *Escherichia coli* O-75. The contralateral kidneys, normal kidneys, normal spleens, and kidneys from rabbits with nephrotoxic serum nephritis were studied as controls. Synthesis was measured by ^{14}C-amino acid incorporation into protein during incubation of tissue slices in sterile culture medium. Soluble protein was separated by elution from diethylaminoethyl cellulose, and immunoglobulin was measured by precipitation with antirabbit immunoglobulin G (IgG) and immunoglobulin A (IgA). In five experiments the mean soluble protein synthesis in pyelonephritic kidneys (48,950 cpm/g) was 19 times greater than in the contralateral kidneys (2540); in individual experiments pyelonephritic kidneys demonstrated 5 to 100 times more protein synthesis. In pyelonephritic kidneys 71% (35,040/48,950) of synthesized protein was IgG as compared with 11% (292/2540) in contralateral kidneys. IgA accounted for 11% (5700/48,950) synthesized protein in pyelonephritic kidneys as compared with 6% (168/2540) in contralateral kidneys. Synthesis of IgG and IgA by normal kidneys (four experiments) was comparable to that by contralateral kidneys. This enhanced immunoglobulin response was present from days 14 through 40 but absent at days 2 and 7. Approximately 10% of the IgG was precipitated by *E. coli* O-75 somatic antigen. These studies provide evidence that in experimental pyelonephritis a significant local immunoglobulin response occurs which is represented primarily by the production of IgG, suggesting that local immunoglobulin formation and specific antibody synthesis may be important factors in determining patterns of host resistance.

178. Carbohydrate-Induced Calciuria and Kidney Stone Formation. J. LEMANN, JR.,* W. F. PIERING,* AND E. J. LENNON, Milwaukee, Wis.

Daily total urinary calcium excretion does not exceed the "normal" range in some patients who have formed calcium-containing kidney stones. We wondered whether intermittent bursts in the rate of calcium excretion ($U_{Ca}V$) might contribute to the pathogenesis of calculi. Since ingestion of various nutrients augments $U_{Ca}V$ in normal subjects, we gave 100 g glucose orally to six normal subjects (Normals), six patients with histories of calcium stones without infection (Stones), and four close relatives of patients with stones (Relatives). Renal clearances were performed during water diuresis. After four control periods, glucose was given and six 20-min periods were observed. During control periods mean serum ultrafiltrate calcium (UF_{Ca}) and magnesium (UF_{Mg}) concentrations, inulin clearances, and excretion rates of magnesium, sodium, and complexing anions did not differ as between the groups. $U_{Ca}V$ averaged ($\pm$ SEM) 2.2 ± 0.4 μmoles/min in Normals and was significantly higher in Stones and Relatives (5.0 ± 0.8 and 6.9 ± 1.1 μmoles/min; $P < 0.001$) although overlap between groups existed. After glucose, UF_{Ca} was unaltered, inulin clearance fell, and $U_{Ca}V$ rose in all subjects, indicating diminished net tubular calcium reabsorption. The peak increment in $U_{Ca}V$ for Stones and Relatives together ($+ 5.5 \pm 0.6$ μmoles/min) was greater than for Normals ($+ 3.6 \pm 0.5$ μmoles/min; $P < 0.05$), and the peak concentration of calcium in the urine for Stones and Relatives (1.07 ± 0.19 mmoles/liter) was also greater than in Normals (0.38 ± 0.02 mmole/liter; $P < 0.025$). Changes in magnesium, sodium, potassium, acid, and complexing anion excretion rates were not different as between groups. We conclude that Stones and Relatives have an exaggerated augmentation of $U_{Ca}V$ after glucose ingestion. Coupled with higher basal rates of $U_{Ca}V$, high urinary calcium concentrations result that could favor calcium salt precipitation. Thus, augmentation of $U_{Ca}V$ after meals or ingestion of carbohydrate-rich foods or beverages might induce stone nidation in susceptible individuals.

179. Variable Responsiveness in von Willebrand's Disease. JACK LEVIN* AND DUDLEY P. JACKSON, Baltimore, Md.

Patients with von Willebrand's disease are reported to respond reproducibly to transfusion by a delayed increase in the level of AHG (antihemophilic globulin, factor VIII). AHG levels were measured serially after 66 transfusions of FFP (fresh-frozen plasma) to five patients with von Willebrand's disease. Two women received infusions of 400–600 ml FFP on 8 and 12 separate occasions. AHG reproducibly rose from 3% of normal to average levels of 43% and 65% respectively, 6–20 hr after transfusion. AHG level of another patient (pretreatment AHG 5%) was maintained at approximately 100% by daily transfusion of FFP, and was > 60% 60 hr after the last transfusion. Two patients acquired a transient refractory state after multiple transfusions. In one, the delayed rise of AHG after transfusions of 1000 ml FFP or its equivalent, administered every 3rd day, progressively declined from 36% to < 5% over 12 days. 1 wk later, transfusion of 800 ml FFP increased his

AHG from 4% to 30%. The other patient, when first seen, increased his AHG from 1% to 80% and 100% after transfusions of 600 ml FFP on two occasions. Multiple transfusions were required during the next month, and a delayed rise in AHG could not be demonstrated on 13 occasions during the subsequent 9 months. Responsiveness was not altered by prednisone or Enovid. After 1 yr, during which no transfusions were administered, 600 ml FFP increased AHG from 1% to 32% and 37%, respectively. Circulating anticoagulants (anti-AHG) could not be demonstrated by in vitro tests. Results demonstrate the variability of response to transfusion of patients with von Willebrand's disease. Some patients acquire a transient refractory state possibly attributable to an inhibitor of the von Willebrand stimulating factor or to "exhaustion" of the mechanism necessary for in vivo production of new AHG.

180. Inhibition of Infectivity of Reovirus, Type 2, by Glycoside-Cleaving Enzymes. A. MARTIN LERNER AND Q. R. MIRANDA,* Detroit, Mich.

The primary and necessary first phase of virus infection is absorption of an active site on the surface of the virus to a suitable receptor on the cellular membrane. Since animal viruses exhibit marked species and tissue specificities, this initial stage of virus-cell union is an appropriate stereochemical interaction. Recently, it has become evident that capsids of several hemagglutinating enteroviruses and reovirus, type 2, contain not only protein, but also saccharides. Moreover, the carbohydrate moieties are necessary for at least one type of virus-cell union; namely, hemagglutination (HA). Glucose, galactose, glucuronic acid, and hyaluronic acid are units of the capsids of echoviruses, types 3, 7, 11, 12, and 19; galactose, glucose, glucuronic acid, and *n*-acetyl-*d*-glucosamine are components of the protein of reovirus, type 2. Echovirus, type 12, and reovirus, type 2, were grown in tissue cultures, and partially purified by differential centrifugation, successive treatments with DNase, RNase, and trypsin, and finally by column chromatography with Sephadex G-200. 16 HA units of each virus was incubated with 1% solutions of β-glucosidase and lysozyme (*n*-acetyl-*d*-glucosaminidase) at 4°C for 24 hr. Appropriate albumin controls accompanied each experiment. Lysozyme was a chromatographically pure crystalline molecule, and both enzymes were free of contaminating proteolytic activity. Infectivities of β-glucosidase–treated and control preparations of echovirus, type 12, were similar (1.26×10^6 TCD_{50}). On the other hand, reovirus-treated preparations decreased from 7.95×10^3 to 3.99×10^1 with lysozyme, and to 3.17×10^3 with β-glucosidase. The carbohydrases destroyed 99.6% and 96.7% of reovirus infectivity, respectively. These data suggest that *n*-acetyl-*d*-glucosamine is the terminal unit of a saccharide chain containing glucose on the protein of reovirus, type 2. Attachment leading to infection occurs by means of the saccharide of reovirus, but this is not the mechanism of infection with echovirus, type 12.

181. Two Cytoplasmic Proteins from Rat Liver and Their Role in Hepatic Uptake of Sulfobromophthalein (BSP) and Bilirubin. A. JONATHAN LEVI,* ZENAIDA GATMAITAN,* AND IRWIN M. ARIAS, New York, N. Y.

After intravenous administration of BSP or bilirubin to Wistar rats, gel filtration (Sephadex G-75) of 100,000 *g* liver supernatant yielded two albumin-free protein fractions (Y and Z) with high affinity for these organic anions. These fractions were purified by ion exchange chromatography and electrofocusing. A single protein, identified by gel electrophoresis and analytical ultracentrifugation, was isolated from the Z fraction. This protein has a molecular weight of 12,000, sedimentation coefficient of 1.9S, an isoelectric point of 5.8, and a high affinity for BSP. A second binding protein has been partially purified from the Y fraction. Preliminary studies reveal a molecular weight of 32,000 and an isoelectric point of 6.7. Mean protein turnover in Y and Z fractions was determined from serial changes in specific activity after pulse labeling in vivo with ^{14}C-guanidino-arginine. Half-times of approximately 17 and 2 days respectively were calculated. BSP added to liver supernatant was preferentially bound to the Y fraction until 0.01 mg of BSP per mg protein was attained, after which BSP was progressively bound to Z. Similar patterns of dye binding to Y and Z fractions were obtained with liver from Gunn rats, sheep, and the single human studied. These fractions were not found in rat brain, kidney, or heart. A bile-secreting hepatoma (H-35) had both Y and Z fractions; however, a non–bile-secreting hepatoma (Morris 5123) lacked Z fraction, and the Z protein was absent on gel electrophoresis. Flavaspidic acid and bunamiodyl cause bilirubin and BSP retention in man and rats by interfering with hepatic uptake mechanisms. The effect of these agents was studied in vivo and in vitro. Selective blocking of bilirubin and BSP binding to the Z fraction was produced by each agent. 1 mole of flavaspidic acid prevented the binding of 1 mole of BSP to the Z fraction in vitro. This study suggests that these proteins are important in hepatic transfer mechanisms and in the pathogenesis of jaundice resulting from defective hepatic uptake of bilirubin.

182. Penicillin Hypersensitivity and the Doctrine of Original Antigenic Sin. BERNARD B. LEVINE, VERA LEVYTSKA,* AND DAVID M. ZOLOV,* New York, N. Y.

Humans vaccinated with influenza antigens produce antibodies reactive in higher titer with the influenza strain first experienced in childhood than with the influenza strain used for vaccination, hence the term "original antigenic sin." We have observed a patient who, after a long course of dimethoxyphenylpenicillin (methicillin) therapy, was found to have serum (IgG and IgM) and skin-sensitizing antibodies specific for the benzylpenicilloyl (BPO) hapten rather than for the dimethoxyphenylpenicilloyl (DPO) hapten. This was demonstrated by passive hemagglutination, direct skin tests, and Prausnitz-Kustner tests and by specific hapten

inhibition of these reactions. This patient had received benzylpenicillin three years previously. In order to investigate this phenomenon further, an animal model was established. Guinea pigs were immunized with 50 μg of DPO-poly-L-lysine (DPO_{27}-PLL_{535}) in complete adjuvant. 4 months later one-half were boosted with 1.0 mg DPO_{27}-PLL_{535} and the other half with 1.0 mg of BPO_{29}-PLL_{535} (free of detectable DPO conjugate). Both groups made anamnestic responses. Both groups made antibodies specific for the DPO (priming) hapten as demonstrated by hemagglutination and quantitative precipitation reactions, and by quantitative hapten inhibition with monovalent BPO and DPO haptens. However, several differences between the two groups were noted. Maximal titers were reached at 7 days for the DPO-boosted group and at 14 days for the BPO-boosted group. Quantitative precipitin experiments indicated that, as compared with the DPO-boosted antisera, BPO-boosted antisera were more cross-reactive, and the antibodies appeared to have lower average affinity for the DPO hapten. These results indicate that the doctrine of original antigenic sin applies also to drug allergy, and apparently applies to the production of IgM and skin-sensitizing antibodies as well as to IgG antibodies. These results support a selective role for antigen in the anamnestic response.

183. Stimulation of Plasma Insulin and Growth Hormone in Man by Cyclic 3′,5′-AMP. ROBERT A. LEVINE,* Brooklyn, N. Y. (introduced by John F. Mueller).

Exogenous cyclic adenosine-3′,5′-monophosphate (3′,5′-AMP) and theophylline have been shown to stimulate insulin release from isolated perfused rat pancreas, and growth hormone (GH) output from heifer pituitary slices. This study was undertaken to determine whether 3′,5′-AMP administration influences the level of fasting venous plasma immunoreactive insulin and GH in man. After 12 hr fasting, one healthy volunteer subject received an acute injection of 3′,5′-AMP (4 mg/kg), three subjects were given infusions of 3′,5′-AMP (0.25–0.5 mg/kg per min for 30–60 min), and one other normal subject and one adrenalectomized patient received the *N*^{6}-*2′-O*-dibutyryl derivative of 3′,5′-AMP (DBC) (0.2 mg/kg per min for 60–90 min). Plasma GH and insulin concentrations were measured by radioimmunoassay procedures and also by a coated-charcoal modification of the radioimmunoassay procedure for insulin. Following 3′,5′-AMP or DBC infusion, a prompt rise in plasma insulin was observed, followed by slower increases in plasma GH and glucose. Circulating GH rose from an initial mean value of 1 ± 0 mμg/ml to a maximum mean level of 14 ± 10 mμg/ml ($P < 0.005$), insulin from 13 ± 3 μU/ml to 60 ± 12 μU/ml ($P < 0.001$), and glucose from 93 ± 18 mg/100 ml to 169 ± 75 mg/100 ml ($P < 0.05$). The variable hyperglycemia induced by 3′,5′-AMP or DBC apparently had no suppressive effect on plasma GH. After cessation of nucleotide infusion, plasma insulin and GH decreased to preinfusion levels, the former rapidly and the latter more slowly, while plasma glucose remained elevated or continued to rise for 15 min and then decreased. Mild headache and abdominal pain developed in two of four subjects during 3′,5′-AMP administration, but DBC infusion was unaccompanied by any uncomfortable side effects in two others. These results in man support previous in vitro data indicating that 3′,5′-AMP may be the intracellular mediator involved in the mechanism of insulin and GH release.

184. Dialysis as a Tool in the Study of Erythropoietin (EP). JASPER P. LEWIS,* DOROTHY A. ALFORD,* MARY M. DICKINSON,* RUSSELL R. MOORES,* EDWARD GARDNER, JR.,* CLAUDE-STARR WRIGHT,* AND LINDA L. SMITH,* Augusta, Ga.

Although several groups of investigators have demonstrated EP to be dialyzable under certain conditions, the use of the dialysis technique in the study of EP has been limited. This report reexamines the application of dialysis to the purification and characterization of EP. Equilibrium dialysis was done on a urinary concentrate obtained by column chromatography using a membrane that gave a 10-fold increase in EP specific activity in the dialysate. Physicochemical and potentiation studies were done on the fractions obtained. By dialyzing against a 0.5 M NaCl solution, most of the dialyzable EP was removed in 9 days, leaving an appreciable amount of a nondialyzable EP factor. The sedimentation coefficients of the nondialyzable EP fraction ranged from 2.7 to 4.5S. The most highly purified dialyzable fraction was about 2S and by immunoelectrophoresis contained three distinct lines in the α-globulin–albumin region and one vague line in the γ-globulin region. With the membrane and urine concentrate used, the passage of EP appeared to be dependent on ionic strength and pH. Incubation of the nondialyzable EP fraction with normal rabbit serum generated 15 units/ml of EP activity during 30 min, whereas the dialyzable EP fraction generated 2.7 units. Inhibition was demonstrated by increasing the amount of serum used. The Lineweaver-Burk plot of the data for the nondialyzable fraction gave a curve typical of enzymatic inhibition by excess substrate.

185. Hepatic Microsomes: A New Site for Ethanol Oxidation. CHARLES S. LIEBER AND LEONORE M. DECARLI,* New York, N. Y.

It is generally accepted that alcohol dehydrogenase (ADH), a soluble enzyme of the cell sap, is responsible for hepatic oxidation of ethanol to acetaldehyde. ADH, however, markedly favors the reverse reaction at physiological pH. Therefore, we searched for other ethanol-oxidizing systems. Hepatic microsomes were isolated from 60 rats (fed various diets) and from three human surgical biopsy specimens; upon incubation with NADPH generating systems, all preparations actively oxidized ethanol to acetaldehyde at pH 7.4. Comparable results were observed with hepatic supernatants (containing microsomes and cell sap) obtained from normal rats and from needle biopsy specimens of three patients with normal or alcoholic fatty livers. Cell sap alone or purified

ADH was inactive at pH 7.4. Rat kidney and brain had a negligible microsomal ethanol oxidative system (MEOS). To compare adaptive properties of MEOS and ADH, 18 rats were pair-fed diets containing 36% of calories as ethanol or carbohydrate (controls). After 24 days of ethanol feeding, MEOS increased 50% per g of liver ($P < 0.01$), with a 30% rise in "specific activity" (calculated per mg of microsomal protein; $P < 0.05$). In contrast, ADH showed no significant adaptation. MEOS had characteristics commonly found among microsomal drug-detoxifying enzymes: requirement for O_2 and NADPH, partial inhibition by CO, and upon electron microscope examination, proliferation of smooth endoplasmic reticulum (SER) after induction by substrate administration. MEOS, however, did not significantly change when marked SER proliferation was induced by drugs other than ethanol, such as phenobarbital or butylated hydroxytoluene; this further illustrates the specificity of the increase in MEOS after ethanol feeding. In conclusion: a hitherto unrecognized hepatic microsomal ethanol-oxidizing system is described which is capable of adaptation to ethanol feeding and, unlike the classic ADH, is active at pH 7.4; therefore this microsomal system is a likely pathway for the "physiological" oxidation of ethanol.

186. Natriuresis during Pregnancy: Roles of Tubular Reabsorption (T) and Glomerular Filtration Rate (GFR). MARSHALL D. LINDHEIMER* AND PETER V. WESTON,* Cleveland, Ohio (introduced by Charles H. Rammelkamp**).

During pregnancy acute saline loading is followed by increases in filtered (F_{Na}) and excreted sodium ($U_{Na}V$). Since in the third trimester changing from the lateral to the supine position reduced GFR, this maneuver was used to determine the relative contribution of decreased T_{Na} and increased F_{Na} to postsaline natriuresis. Control observations were made in 15 mineralocorticoid-treated volunteers positioned in lateral recumbency. F_{Na} averaged 17.8 mEq/min and $U_{Na}V$ 220 μEq/min. Thereafter 4 liters of 0.5 N saline was administered over 2 hr, and measurements were repeated during the 2nd hour. F_{Na} was 19.6 mEq/min and $U_{Na}V$ was 415 μEq/min (measured in 13 subjects). After the load the patients assumed the supine position. F_{Na} fell to 16.1 mEq/min and $U_{Na}V$ to 179 μEq/min. F_{Na} decreased below control in 11 supine subjects after saline loading (-0.3 to -6.8 mEq/min). $U_{Na}V$ fell to or below control in 10 of these. In the other 4 subjects F_{Na} remained 0.8–2.6 mEq/min above control. $U_{Na}V$ still decreased to control levels. Thus assumption of the supine position blunted natriuresis despite volume expansion and independently of F_{Na}. During maximal water diuresis, changes in C_{urea}/C_{inulin} (C_{ur}/C_{in}) and in the portion of the filtrate excreted as free water ($C_{H_2O} \times 100/GFR$) relate inversely to the fraction of the GFR reabsorbed proximally. A decrease in this fraction increases the percentage of F_{urea} and F_{Na} that arrives distally where urea reabsorption virtually ceases and diluting sites are located. Consequently, C_{ur}/C_{in} and $C_{H_2O} \times 100/GFR$ increase. In our study, during the 2nd hour of infusion of 12 maximally hydrated subjects C_{ur}/C_{in} increased in 10 ($+0.03$ to $+0.15$), and $C_{H_2O} \times 100/GFR$ increased in all ($+1.49$ to $+7.45$ ml/min). Therefore salt loading of lateral-recumbent subjects resulted in a natriuresis and a decrease in the fractional reabsorption of filtrate at proximal nephron sites. Thus tubular adjustments independent of mineralocorticoid activity may be involved in renal sodium homeostasis during pregnancy.

187. Neoplasia Induced by Mycotoxins. DONALD B. LOURIA, J. KELLY SMITH,* AND GERALD C. FINKEL,* New York, N. Y.

Groups of CFW mice were given a combination of aflatoxins B-1 and G-1 by injection (100 μg), force feeding (100 μg), or aerosol (1 mg/ml) daily for 1 yr. Because *Alternaria* is readily visualized in all cigarette tobaccos on direct smear, other groups of mice were given 14 day culture supernatants of *Alternaria* or sonicated mycelia daily by aerosol. One of nine untreated control mice (11%) developed lymphatic leukemia during the 12 month experimental period. Of those treated by aerosol or force feeding, 15 died or because of sickness were sacrificed between the 5th and 12th months; the other 26 were sacrificed at the end of the 1 yr experimental period. Leukemia and/or lymphoma developed in 10 of 17 mice (59%) given aflatoxin aerosols and in 5 of 7 (71%) administered aflatoxins by force feeding; interestingly, none of the latter showed evidence of liver damage. Of those given sonicated *Alternaria,* 7 of 10 (70%) developed leukemia, as did all 7 mice given *Alternaria* medium supernatants. The increased incidence of leukemia is statistically highly significant ($P < 0.01$). Each of 7 mice given aflatoxin subcutaneously developed local fibrosarcomas in 5–10 months. Aflatoxins thus are capable of inducing local tumors directly, and both aflatoxins and *Alternaria* appear capable of increasing profoundly the incidence of neoplasia in a leukemia-lymphoma-bearing mouse strain, presumably by activation of tumor viruses. Analysis of tobaccos by chloroform-acetone extraction, preparative and thin-layer chromatography, ultraviolet and fluorescence emission analysis, and melting-point determinations has revealed that no aflatoxins are present. However, many tobaccos studied do contain a substance similar to, but not identical with, aflatoxin B-2. This substance is lethal for chicken embryos, its potency according to MLD determinations being approximately 1/89 that of aflatoxin B-1 and 2/3 of B-2.

188. Renal Interstitial Pressure during Exaggerated Natriuresis in Essential Hypertension. JEROME LOWENSTEIN,* ELLIOT BERANBAUM,* RICHARD H. MCSHANE,* HERBERT CHASIS,** AND DAVID S. BALDWIN,** New York, N. Y.

The mechanism responsible for decreased tubular reabsorption of sodium during exaggerated natriuresis in hypertensive man is unknown. Studies in animals have

related increased renal interstitial pressure to reduced net sodium transport in the proximal tubule. In the present study, we have found that calculated glomerular pressure and renal interstitial pressure as measured by wedged renal vein pressure (WRVP) are increased during exaggerated natriuresis in man. In five patients with early essential hypertension, retrograde catheterization of the renal vein was performed and a subselective catheter was "wedged" in a small venous branch. Control GFR and renal plasma flow averaged 113 ml/min and 413 ml/min respectively; WRVP averaged 26 mm Hg (range 23–27 mm Hg). After rapid intravenous administration (17.5 ml/min) of 2.5% saline, average sodium excretion increased from 305 μEq/min to 1680 μEq/min. GFR increased in four patients and was unchanged in one. Renal plasma flow increased in four. WRVP increased in each patient, averaging 53 mm Hg (range 34–66 mm Hg) at the peak of natriuresis. Mean arterial pressure increased from 125 to 136 mm Hg, while afferent arteriolar resistance decreased from 4911 to 3126 dyne sec cm^{-5}, and efferent resistance was unchanged. Calculated glomerular pressure increased from a mean of 74 mm Hg to 96 mm Hg. We have previously shown during solute diuresis with mannitol that renal interstitial pressure is increased and GFR is decreased, presumably as a consequence of increased tubular hydrostatic pressure. The hemodynamic response to saline differs in that a marked increase in glomerular pressure occurs and GFR does not fall, indicating that the increase in interstitial pressure during saline is due, at least in part, to a decrease in resistance between the systemic circulation and the renal interstitium. The demonstration that the renal hemodynamic response to saline is characterized by increased glomerular and renal interstitial pressures provides a possible explanation for the decreased reabsorption of sodium during exaggerated natriuresis in hypertensive man.

189. Relationship between Inotropic Activity and Myocardial Concentration of Ouabain. ROBERT J. LUCHI,* C. DICK PARK,* AND JOHN A. WALDHAUSEN,* Philadelphia, Pa. (introduced by Hadley L. Conn, Jr.**).

The purpose of this investigation was to measure myocardial concentration of ^{3}H-ouabain in relation to ouabain-induced increases in myocardial contractility in the empty paced heart. Eight dogs were studied. Cardiopulmonary bypass was instituted, with flow maintained at 100 cc/min per kg. Contractility was measured by a strain gauge (SG) sutured to the left ventricle (LV). ^{3}H-ouabain (0.046 mg/kg and 0.14 mg/kg) was injected into the oxygenator. Tritium was determined in LV biopsies and arterial plasma. An inotropic response was defined as an increase in SG amplitude 10% above control; "toxic" effects as sustained ventricular tachycardia or ventricular fibrillation (VF). Both maximum myocardial concentration of ouabain and maximum inotropic effect occurred in 20–30 min after drug injection. Minimum myocardial ouabain concentration associated with an inotropic effect was 7×10^{-7} mmole/g dry weight (gdw); maximum inotropic effect with $8–10 \times 10^{-7}$ mmole/gdw; tissue:plasma ratio of ouabain approximated 3:1 at 30 min. Toxicity was associated with 21×10^{-7} mmole ouabain/gdw. Thus, minimum "therapeutic" concentration of ouabain was approximately 30% of the "toxic" concentration. Acute reduction (25–50%) in ouabain concentration occurred with VF or AC countershock. Return of coordinated contraction in VF or cessation of countershock was accompanied by restoration of concentration of ouabain within 10 min. It has been inferred that digitalis acts in the area where myosin and actin filaments overlap. Ouabain concentration 7×10^{-7} mmole/gdw is approximately 20 times less than estimated myosin cross-bridge concentration in the myocardium. Assuming ouabain binding at cardiac membrane and other intracellular loci, the observed myocardial concentration of ouabain would seem to be too small to explain ouabain action at the myosin cross-bridge sites.

190. Depletion of Thyroidal ATP Accompanying Active Transport of Iodide. MORELLY L. MAAYAN,* Boston, Mass. (introduced by William B. Castle**).

Indirect evidence has indicated that the thyroidal active transport of iodide is somehow related to the metabolism of adenosine triphosphate (ATP), but direct evidence of this relationship and its nature has been lacking. In the present experiments, the relation between iodide transport and thyroidal concentration of ATP, as well as pyridine nucleotides, has been evaluated. In hypophysectomized rats, with or without exogenous TSH, ATP concentrations in thyroids of animals given a low iodine diet were higher than in those given a high iodine diet. In both intact and hypophysectomized rats, 250 μg of iodide given acutely decreased thyroidal ATP by approximately 50% in 30 min. This effect was evident, though less marked, within 5 min of iodide injection. Consistent, though lesser, effects followed administration of only 25 μg of iodide. The effect of acute iodide was not prevented by prior administration of propylthiouracil in doses sufficient to block organic binding. However, doses of thiocyanate or perchlorate sufficient to inhibit iodide transport, though themselves without effect on ATP, did prevent the ATP-lowering effect of acute iodide loads. Changes in pyridine nucleotides, principally in NADP, followed a pattern similar to that of ATP, suggesting that they may be secondary to ATP depletion. The findings indicate that increasing rates of active thyroidal iodide transport are associated with increasing depletion of thyroidal ATP. This effect, which is independent of TSH, may reflect the utilization of ATP during active iodide transport.

191. Hypervolemia in the Hyperviscosity Syndrome of Macroglobulinemia. M. R. MACKENZIE,* E. BROWN,* AND H. H. FUDENBERG, San Francisco, Calif.

Hyperviscosity syndrome (HVS) (mucus membrane bleeding, retinopathy, hearing loss, vertigo) occurs in 40% of patients with Waldenström's macroglobulinemia (WM). Expanded blood volume is a previously unrecognized manifestation of this syndrome. We measured

relative serum viscosity (RSV) and blood volume in 17 patients with WM, 6 without, and 11 with HVS. Of the latter, 6 were studied before and after therapy lowered their viscosity. The RSV was determined with an Ostwald viscometer. The ^{51}Cr-labeled cells and T1824 or ^{131}I-HSA were used to measure cell and plasma volumes respectively; 54 measurements were made in the 17 patients; none had cardiac failure. On the average, the patients with HVS and RSV greater than 4.0 had significantly larger mean total blood volume (5.13 ± 0.22 liters) than predicted for their sex, height, and weight (3.83 ± 0.07 liters) ($P < 0.001$). This was due to increased plasma volume, mean of 4.48 ± 0.22 liters, as compared with a predicted mean of 2.54 ± 0.05 liters ($P < 0.001$). The mean increased plasma volume in HVS was $79 \pm 5.3\%$ over predicted, whereas those WM patients without HVS had a plasma volume increase of only $38 \pm 7.2\%$ ($P < 0.001$). When RSV was plotted against plasma volume, a striking correlation was obtained, i.e., the higher the RSV, the greater the plasma volume. When the mean plasma volume of 6 patients with HVS was determined before and after the RVS had been lowered by plasmapheresis or drug therapy, there was a mean decrease of 45% ($P < 0.001$). Hyperviscosity symptoms were present only in patients with increased plasma volume. These data establish a relationship between high RSV, high plasma volume, and symptoms of HVS in Waldenström's macroglobulinemia.

192. The Regulation of Fatty Acid Biosynthesis in Rat Hepatomas. PHILIP W. MAJERUS* AND H. P. MORRIS,* St. Louis, Mo. (introduced by Carl V. Moore**).

The regulation of fatty acid synthesis in "minimum deviation" hepatomas 7777 and 9618A has been studied by comparing the acetyl CoA carboxylase (AC) and fatty acid synthetase (FAS) activities of tumor and host liver, both in animals starved 48 hr and in those subsequently refed a fat-free diet for 48 hr. These experiments demonstrate that neither AC nor FAS in hepatomas is subject to the normal dietary alterations observed in host liver. Thus, in host liver AC activity was stimulated 12- to 29-fold when starved animals were refed. In both tumors studied there was no difference in AC levels (1.5–1.3 mμmoles malonyl CoA formed per min per mg protein) as between starved and refed animals, the levels being 5- to 10-fold greater than starvation levels in host liver. Similarly, FAS levels in host livers increased 12- to 20-fold after refeeding, whereas in tumors no changes in FAS occurred. AC was purified 164-fold from rat liver and 67-fold from hepatoma to define this lack of regulation further. Studies of the purified enzymes disclosed that the tumor enzyme was essentially identical with that derived from liver in terms of heat inactivation, affinities for acetyl CoA and ATP, activation and aggregation by citrate, product inhibition by malonyl CoA, pH optima, intrinsic specific activity as determined from biotin content, and "feedback" inhibition by palmityl CoA. These experiments suggest that the AC from tumors is identical with enzyme from host liver and that the increased activity in the tumors results from an increased amount of enzyme rather than from a structurally altered enzyme. Therefore, the defective regulation of fatty acid synthesis may result from either altered rates of enzyme synthesis or degradation. The coordinate lack of regulation of all the enzymes in the pathway suggests that the alteration may be at the level of repressor control of enzyme synthesis.

193. Immunogenic Properties of Soluble Transplantation Antigens. DEAN L. MANN,* JOHN L. FAHEY, AND STANLEY NATHENSON,* Bethesda, Md., and New York, N. Y.

Previous studies of the biologic and biochemical properties of transplantation antigens have been hampered by being restricted to suspensions of lipoprotein membrane material. Recently, soluble isoantigens have been prepared from both mouse spleen cells and human lymphoid cell membranes by treatment with papain. The specific isoantigenic activity of the histocompatibility locus of the mouse (H-2) and of man (HL-A) is identified by cytotoxic inhibition tests. The two species show their respective H-2 and HL-A isoantigenic activity to be associated with glycoproteins of 60,000 to 70,000 molecular weight and have many other similar physical-chemical properties. Investigation of biologic (immunogenic) activity in these purified isoantigens was undertaken by testing the ability of mouse H-2 isoantigens to accelerate graft rejection. Soluble isoantigenic material prepared from C57B1/6 spleen cells (H-2^b) was given to B10D2 (H-2^d) mice which subsequently were challenged with C57B1/6 skin grafts. Single doses of 25 μg and 2.5 μg given intraperitoneally with Freund's adjuvant caused accelerated graft rejection (7.7 ± 1.37 and 8.1 ± 1.2 days respectively) as compared with control animals (11.3 ± 1.04 days). Intravenous injections 3 times per week for 3 wk and 6 wk also accelerated graft rejection. No accelerated rejection was observed when mice were challenged with a different H-2 skin graft (A/HeJ, H-2^a). This evidence clearly indicates that in addition to being isoantigens, these materials from mouse function with immunogenic specificity as transplantation antigens.

194. Lipid Absorption in Hamsters with Reduced Intraduodenal pH. CHARLES M. MANSBACH, II,* AND MALCOLM P. TYOR, Durham, N. C.

The effect of a reduced intraduodenal (ID) pH on intestinal lipid absorption was studied in hamsters given 1 N HCl in 0.15 M NaCl, 0.0068 ml/min, via gastrostomy before and during administration of corn oil + ^{14}C-triolein. The ID pH was 1–4, as compared with 7.5 in paired controls. Luminal pH in the distal half of acid-perfused hamster (APH) small intestine was 7.5. Triglyceride (TG) absorption, measured from ^{14}C-triolein radioactivity of intestinal mucosal scrapings, was reduced in the proximal and distal halves of APH. Residual ^{14}C-triolein in the lumen of the entire gastrointestinal tract was increased in APH. Absorption was maximal in the distal half of APH, whereas controls absorbed maximally

in the proximal half. Proximal mucosal homogenates from APH esterified ^{14}C-palmitate to ^{14}C-triglyceride normally. Despite similar concentrations of total lipid and trihydroxy bile salts in the two groups, there was a markedly decreased lipid content in aqueous phase of ultracentrifuged ID samples from APH. When the pH was raised from 2.0 to 7.5 in vitro, more lipid distributed into the aqueous. ID samples from APH showed a marked decrease in fatty acid content. However, raising the pH to 7.5 by addition of 5 N NaOH in vitro resulted in increased lipolytic activity of samples with an initial pH of 3.5–4.5, but not in those with an initial pH of 1.0–2.0. When aliquots of pooled ID contents (pH 7.5) obtained from saline-perfused hamsters were reduced to pH 3.5–4.5 and pH subsequently returned to 7.5, lipolytic activity was considerably less than that in fluid which had remained at 7.5. Impaired lipid absorption in hamsters with low ID pH may be attributed to decreased activity of lipase and a pH effect on the solubilization process. The observed recovery of lipid absorption in the distal small bowel of APH may be ascribed to the demonstrated ability to significantly reactivate lipase activity and to more efficient solubilization of the products of lipolysis.

195. The Profile of Tubular Sodium Reabsorption in Normal Man before and after Escape from a Mineralocorticoid and in Patients with Pathologic Sodium Retention. JOSEPH A. MARTINO* AND LAURENCE E. EARLEY, Boston, Mass.

Micropuncture studies in rats and dogs have demonstrated that fractional proximal tubular sodium reabsorption decreases independently of mineralocorticoids and GFR during volume expansion natriuresis, whereas distal reabsorption is enhanced. To determine whether similar changes in fractional reabsorption occur in man, studies were performed in seven normal volunteers using the technique of "distal blockade," which in the dog yields results similar to those of micropuncture. By sequentially administering chlorothiazide and ethacrynic acid with replacement of urinary losses, increments in sodium excretion were used to calculate fractional sodium reabsorption distal to the medullary loop of Henle (T_{Na}^{D}), in the medullary loop (T_{Na}^{L}), and in the proximal tubule (T_{Na}^{P}). On 50 mEq sodium diets, T_{Na}^{P} was 0.69 ± 0.04 (SD); T_{Na}^{L}, 0.24 ± 0.02; and T_{Na}^{D}, 0.05 ± 0.01. GFR averaged 119 ± 25 ml/min. During saline infusion after "escape" from 9-α-fluorohydrocortisone, T_{Na}^{P} decreased to 0.63 ± 0.02, but T_{Na}^{D} increased to 0.10 ± 0.03. T_{Na}^{L} was unchanged at 0.22 ± 0.04. GFR increased to 146 ± 18 ml/min. Quiet standing was antinatriuretic, as T_{Na}^{P} increased from 0.65 to 0.74, and GFR fell 14%. In seven cirrhotics retaining sodium, T_{Na}^{P} was 0.76 ± 0.04; T_{Na}^{L}, 0.20 ± 0.04; and T_{Na}^{D}, 0.04 ± 0.01. GFR averaged 79 ml/min. T_{Na}^{P} was 21% higher than in normals during mineralocorticoid escape. Restudy after forced sodium losses in two cirrhotics showed T_{Na}^{P} increased an additional 10% and 13%. Although GFR as a determinant of sodium excretion cannot be eliminated, these studies do indicate that fractional proximal reabsorption is increased in patients with cirrhosis retaining sodium, and depressed in normal man during mineralocorticoid "escape." During saline diuresis in the presence of a mineralocorticoid, most of the proximally rejected sodium is recaptured by reabsorption at the chlorothiazide-sensitive site distal to the medullary loop of Henle.

196. Heme and Methemoglobin: Naturally Occurring Inhibitors of Drug Metabolism. HARVEY S. MARVER,* San Francisco, Calif. (introduced by Rudi Schmid).

An oxidative pathway in liver microsomes governs duration and intensity of action of many drugs and endogenous steroids. In turn, the pathway is induced by these very compounds. We observed that hemin, methemalbumin, and methemoglobin reversibly depress normal levels, and block drug-mediated stimulation, of microsomal oxidation. In vivo, hemin prevents drug-stimulated adaptation as shown by prolonged sleeping times and reduced LD_{50}'s. Pretreatment of rats resulted in the following sleeping times after hexobarbital, in minutes: pretreatment with phenobarbital, 20; with phenobarbital and 4 μmoles/100 g hemin, 128; with saline, 65; with hemin, 136. Similarly, hemin reduced the LD_{50} of both hexobarbital and phenobarbital. This effect of heme results from repression of hepatic microsomal oxidation, as evidenced by studies in vitro. Aminopyrine demethylation in livers of treated rats ($\times$ controls) was: phenobarbital, 3.5×; phenobarbital and hemin, 0.9×; hemin, 0.6×. Parallel results were obtained with aniline, hexobarbital, and β-estradiol. These responses result from suppression by hemin in vivo of all measurable components of microsomal oxidation. These changes of cytochrome p-450 were observed ($\times$ controls): phenobarbital, 3.8×; hemin and phenobarbital, 0.8×; hemin, 0.6×. Cytochrome b_5, NADPH cytochrome c reductase, microsomal protein and phospholipid, and expansion of smooth endoplasmic reticulum were repressed analogously. Hemin also blocks stimulation of this pathway by chlordane, phenylbutazone, and 3,4-benzpyrene. The response to heme is initiated in microsomes. In vivo, microsomes both concentrate exogenous ^{59}Fe-heme and are the only organelles displaying reduced uptake of 2-^{14}C-phenobarbital after heme administration. Heme also represses drug-mediated induction of δ-aminolevulinic acid synthetase, which controls heme biosynthesis. Since this enzyme is induced by drugs to provide heme for cytochrome p-450, some consequences of heme administration may be linked to this effect. However, the multifaceted repression (including nonheme proteins) suggests that heme acts by interfering with uptake of inducer at extranuclear binding sites. This may represent an important mechanism for enzyme regulation.

197. Suppression of Parathyroid Activity by Magnesium Infusion. S. G. MASSRY, J. W. COBURN, L. W. CHAPMAN, AND C. R. KLEEMAN,* Los Angeles, Calif.

An interrelation between magnesium metabolism and parathyroid activity has been postulated. The effect of

elevated serum magnesium on parathyroid function, assessed by changes in (1) phosphate/creatinine clearance ratios (C_p/C_{cr}) and (2) serum calcium levels, was studied in 13 dogs given $MgCl_2$ infusions. The influence of diurnal variation on C_p was excluded by pairing each study with a control infusion without $MgCl_2$ and by performing experiments both in the morning (seven dogs) and in the afternoon (four dogs) hours. During magnesium infusion, serum phosphate rose progressively. Despite this rise, C_p/C_{cr} fell in all experiments, reaching 5–20% of the value observed in the paired control experiment. Serum calcium fell by 1–2 mg/100 ml during magnesium infusion, with proportionate changes in the diffusible and ionized fractions. A significant fall in C_p/C_{cr} occurred at a serum magnesium of 3.5–4.0 mg/100 ml, a rise of only 2.0 mg/100 ml. The pattern of fall in C_p/C_{cr} during magnesium infusion was identical with that observed during $CaCl_2$ infusion (seven dogs) or in the hours immediately following thyroparathyroidectomy (seven dogs). When parathyroid extract was injected into four dogs receiving $MgCl_2$ infusion, both C_p/C_{cr} and serum calcium rose. In two thyroparathyroidectomized dogs, magnesium infusion did not affect C_p/C_{cr} or serum calcium. These data indicate that hypermagnesemia, like hypercalcemia, suppresses parathyroid hormone activity by inhibiting production and/or release of the hormone without interfering with end organ response. The possibility that other divalent cations may share this effect is under investigation.

198. Cholesterol Loading of Acanthocytic Red Cell Membranes Causing Hemolytic Anemia in Experimental and Genetic Abetalipoproteinemia. JOHN A. McBRIDE* AND HARRY S. JACOB, Boston, Mass., and London, England.

Lipid fluxes between plasma and RBC membranes may critically affect RBC shape and survival. Altered lipid interchange possibly underlies acanthocytosis in liver disease, in hereditary abetalipoproteinemia, and in fat-deprived newborns. In one such infant, studied with F. A. Oski, plasma betalipoprotein remained at fetal levels (one-fifth normal), and acanthocytosis with severe hemolytic anemia ensued after 1 month of lipid-free intravenous feedings. Accompanying acanthocytogenesis, RBC cholesterol increased 25%; other lipids were unaffected. Acanthocytes from two hereditary abetalipoproteinemics were similarly cholesterol laden. Mechanisms for these changes were sought in rats rendered abetalipoproteinemic by orotic acid (OA). Confirming the findings of others, immunoreactive betalipoprotein disappeared 5 days after feeding 1% OA; other lipoproteins were unaltered. Biconcave RBC progressed through target forms to typical acanthocytic forms during the following month. Osmotic fragilities decreased 4 SD, and ^{51}Cr-labeled RBC survivals diminished 35% with enhanced splenic entrapment. Of RBC membrane lipids, only cholesterol changed (increased 20–50%). The resulting wrinkled RBC lost deformability, manifested as increased cone plate viscosity. Reflecting the cholesteroltropism of polyene antibiotics, amphotericin quadrupled sodium accumulation and osmotic fragility in acanthocytic, relative to normal, RBC. Rabbits fed excessive cholesterol demonstrated identical cholesterol-loaded acanthocytes and hemolytic anemia. Rates of RBC ^{14}C-cholesterol efflux slowed 30% in abetalipoproteinemic rat or human plasmas. Incubating rat acanthocytes in normal plasma normalized membrane cholesterol and osmotic fragility. Cholesterol efflux also diminished from OA rat livers, increasing hepatic cholesterol 50%. We conclude that membrane cholesterol accumulation, by generating increased surface, produces wrinkling, altered rheology, and thereby premature reticuloendothelial destruction of RBC. Plasma betalipoprotein, reflecting its marked cholesterol affinity, prevents excessive RBC membrane (and hepatic) sterol accumulation. Its deficiency, genetically or in infants ingesting no fats, or its alteration causing spur RBC in cirrhosis, hampers cholesterol efflux from RBC membranes with attendant acanthocytogenesis and hemolytic anemia.

199. Alterations in the Ultrastructure and Lysosomal Activity of Human Neutrophils during Bacterial Infections. CHARLES E. McCALL,* ISA KATAYAMA,* AND RAMZI COTRAN,* Boston, Mass. (introduced by Maxwell Finland**).

Morphologic alterations in blood neutrophils frequently accompany serious bacterial infections. Identified by a Romanowsky stain, these "toxic" neutrophils contain combinations of Döhle bodies, dark blue inclusions known as "toxic" granules, and vacuoles. Studies of toxic neutrophils from 12 patients with bacterial infection have revealed changes in cytoplasmic ultrastructure and lysosome activity. Serial sections studied by light and electron microscopy facilitated identification of specific inclusions. Toxic neutrophils often contained lamellar aggregates of rough endoplasmic reticulum, which in some cells could be identified as Döhle bodies. A prominent Golgi complex was also frequently present. Some vacuoles were autophagic bodies. Toxic granules consisted of large electron-opaque granules which could be differentiated from the smaller, less dense "specific" granules. Such large granules were also present in neutrophils of controls and in neutrophil precursors, suggesting that alterations in the membranes or internal composition of these granules might result in their light-microscope staining characteristics in toxic neutrophils. Leukocytes were lysed and homogenized in 0.34 M sucrose and separated by ultracentrifugation into lysosomal and soluble fractions. Alkaline phosphatase of toxic cells was 8 times the control; 80% of the activity of both was located in the lysosomal fraction. Beta glucuronidase was normal. Total acid phosphatase was normal, but the percentage located in the nonlysosomal fraction of toxic neutrophils was increased, suggesting that lysosomes were "labilized." Formation of neutral red vacuoles in supravitally stained preparations, an index of lysosome activity, occurred more rapidly in toxic neutrophils than in controls. This reaction paralleled the formation of clear vacuoles and degranulation in unstained wet mounts and could be blocked by colchicine, a lysosome stabilizer. These observations suggest that altered lysosome pro-

duction and heterogeneity of lysosomal composition and activity are characteristic of toxic neutrophils, infection resulting in either immaturity or transformation of neutrophilic cytoplasm.

200. Genetic Controls in the Immune Response. HUGH O. MCDEVITT* AND MARVIN L. TYAN,* Palo Alto and San Francisco, Calif. (introduced by Halsted R. Holman).

The occurrence of immunological abnormalities in relatives of patients with lupus erythematosus, and the genetic predisposition to a similar disease in NZB mice, suggest that genetic factors underlie certain immunological diseases. However, the exact nature of these genetic factors is unknown. It has been shown that the ability of C3H, CBA, and C57 mice to respond to branched, multichain synthetic polypeptide antigens with antigenic determinants composed of tyrosine and glutamic acid ((T,G)-A—L) or histidine and glutamic acid ((H,G)-A—L) is a dominant genetic trait which is specific for the amino acid composition of the antigenic determinant. In the present study, the ability to respond well to (T,G)-A—L was successfully transferred in 19 of 28 cases by the transfer of 125×10^6 high-responder (C3H $\times$ C57B1/6) F_1 normal spleen cells into lethally irradiated low-responder C3H parental recipients. The transfer of a secondary immune response to (T,G)-A—L by similar transfer of preimmunized spleen cells was uniformly successful. The anti-(T,G)-A—L response of several inbred strains of mice revealed a correlation between the major histocompatibility (H-2) type and high response. Linkage tests in a segregating (CBA $\times$ C57) $F_1 \times$ CBA backcross population showed close linkage between anti-(T,G)-A—L response and H-2 type. These results indicate that the mechanism of genetic control of the immune response to (T,G)-A—L is directly related to the process of antibody formation and is a property of one of the cell types in the spleen cell population. Genetic differences in immune response to specific antigens do exist, and these genetic differences operate at the level of antibody formation. Such genetic factors may be of great importance in susceptibility to infectious disease, and in the development of diseases of immunological abnormality.

201. Intolerance of Diabetic Patients to Positive K^+ Balance Despite Enhancement of Glucose Disappearance Rate. JOHN MCNAY,* K. ROGER HORNBROOK,* AND ERDOGAN ORAN,* Atlanta, Ga. (introduced by Leon I. Goldberg).

K^+ administration has been shown to improve glucose tolerance in conditions characterized by potassium depletion, such as hyperaldosteronism and chronic thiazide administration. Since reduced total body K^+ is also found in diabetes mellitus, we compared the effects of the K^+-retaining diuretic MK 870 in 18 hypertensive patients receiving hydrochlorothiazide, 4 normal patients, and 4 adult-onset diabetics. The latter group was characterized by insulin secretion significantly lower than that of the other two groups. Insulin secretion was assessed by calculating the ratio of serum immunoreactive insulin to mg blood glucose (IRI/G), 20 min after intravenous glucose injection. Metabolic ward balance studies confirmed positive K^+ balance in each type of patient. There was an increase in the fractional disappearance rate of intravenous glucose (Kg) in 22 of 25 patients, mean 0.29 min^{-1}, $P < 0.001$. A relationship between increased IRI/G and increased Kg was suggested by the following: Kg increased in 10 of 12 patients who had an increase in IRI/G; IRI/G increased in 10 of 13 patients with increased Kg. During MK 870 administration, hyperkalemia (serum $[K^+]$ 6.3, 6.8, and 7.2 mEq/liter) developed in 3 of the 4 diabetic patients, none of whom had significant renal impairment. The mechanism of this effect may be related to the observation that the serum $[K^+]$ response of the normal and diabetic patients to glucose was altered during MK 870 administration. Rather than a decrease in serum $[K^+]$, a significant increase ($P < 0.05$) was seen, amounting to 1.8 and 1.3 mEq/liter in 2 of the diabetic patients. This effect was not observed in the presumably potassium-depleted hydrochlorothiazide-treated patients. The known K^+ depletion of diabetic patients is probably secondary to abnormal carbohydrate metabolism rather than the converse as in hyperaldosteronism and chronic thiazide administration, and attempts to correct it may cause dangerous hyperkalemia.

202. Release of Kinins during Wheal and Flare Allergic Skin Reactions. BENO MICHEL,* THOMAS RUSSELL,* R. K. WINKELMANN,* AND GERALD J. GLEICH,* Rochester, Minn. (introduced by Ward S. Fowler**).

Histamine has long been regarded as the most important mediator of wheal and flare cutaneous reactions in allergic individuals. However, kinin release had been demonstrated by dermal perfusion in dermographism, cold urticaria, and the whealing of urticaria pigmentosa. This raised the possibility that kinin was released in allergic skin reactions as well. We examined wheal and flare skin reactions by dermal perfusion and tested perfusates for histamine (atropinized guinea pig ileum) and for kinins (estrogenized rat uterus). Six allergic subjects were skin tested with allergens, such as *Alternaria,* ragweed, and cat hair, to which they were known to be sensitive. 11 perfusates were examined for biologic activity. All were active on the rat uterus, but none was active on the guinea pig ileum. One perfusate was analyzed by gel filtration on Sephadex G-25; the activity eluted at a volume similar to that of synthetic bradykinin. The kinin-like activity in the perfusate appeared after the wheal reached maximal size (4–18 min). Evans blue injected intravenously before skin testing in four patients appeared in the wheal before kinin could be detected in the perfusates. One reason for the delay might be the time required for generation of a measurable quantity of kinin. These results provide evidence for kinin release during wheal and flare allergic skin reactions in man.

203. Effect of Adrenergic Blockade on Tracheal Smooth Muscle Response to Histamine, Mecholyl, Anaphylaxis, and Catecholamines. ELLIOTT MIDDLETON, JR.,* AND STANLEY R. FINKE,* New York, N. Y. (introduced by Nicholas P. Christy).

Previous investigations demonstrated that pharmacologic beta adrenergic blockade (BAB) may aggravate bronchial asthma and increase sensitivity of guinea pigs to histamine (H), mecholyl (M), and anaphylaxis. The present experiments tested whether these observations hold for isolated guinea pig tracheal smooth muscle. Tracheal segments from Hartley strain animals were suspended from a linear motion transducer in Tyrode's solution containing glucose. Isoproterenol (I; 3×10^{-9} to 2×10^{-7} M), epinephrine (E; 10^{-6} to 10^{-5} M), and norepinephrine (NE; 5×10^{-7} to 10^{-5} M) all induced equivalent relaxation. A semilogarithmic plot of concentration versus maximum slope of relaxation curve showed a linear relationship. Drugs which cause BAB, propanolol (10^{-5} M) and MJ1999 (3×10^{-5} M), competitively blocked catecholamine-induced relaxation. NE (5×10^{-5} M) often caused weak contraction of muscles blocked with MJ1999 (10^{-3} M). Contractions of tracheal muscle by H (0.8×10^{-6} M), M (10^{-7} M), or antigen (BSA-sensitized animals) were not augmented by BAB. Addition of NE to these beta-blocked systems did not enhance contraction. High concentrations of theophyllin (10^{-4} M) relaxed normal trachea, and in the presence of subrelaxing concentrations (10^{-5} M) NE-induced relaxation was augmented. Adenosine-3',5'-monophosphate (cyclic AMP; 4×10^{-5} M) alone had no effect on tracheal muscle, but with theophyllin (10^{-5} M) relaxation occurred. Tracheas which failed to relax with NE in the presence of beta blockade (MJ1999) relaxed when theophyllin (10^{-5} M) and cyclic AMP (4×10^{-5} M) were added sequentially to the bath, indicating that the muscle-relaxing mechanism was intact in the face of BAB. Also hydrocortisone (F; 3×10^{-6} M) partially returned responsiveness to NE in beta-blocked muscle (MJ1999), suggesting that F modulates the activity of the beta adrenergic system leading to relaxation. Alpha adrenergic blockade (AAB) induced with phentolamine (5×10^{-5} M) or dibenzyline (2×10^{-5} M) enhanced relaxation induced by NE, I, or E. AAB partially reversed previously established BAB.

204. The Effect of Tissue Destruction on the Autoimmune Response in NZB Mice. P. A. MIESCHER, H. D. FLAD,* J. H. L. PLAYFAIR,* AND A. GHAFFAR,* London, England, and New York, N. Y.

The present concept of the etiology of SLE entails a genetic predisposition and possibly an exogenous trigger mechanism as well. NZB mice behave in many respects like SLE patients. They form autoantibodies and tend to develop depositions of immunoglobulin and complement components on the glomerular basement membranes. This study has investigated the possible role of a trigger mechanism in the "spontaneous" disease of NZB mice less than 8 wk of age, i.e. before the appearance of immunoglobulin deposits in the kidney. CCl_4 was chosen as a trigger, because it produces tissue destruction, and has been shown to induce a self-limited "autoimmune" response in rats and rabbits. The immune response was assessed by complement fixation test using liver homogenate as the antigen. NZB and Balb/c (nonautoimmune) mice were injected with CCl_4 at 4, 6, and 12 wk of age. In the older mice, identical "autoimmune" titers were obtained in both strains, whereas in the 4 wk old animals, NZB mice gave significantly higher responses than Balb/c mice. A smaller group of C57B1 mice gave responses significantly lower than Balb/c mice. After a second injection of CCl_4, the titers in all three strains rose. In the 12 wk old mice, only the NZB animals showed a rise after the second injection. In mice injected with CCl_4, immunoglobulin deposition in the glomeruli was found in 0 out of 5 C57B1, 4 out of 24 Balb/c, and 21 out of 24 NZB mice. 2 out of 10 nontreated NZB mice exhibited light staining of immunoglobulin in the glomeruli. These studies support the assumption that a trigger mechanism may induce "autoimmune" disease in predisposed subjects.

205. Hypertriglyceridemia of Endogenous Origin Produced by Feeding *Trans*-Isomerized Trilinolein. MAURICE A. MISHKEL* AND NORTON SPRITZ, New York, N. Y.

There is evidence that geometric isomerism of dietary fats plays a role in their effects on plasma lipids. To investigate this phenomenon further, various fats were fed, as 40% of calories in formula feeding, to five subjects maintained at constant weight. On saturated or *cis*-unsaturated fats, their fasting triglyceride concentrations averaged 60–275 mg/100 ml. Isocaloric substitution with 50–100% *trans*-trilinolein (TT), a synthetic oil containing 65% di-*trans* and 35% mono-*trans* octadecadi-9,12-enoic acid, produced triglyceride levels 1.6–3.9 times the control values. During isocaloric fat-free feeding, one subject had a 2-fold triglyceride rise, compared with a 3.9-fold increase with TT. Two types of evidence indicated that hypertriglyceridemia produced by TT was of endogenous origin: (1) In all subjects there was a marked increase in pre-beta lipoproteins. (2) Even when dietary fat was 100% TT, pre-beta lipoprotein triglyceride never exceeded 50% *trans* fatty acids, fasting or postprandially; yet chylomicrons produced by TT closely resembled the dietary fat. During TT feeding, plasma lipoprotein lipase concentration was normal and, in in vitro incubations, the enzyme showed comparable activity against *trans* and other fatty acids. In rats, fat feeding suppresses the high level of hepatic lipogenesis induced by carbohydrate. If *trans* fats lack this suppressive effect, the hypertriglyceridemia seen with TT in man could reflect excessive triglyceride synthesis. To test this possibility, we measured hepatic triglyceride synthesis after the administration of TT or *cis*-trilinolein in rats fed a high carbohydrate diet. However, both fats suppressed conversion of isotopic glycerol and acetate to triglyceride; indeed, TT produced significantly greater suppression. Thus, both high carbohydrate and TT feeding produce endogenous hypertriglyceridemia: the former is associated with maximal and the latter with minimal

lipogenesis. Therefore, the hypertriglyceridemia of TT appears to result from impaired removal of endogenous particles through some pathway other than hydrolysis by lipoprotein lipase.

205. Studies with Ion Exchange Calcium Electrodes: The Distribution of Ionized Calcium between Blood and Cerebrospinal Fluid (CSF) in Man and Dog. EDWARD W. MOORE* AND ANDRE L. BLUM,* Boston, Mass. (introduced by Thomas C. Chalmers**).

Neurologic derangements frequently accompany hyper- and hypocalcemia. The mechanisms governing CSF calcium ion concentrations [Ca^{++}] may therefore have clinical as well as physiological importance. Previous studies have yielded CSF/plasma chloride ratios of about 1.20. If [Cl] is passively distributed, 1.20 represents the Gibbs-Donnan ratio (g), and the expected electrical potential between plasma and CSF is about 5 mv (CSF^+), a figure found experimentally in dog and goat by Held and coworkers. If [Ca^{++}] is passively distributed, the expected CSF/plasma [Ca^{++}] ratio is about 0.69: $g = 1.20 = [Cl]_{CSF}/[Cl]_{pl} = ([Ca^{++}]_{pl}/[Ca^{++}]_{CSF})^{\frac{1}{2}}$. Previous studies, in which only *total* calcium [Ca] was measured, have yielded CSF/plasma ratios of only 0.5; it has therefore been held that calcium is actively transported out of CSF. Studies of venous serum and CSF were made in five hospitalized patients (lumbar fluid) and five anesthetized mongrel dogs (cisternal fluid). As in previous studies, *total* calcium CSF/plasma ratios were 0.52 ± 0.01 (SE) in man and 0.52 ± 0.01 in dog, whereas respective [Cl] ratios were 1.23 ± 0.01 and 1.19 ± 0.01. In contrast, CSF/serum *ionized* calcium ratios were 0.73 ± 0.01 (man) and 0.83 ± 0.02 (dog). Respective g ratios for [Ca^{++}] of 1.17 ± 0.01 and 1.10 ± 0.01 were significantly lower than respective g ratios for [Cl] in both man ($P < 0.05$) and dog ($P < 0.02$). [Ca^{++}] levels in CSF were thus higher than those predicted for a passive distribution. These [Ca^{++}] values were not an electrode artifact, since simultaneous serum ultrafiltration studies yielded similar g ratios for [Ca^{++}] and [Cl]: in man, $g_{Ca^{++}} = 1.21 \pm 0.03$, $g_{Cl} = 1.20 \pm 0.03$; in dog, $g_{Ca^{++}} = 1.15 \pm 0.04$, $g_{Cl} = 1.22 \pm 0.05$. In conclusion, total calcium measurements provide an unreliable index of calcium ion distribution phenomena. Direct measurements of Ca^{++} contradict the previous suggestion that calcium is actively transported out of CSF. Whether observed ratios represent a simple diffusion process is problematical, since soluble calcium complexes possessing electric charge may also be present.

207. The Use of ^{14}C–Sodium Taurocholate to Estimate Hepatic Plasma Flow. THOMAS Q. MORRIS,* New York, N. Y. (introduced by Stanley E. Bradley**).

Measurement of hepatic plasma flow has usually required the administration of dyes or foreign particulate substances which are removed from the plasma by the liver. In certain conditions hepatic uptake of these indicators has proved deficient. ^{14}C–sodium taurocholate was employed to assess the feasibility of using bile salts as test substances in the estimation of hepatic plasma flow. Measurements of hepatic plasma flow and biliary clearance of taurocholate were made under Nembutal anesthesia in dogs which had previously undergone cholecystectomy and implantation of a Thomas duodenal cannula to permit accurate collection of bile. The hepatic vein was catheterized via the external jugular vein. Throughout all studies a 1.3% solution of sodium taurocholate containing tracer amounts of the labeled bile salt was infused continuously. In eight animals the hepatic extraction of taurocholate during infusion rates of 10–12 μmoles/min averaged 87% (range 76–100%). To confirm this avid hepatic uptake of taurocholate, simultaneous measurements of estimated hepatic plasma flow and biliary clearance of taurocholate were made during a steady state in five dogs. If hepatic extraction of taurocholate actually approximated 100%, good agreement between the indirect estimate of hepatic plasma flow and the direct determination of biliary clearance would be found. This proved to be true with an average difference of less than $\pm$ 10%. General usefulness of this technique was underscored by its continued reliability during hemorrhagic hypotension, canine hepatitis, partial biliary obstruction, and glucagon administration. Because of this efficient extraction, measurement of the plasma clearance of ^{14}C–sodium taurocholate, calculated as the hepatic uptake divided by the peripheral concentration, yields an accurate estimate of hepatic plasma flow without requiring anesthesia, hepatic vein characterization, or administration of a foreign indicator.

208. Hypergammaglobulinemic Renal Tubular Acidosis: A Spectrum of Physiological Disturbances. R. CURTIS MORRIS, JR.,* A. SEBASTIAN,* E. MORRIS,* AND I. UEKI,* San Francisco, Calif. (introduced by I. S. Edelman**).

In patients with so-called hypergammaglobulinemic renal tubular acidosis (RTA), the acidification process of the proximal tubule has not been implicated because the measured $TmHCO_3^-$ of patients affected has not been subnormal. Demonstration of a $TmHCO_3^-$ of $< 85\%$ of normal would presumably implicate the proximal tubule, since it accounts for 85–90% of the renal reabsorption of HCO_3^- in the monkey and rat. We measured $TmHCO_3^-$ in five patients with RTA and hypergammaglobulinemia: two women with Sjögren's syndrome (SS_1, SS_2), one with hypergammaglobulinemic purpura (HP), one with idiopathic hypergammaglobulinemia (IH), and a man with multiple myeloma (MM). $TmHCO_3^-$ was only minimally reduced or normal in SS_1, HP, and IH (2.4, 2.6, 2.7 mEq/100 ml glomerular filtrate; normal, 2.6–2.8) but moderately reduced in MM (2.1) and strikingly reduced in SS_2 (1.6). During acidosis, urinary pH persisted higher than 6 in all patients except MM, whose urinary pH decreased to 5.8. The significant reduction of $TmHCO_3^-$ in MM and SS_2 characterizes an acidification defect physiologically distinct from that of SS_1, HP, and IH. The 35% reduction of $TmHCO_3^-$ in SS_2 unequivocally implicates the acidification process of the proximal nephron. Only the patients with significant reduction of $TmHCO_3^-$ (MM, SS_2) had amino-

aciduria and increased clearances of phosphate and urate, indicating associated proximal tubular dysfunction; only these had increased urinary excretion of immunoglobulin light chains and lysozymuria. In SS_2, $TmHCO_3^-$ decreased in 2 months from 2.0 to 1.6 without change in GFR; >3 mEq/kg per day of alkali did not correct acidosis. One mEq/kg per day of alkali corrected the acidosis of SS_1, HP, and IH. MM required an intermediate amount of alkali. These findings indicate that in "hypergammaglobulinemic RTA" more than one kind of physiologic disturbance can operate. The difference in kind has clear pathogenetic and therapeutic implications.

209. Muscle Adaptation to Exercise in Man: Effects on Glycogen and Lipid. THOMAS E. MORGAN,* FLOYD A. SHORT,* AND LEONARD A. COBB,* Seattle, Wash. (introduced by J. Thomas Dowling).

Exercise training in man has been studied for many years, but there have been few studies of specific biochemical adaptations in skeletal muscle. We have studied the effect of unilateral isotonic quadriceps exercise on concentrations of glycogen, total phospholipid, phosphatidyl choline, cholesterol, and triglyceride in the vastus lateralis of 10 normal men. After 4 to 6 wk of daily training, the trained and the contralateral untrained legs were biopsied. Glycogen, phospholipid, phosphatidyl choline, and triglyceride concentrations were higher in trained resting muscle but cholesterol was lower. In each subject the phosphatidyl choline:cholesterol ratio was greater in the trained than in the untrained muscles. All differences were highly significant ($P < 0.01$). Bergstrom and Hultman have shown an increase in muscle glycogen concentration after acute exercise. Our data indicate that this glycogen increase persists during long-term exercise training and that the effect is local rather than humoral. Holloscy has suggested that mitochondria increase in size or number in exercised rats. The lipid changes in our study suggested alterations in cellular membranes in which phosphatidyl choline and cholesterol are major components. Cell fractions prepared from human muscle showed a high phosphatidyl choline: cholesterol ratio for mitochondria. The observed increase in phosphatidyl choline and decrease in cholesterol is therefore compatible with increased mitochondrial size or number and decreased total cell cholesterol. (Simple dilution of cholesterol in an increased cell mass was not demonstrated.) Although there was wide variation in triglyceride concentration, and contamination by adipose tissue is a possibility, triglyceride was consistently higher in trained than in untrained muscle. The results are consistent with the hypothesis that exercise training induces local alterations in muscle mitochondria and in stores of energy substrates.

210. The Relationship of Erythropoietin Effectiveness to the Generative Cycle of the Erythroid Precursor Cell. BERNARD S. MORSE,* NICHOLAS J. RENCRICCA,* AND FREDERICK STOHLMAN, JR., Boston, Mass.

To evaluate the mechanism of action of erythropoietin (EP), the effect of this hormone was studied in hypertransfused animals treated with hydroxyurea (OH). Hydroxyurea is a rapidly acting and metabolized agent which has a selective lethal effect on cells in DNA synthesis (S). A single i.v. dose caused a partial erythroid depopulation and reticulocytopenia in normal mice. ^{3}H-thymidine incorporation into marrow DNA was immediately inhibited, and the erythroid stathmokinetic index remained at or near zero levels for about 7 hr; thereafter colchicine-arrested erythroid mitoses reaccumulated. This interval is consistent with the progression of unaffected cells in G1 through S (6 hr), G2 (1 hr), and prophase, and lends cogent support to the thesis that OH completely eradicates erythroid cells in S. To determine the effect on erythroid precursor cells, OH and EP were given simultaneously to hypertransfused CF mice. A subnormal but prolonged erythroid response was observed in OHEP-treated as compared with EP-treated mice. Marrow nucleated erythroid cells $\times 10^6$ per tibia in untreated controls were 0.1. After EP they were 0.2 on day 1, 2.3 on day 2, 0.7 on day 3, 0.3 on day 4, and after OHEP they were 0.3 on day 1, 1.5 on day 2, 1.5 on day 3, and 0.3 on day 4. The response to EP in OH-treated hypertransfused mice suggests that the immediate precursor compartment is in cycle in the hypertransfused animal; and that a small portion of such cells in the erythropoietin-responsive state are destroyed, but that the major portion of such cells are in either a prolonged G1 or G0.

211. Clinical Experience with a New Assay for Serum Testosterone. B. E. P. MURPHY,* Montreal, Canada (introduced by J. S. L. Browne**).

Serum is known to contain a globulin which binds only estradiol and testosterone and, to a lesser extent, some other closely related steroids, but not cortisol, progesterone, or aldosterone. This property has permitted it to be used to measure subnanogram amounts of the sex hormones by competitive protein-binding radioassay. The testosterone of 0.3 ml of plasma was extracted into ether and assayed using tritiated testosterone as the tracer, and late pregnancy plasma as the source of binding protein. Florisil was used to adsorb the unbound fraction. In serum the mean $\pm$ SD in 18 healthy men was 0.69 ± 0.19 μg/100 ml; in 18 hospitalized elderly men, 0.63 ± 0.15 μg/100 ml; in 15 healthy premenopausal women, 0.18 ± 0.06 μg/100 ml; and in 10 women with idiopathic hirsutism, 0.44 ± 0.13 μg/100 ml. Two women in whom adrenocortical function was suppressed with dexamethasone had values indistinguishable from zero. High values were found in one woman with congenital adrenal hyperplasia, in two women with Cushing's syndrome, and in pregnant women. Similar studies were carried out in urine, but the differentiation between groups was less satisfactory. The values for serum testosterone in men compare well with those obtained by other methods, but those for women are slightly higher. This assay can be carried out in 3 hr, and 20 samples can be processed by one technician in one day. Since the very small volumes of blood required permit serial sampling, hitherto impossible, this assay promises to be useful in physiologic

studies in animals and man besides providing a diagnostic tool.

212. **Deleterious Effect of Low Temperature on Platelet Viability.** SCOTT MURPHY* AND FRANK H. GARDNER,** Philadelphia, Pa.

Previous studies have indicated that the human platelet rapidly loses viability when stored at 4°C either as platelet-rich plasma (PRP) or as a concentrate. The following data indicate that low temperature in itself is harmful and that storage at 22°C permits longer maintenance of viability. PRP, obtained from ACD whole blood, was stored in plastic bags at 22°C and 4°C. Percentage yield of platelets circulating in normal volunteers during the first 3 hr after infusion, and platelet life-span, were determined after infusion of ^{51}chromium-labeled autologous platelets prepared from acidified PRP. Control studies without a storage interval demonstrated an average yield of 66% (seven studies) followed by a linear life-span to a $t_{\frac{1}{2}}$ of 3.0–4.5 days. 10% of circulating ^{51}chromium remained at 7–8 days. When PRP was stored at 22°C for 6 and 16 hr (six studies each), average yields were reduced to 57 and 58% respectively with life-spans identical with that of the control. When PRP was stored at 4°C for 6 hr (six studies) and 16 hr (eight studies), yields were reduced to 52 and 45% respectively. With 6 hr storage at 4°C, the life-span was exponential, reaching $t_{\frac{1}{2}}$ at 1.7–2.8 days and 10% on days 7–8. With 16 hr storage at 4°C, life-span was markedly shortened to a $t_{\frac{1}{2}}$ less than 2 days, and always reached 10% before day 3. Platelet ATP levels declined over 16 hr by an average of 12% both at 4°C and at 22°C, suggesting that the damage induced by low temperature is not related to inadequate maintenance of high-energy phosphate stores. When platelets were stored for 40 hr at 22°C (nine studies), the average yield was 33%, followed in six cases by a normal life-span and in three cases by a shortened life-span reaching 10% in 3–4 days.

213. **Afterload and Force-Velocity Relationships in Acute Experimental Anemia.** JOHN F. MURRAY, San Francisco, Calif.

Afterload can be defined as the total force encountered by the left ventricle during mechanical systole. Variations in afterload might occur in acute normovolemic anemia secondary to changes in ventricular wall dimensions, which affect tension, and the decreased outflow resistance from the reduction in whole-blood viscosity. The purpose of this study was to measure afterload and to separate the influence of changes in diastolic volume, which would affect force developed during isometric contraction, from the influence of changes in outflow resistance, which should affect the rate of decline in force during the ejection phase. Mercury strain gauges were sutured around the left ventricle (LV) and Hefner force gauges, designed to measure force during a changing load, were sutured over a deep slit in the LV of seven dogs. Two additional dogs were studied intact several weeks after implantation of flow probes, pressure transducers, cannulas, and, in one, tantalum screws to define the inside dimensions of the LV. Two stages of anemia were studied (A1 and A2). Systolic force reached a peak during isometric contraction and then declined during the ejection phase. Because end diastolic volume increased, peak systolic force increased 35% (A1) and 67% (A2). The rate of decline of force during ejection and total area under the force-time curve (afterload) increased in anemia. In the three animals force-velocity relations could be compared at isosegment lengths, and no evidence of an increase in contractility was observed. These data show that in acute experimental anemia (1) an increase in end diastolic fiber length causes an increase in peak systolic force during isomeric contraction, (2) systolic force rapidly diminishes during the ejection phase, reflecting the reduction in outflow resistance, and (3) total systolic force (afterload) increases rather than decreases as has been reported.

214. **The Effect of Gastric Secretions on Iron Absorption.** M. J. MURRAY* AND NELL STEIN,* Minneapolis, Minn. (introduced by S. Schwartz**).

Gastric juice from patients with iron-deficiency anemia and hemochromatosis has been observed to promote iron absorption in biological systems. The present study investigated the effects of gastric juice from other types of anemia and liver disease. Gastric juice was collected after stimulation with betazole from normal humans and from patients with pernicious anemia, hypoplastic anemia with hemosiderosis, hemolytic anemia, iron-deficiency anemia, and nonanemic portal cirrhosis, and was brought to a standard pH of 1.2 by the addition of 0.1 N HCl. Each sample was tested for its effect on iron absorption by adding 2 ml to 0.25 mc of ^{59}Fe as ferrous citrate and carrier and then giving the mixture by intubation to five totally gastrectomized rats previously bled repeatedly to hemoglobin level near 9 g. The per cent absorption of ^{59}Fe was determined 10 days later by whole-body counting. The results expressed as mean percentage absorption ± SEM were: without juice 6.7 ± 0.6, with normal juice 15.2 ± 1.1 ($P = 0.01$), with pernicious anemia 5.9 ± 0.4 ($P = 0.7$), with hypoplastic anemia juice 6.3 ± 0.7 ($P = 0.5$), with hemolytic anemia juice 31.2 ± 2.3 ($P = 0.001$), with iron-deficiency juice 47.7 ± 2.9 ($P < 0.001$), and with cirrhosis juice 27.2 ± 2.3 ($P = 0.001$). Thus gastric juice from iron-deficiency anemia, hemolytic anemia, and cirrhosis, but not from pernicious anemia or hypoplastic anemia, contains a factor which partially corrects the absorption defect for ^{59}Fe in totally gastrectomized anemic rats.

215. **Cell Fractionation and Polyribsome Profiles in Phytohemagglutinin-Stimulated Lymphocytes.** HENRY L. NADLER,* DAVID YI-YUNG HSIA,* AND ROBERT M. DOWBEN, Chicago, Ill., and Cambridge, Mass.

Pure lymphocytes separated from whole blood were homogenized by nitrogen cavitation, a method which ruptures virtually all cells while intracellular organelles

remain intact. Differential centrifugation yielded clearer separation of cell fractions and greater enzyme recoveries as compared with other methods of homogenization. Lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PD) appeared almost entirely in the high-speed supernatant; acid phosphatase (AcP) was confined to the lysosomal fraction. β-Glucuronidase (βGUR) and α-glucosidase (αGLU) were distributed in all cell fractions. Pooled pure lymphocytes were cultured with and without phytohemagglutinin (PHA). Blastic transformation, cell division, and greater ^{3}H-thymidine incorporation were found in the PHA-treated cells. When stimulated cells were compared with control cells, LDH, G-6-PD, AcP, αGLU, and protein decreased slightly during the first 6 hr and subsequently increased linearly to a maximum at 65 hr. The changes in βGUR were not consistent. Within 6 hr after stimulation with PHA, AcP was not segregated to the lysosomal fraction as it was in control cells; AcP remained widely distributed in all cell fractions in PHA-treated cells for the remainder of the cell cycle. This redistribution of enzyme suggests a direct effect of PHA upon lysosomal membranes. Undegraded polyribosomes have been obtained from cells ruptured by nitrogen cavitation and the profiles examined at various intervals after PHA stimulation. The system permits study of the changes in the pattern of protein synthesis which occur during the cell cycle.

216. Partial Purification and Characterization of the Coagulant Protein of Tissue Factor: Control by Exogenous Lipid. Y. NEMERSON,* New Haven, Conn. (introduced by Stuart Finch**).

The mechanism by which tissue factor activates blood coagulation at the site of vascular injury has not been amenable to study because of a lack of knowledge of the nature of tissue factor, and, indeed, knowledge of whether it is a specific entity. To this end, we have partially purified and characterized the protein moiety of this lipoprotein. The protein was obtained in water-soluble form from an acetone powder of brain tissue by (1) delipidation with heptane:butanol, (2) solubilization in deoxycholate (0.25%), and (3) precipitation with $(NH_4)_2SO_4$ (30–60% sat.). The soluble protein contained less than 1% phospholipid and had minimal biological activity. When it was combined with phospholipid micelles, however, activity increased by a factor of several hundred. The protein has been characterized by Sephadex chromatography (G-200) and elutes as a single peak with a Ve/Vo of 1.2, indicating an apparent molecular weight of 450,000 ± 10% (method of Whitaker), assuming a globular protein not absorbed to the gel. The coagulant protein also behaves as a single species on DEAE cellulose, as it elutes as a single peak. The degree of purification cannot be estimated with certainty owing to recoveries in excess of 100% at certain steps (perhaps due to the removal of an inhibitor). The total recovery of activity after gel filtration has averaged about 40% of the particulate preparation. The degree of lipid dependence is striking, and the ratio of protein to lipid critical. Titration of DEAE-purified protein with exogenous lipid micelles showed that maximum activity was achieved at ratios about 1:1 (weight/weight), and deviation from the optimum ratio resulted in marked loss of activity. At optimum lipid concentration, 500-fold increases in biological activity have been observed. On the basis of these data, we suggest that there is at least one specific protein that activates the tissue factor ("extrinsic") pathway of blood coagulation. A soluble protein has been extracted from lung, and studies have been undertaken to determine whether the tissue factor protein is organ specific.

217. Intestinal Lymph Very Low Density Lipoprotein: Role in Cholesterol Metabolism. ROBERT K. OCKNER* AND KURT J. ISSELBACHER, Boston, Mass.

Most previous studies have focused on the chylomicron (CHYLO) as the major means of transport of cholesterol from the intestine to lymph and the systemic circulation. In rats with intestinal lymph fistulas, we have found that a nonchylomicron very low density lipoprotein (VLDL) carries significant quantities of the endogenous lymph cholesterol. Some of the physical, chemical, and metabolic properties of this lipoprotein have been studied. VLDL differed from CHYLO in density, lipid composition, and electrophoresis on agarose. In the fasting state the approximate distribution of lymph cholesterol was: CHYLO 15%, VLDL 50%, and D > 1.006 ("low density" and "high density" lipoproteins) 35%. Intraduodenal administration of cholestyramine or bile fistula resulted in a prompt and marked fall in lymph total cholesterol levels, with virtual disappearance of cholesterol in VLDL and CHYLO. This indicates that VLDL cholesterol is derived from the intestine, rather than from circulating lipoproteins. Furthermore, the fall in lymph cholesterol exceeded the amount diverted from the intestine in bile fistula rats, supporting the concept that a portion of lymph cholesterol is synthesized by the intestine. The distribution of cholesterol in lymph lipoproteins was significantly influenced by the intraduodenal administration of fatty acids (FA) in mixed micelles, with minimal effect on total lymph cholesterol. With unsaturated FA (oleic or linoleic) the mean cholesterol distribution was CHYLO 52%, VLDL 20%, D > 1.006 28%. However, with comparable amounts of saturated FA (palmitic) the distribution was CHYLO 39%, VLDL 42%, D > 1.006 19%. Thus, absorption of saturated FA led to a 2-fold increase in cholesterol of lymph VLDL as compared with unsaturated FA. Differences in plasma disappearance rates between VLDL and CHYLO cholesterol were also noted. After intravenous injection of ^{14}C-cholesterol lipoproteins, the rate of removal of CHYLO radioactivity was twice that of VLDL. These and other studies have shown that VLDL is mainly responsible for the transport of endogenous cholesterol from the intestine to the circulation during the fasting state and during the absorption of saturated fat. The chemical, physical, and metabolic differences between VLDL and CHYLO suggest that these two transport forms of cholesterol may have differing roles in mammalian cholesterol metabolism.

218. Therapeutic Effects of L-Asparaginase on Asparagine-Dependent Neoplasms; Laboratory and Clinical Studies. HERBERT F. OETTGEN,* LLOYD J. OLD,* EDWARD A. BOYSE,* AND MORTON K. SCHWARTZ,* New York, N. Y. (introduced by David A. Karnofsky**).

L-Asparaginase, an enzyme from *E. coli,* inhibits some murine and canine lymphomas. Asparaginase-sensitive tumor cells have an absolute requirement for exogenous L-asparagine, whereas normal cells do not. 32 of 57 patients received asparaginase in a therapeutic trial. An in vitro test, measuring the incorporation of tritiated uridine and valine, was used in each patient; protein and nucleic acid synthesis were inhibited in sensitive cells deprived of asparagine. An automated method was developed to measure asparaginase levels in biological materials. 20 hr after a single intravenous injection, 50–80% of the enzyme remained in the circulating plasma. With daily intravenous injection of 200, 50, and 10 IU/kg, plasma levels were 3–8, 0.5–1.1, and 0.1–0.2 IU/ml, respectively. Plasma concentrations fell to nondetectable levels 4–5 days after the last dose of 200 IU/kg. When plasma enzyme levels were measurable, asparagine was not detectable. The asparaginase preparation used clinically contained, by weight, 60–80% impurities. Mild side effects (fever, weight loss, abnormal liver tests, decrease in serum lipids, anemia, and sensitization) were not necessarily attributable to asparaginase. At 200 and 10 IU/kg per day, bone marrow remissions have been produced in 15 of 21 patients with acute lymphoblastic leukemia (ALL) for 3 wk to 8 months, and some patients remain on maintenance therapy. Subcutaneous nodules regressed in 1 of 3 patients with malignant melanoma. With rare exceptions, a positive in vitro sensitivity test correlated with therapeutic responses. Resistance to treatment after remission, observed in 3 patients, was evident both in vivo and in vitro. Asparaginase is a unique antineoplastic drug, since the therapeutic response depends on a qualitative metabolic defect in the cancer cell. Asparagine dependence may be due to an enzymatic defect in certain cancers; the high incidence of bone marrow remissions produced by asparaginase in ALL may have a direct relationship to the origin and pathogenesis of the disease.

219. Aminogenic Hyperglucagonemia: Demonstration of a New Physiologic Role of Pancreatic Glucagon. A. OHNEDA,* E. PARADA,* A. EISENTRAUT,* AND R. UNGER, Dallas, Texas.

The response of glucagon secretion to hyperaminoacidemia has received little study, despite the ability of this gluconeogenic hormone to lower plasma amino acids. An aminogenic glucagon rise might explain the absence of hypoglycemia during amino acid–stimulated hyperinsulinemia. Pancreaticoduodenal vein (PV) glucagon was, therefore, measured radioimmunochemically during i.v. infusion of Floyd's 10 amino acid mixture (340 mg/min) in 16 conscious dogs with PV catheters. During hyperaminoacidemia, mean PV glucagon rose 2.1 mμg/ml (SEM ± 0.6), paralleling a 350 μU/ml PV insulin rise, while maximal glucose fall averaged only 7 mg/100 ml (±1.9). A significant negative correlation ($r = -0.588$; $P < 0.025$) between glucagon rise and glucose fall was noted, suggesting its possible role in maintaining relative normoglycemia. If so, preventing the aminogenic hyperglucagonemia would cause hypoglycemia. Since neither alpha cytotoxins nor selective pancreatectomy produced complete hypoglucagonism, physiologic suppression of glucagon was attempted by infusing glucose (300 mg/min) 60 min before and throughout the hyperaminoacidemic period. This effectively prevented aminogenic hyperglucagonemia, and glucose fell an average of 35 mg/100 ml (±3.7). To determine whether the fall was due to glucagon lack, hyperglucagonemia was restored during glucose suppression, either by infusing exogenous glucagon endoportally (50 mμg/min), or by overcoming suppression with the alphacytotropic hormone pancreozymin (8 U/min). Restoration of hyperglucagonemia by either means prevented or delayed hypoglycemia. The results demonstrate stimulation of glucagon secretion by hyperaminoacidemia and suggest that a major and hitherto unrecognized function of glucagon is to prevent hypoglycemia during carbohydrate-free protein meals through hepatic replacement of glucose presumed to enter insulin-sensitive cells as a consequence of aminogenic hyperinsulinemia. The results further suggest that, if clinical glucagon deficiency exists, it could be diagnosed by hypoglycemia and absence of hyperglucagonemia during amino acid–pancreozymin infusion.

220. The Intestinal Response to Diabetes: A General Stimulation of Sodium-Dependent Transport Systems. WARD A. OLSEN* AND IRWIN H. ROSENBERG,* Boston, Mass. (introduced by Charles S. Davidson**).

Whereas peripheral glucose uptake is diminished in diabetes, intestinal glucose absorption is increased. To study this intestinal response, we compared accumulation of ^{14}C-labeled sugars and amino acids by jejunal rings from pair-fed control and alloxan diabetic rats. Higher steady-state intracellular concentrations of nonmetabolizable 3-*O*-methyl glucose (3MG) were found in diabetes. Although efflux was not measured, striking increase in influx rates of 3MG appeared adequate to explain higher concentrations at equilibrium. Kinetic studies revealed decreased Michaelis constant (K_m) without change in maximum velocity (V_{max}), indicating increased affinity of "carrier" for 3MG. The diabetic effect increased at higher blood sugar levels, but increased transport of 3MG was not reproduced in controls by hyperglycemia from prolonged glucose infusion or by preincubation with glucose in vitro. The effects of diabetes were not limited to 3MG; all actively transported sugars and amino acids tested (D-galactose, lysine, cycloleucine, and aminoisobutyric acid) showed increased steady-state concentrations and decreased K_m without change in V_{max}. In contrast, diabetes did not affect absorption of passively transported D-arabinose and D-ribose. The diabetic effect was dependent on medium sodium concentration. Complete replacement of sodium by choline abolished differences between diabetics and controls, but as sodium concentration was increased, a progressive difference between diabetic and control transport occurred. Diabetes, therefore, stim-

ulated active transport of amino acids as well as sugars, increasing the affinity of the intestinal cell membrane for a variety of substrates. Since all transport systems stimulated by diabetes have in common their dependence on sodium, the observed changes in transport may relate to a fundamental change in the responsiveness of the intestinal membrane to sodium ion.

221. Mechanism of Estrogen-Induced Tissue Differentiation: Regulation of Nuclear Transcription. BERT W. O'MALLEY* AND WILLIAM L. MCGUIRE,* Bethesda, Md. (introduced by Jacob Robbins).

Hormones may initiate tissue growth and differentiation by directly affecting protein synthesis at transcriptional (nuclear) or translational (cytoplasmic) levels. The specialization of vertebrate reproductive tissues after hormonal stimulation during sexual maturation is one of the few instances where functional and morphological differentiation is not restricted to the period of embryonic development. The present studies are designed to study the mechanism of steroid action in such a model system. Estrogen stimulates oviduct growth (1000-fold) in newborn chicks, with the appearance of three distinct cell types and production of new tissue-specific proteins. We have employed DNA-RNA hybridization techniques to study messenger RNA synthesis in the differentiating oviduct cell nucleus. Competition studies between oviduct nuclear RNA's at various stages of estrogen-mediated differentiation revealed progressive formation of new "species" of messenger RNA. These changes correlated with oviduct epithelial organization and synthesis of new tissue-specific proteins (ovalbumin, lysozyme). RNA was also synthesized in vitro using exogenous *E. coli* polymerase and DNA (chromatin) prepared from immature and differentiated oviducts. Nearest-neighbor analysis of the RNA from differentiated oviducts revealed increased frequency of cytosine and guanosine (CpG and GpC)-containing dinucleotide pairs. Purified oviduct acylating enzyme was used to assay transfer RNA amino acid acceptor activity. Functional tRNA in the estrogen-stimulated cell increased out of proportion to other types of cytoplasmic RNA. In summary: (1) estrogen causes chick oviduct growth and differentiation; (2) DES stimulated production of new species of messenger RNA; (3) nearest-neighbor analysis of nuclear RNA from immature and differentiated oviducts suggested selective alteration of the DNA template; (4) tRNA activity increased coincidentally with hormone-induced differentiation; (5) these observations suggest that estrogen acts on the oviduct cell to influence nuclear transcription and promote differentiation. This model system permits the study of steroid-induced differentiation and biochemical specialization in a defined in vivo animal system.

222. Aldosterone Secretion and the Pituitary Gland. W. P. PALMORE,* R. C. ANDERSON,* AND P. J. MULROW, New Haven, Conn.

The pituitary gland has been considered unimportant in the control of aldosterone secretion. This belief dates back to the histological studies of Deane and coworkers in 1948, which showed widening of the zona glomerulosa after Na^+ depletion in hypophysectomized (hypox) rats, and was strengthened by animal experiments which demonstrated that aldosterone increased in response to acute stimuli in acutely hypox animals. Review of the literature, however, reveals that the response to a chronic stimulus such as sodium depletion is greatly reduced in the hypophysectomized man, dog, and rat. We have studied the role of the pituitary gland in the control of aldosterone secretion in the rat. In contrast to intact rats, hypox rats do not have an increase in aldosterone secretion in response to a low Na^+ diet for 9–14 days, despite a widening of the zona glomerulosa (Aldo SR, $m\mu g/min \pm SE$: intact, 19 ± 2; hypox, 1.4 ± 0.5). The response to Na^+ depletion cannot be restored by daily administration of ACTH. However, daily administration of rat pituitary glands partially restored the response (Aldo SR, 10 ± 1), yet showed no ACTH activity as judged by B secretion, adrenal weight, and morphology. To avoid the effects of prolonged hypophysectomy and the trauma of adrenal vein cannulation, rats were first Na^+ depleted, then hypophysectomized, and the adrenal glands incubated in vitro. 48 hr after hypophysectomy, aldosterone production was markedly reduced. Injections of rat pituitary glands completely prevented the fall in aldosterone production but had no effect on B production. Injections of ACTH alone or growth hormone (GH) alone are ineffective, whereas a combination of ACTH and GH completely prevents the fall. We conclude that the pituitary gland is necessary for the stimulation of aldosterone secretion by Na^+ depletion. ACTH alone is not the essential factor. A combination of hormones ACTH and GH may be the necessary factor, although the lack of ACTH activity in the pituitary gland injections suggests that other hormones may be involved. This mechanism could also play an important role in dog and man.

223. Slow Turnover of Manganese in Active Rheumatoid Arthritis Accelerated by Prednisone. P. S. PAPAVASILIOU,* E. R. HUGHES,* D. C. BORG,* AND G. C. COTZIAS,** Upton, N. Y.

In vitro, manganese activates enzymes critical to mucoprotein synthesis. In animals, manganese deficiency induces defective mucopolysaccharide synthesis coupled with gross mesenchymal aberrations. In man, rheumatoid arthritis displays both abnormal metabolism of mucopolysaccharides and mesenchymal lesions. Still, a connection between manganese and collagen disease appeared probable only when the turnover of ^{54}Mn became slow in one of our patients who developed arthritis from hydralazine. Consequently, the uptake and disappearance of ^{54}Mn were measured over the whole body, the liver, the thyroid, the ear, and the midthigh in 28 patients. The first two body segments represented tissues with vigorous mitochondrial metabolism, the latter primarily mesenchymal pools. The uptakes were similar in 16 controls and 10 arthritics. The rates of loss varied among individuals and were accelerated by manganous sulfate. A highly significant difference emerged between controls and patients with rheuma-

toid arthritis: in the latter, loss of the tracer was slower from the whole body, the thyroid, and, most strikingly, the liver. One arthritic in recent, impressive remission displayed very fast rates. In the others, the slow rates were readily accelerated by therapeutic doses of prednisone. Since a similar slowness has characterized our manganese-deficient animals, manganese analyses were performed by neutron activation. Normal concentrations were found in the blood and serum of the controls, significantly elevated concentrations in the red cells of the arthritics. This argued against classical nutritional deficiency. Suboptimal amounts of manganese might be reaching mesenchymal structures in rheumatoid arthritis, owing to the metal's accumulation in parenchymatous structures, notably the liver. The latter is rich in mitochondria, which normally accumulate manganese. Hence, these organelles are suspected of delaying the transit of manganese in rheumatoid arthritis. Conversely, prednisone appears to exert one of its actions by redistributing this and probably other trace metals.

224. Nychthemeral Variation of Human Growth Hormone in Plasma. DONAL C. PARKER,* J. W. MACE,* R. W. GOTLIN,* AND L. G. ROSSMAN,* La Jolla, Calif. (introduced by W. P. VanderLaan**).

Early observations suggested that significant nocturnal rises in plasma concentration of human growth hormone (HGH) did not occur. We sought to demonstrate nychthemeral variation by hourly sampling via intracatheters placed in arm veins of 16 nonobese healthy young adult males. They were all sampled during a single 24 hr period at bed rest on a uniform diet. Night samples were drawn without rousing the patient. HGH and insulin were measured by radioimmunoassay. HGH rose markedly during sleep in 14 of 16 subjects to a mean value of 17.2 ng/ml $\pm$ 2.6 SE (range 8.1–42.8), which is comparable to adult male arginine responses (9.4 $\pm$ 2.6 SE) and far exceeds fasting levels (2.3 $\pm$ 0.2). 2 others had only minor sleeping HGH elevations. The HGH elevations occurred as single (11) or double (5) peaks. These HGH rises occurred without respect to time of onset of sleep (8 p.m.–3 a.m.) or its duration (2–10 hr). Peaks were most frequent in early and mid sleep periods, lasting 1–3 hr. Daytime HGH mean values were less than 2 ng except for 9 postcibal rises in 7 subjects, mean 8.3 $\pm$ 0.8 SE. In only two did a waking-hour response exceed the HGH maximum of sleep. Insulin concentrations varied appropriately during the day, fell slowly during the late evening, and stabilized at a mean of 5 μU/ml during the rest of the night. Insulin did not vary with sleep or HGH peaks. Thus nychthemeral variation in growth hormone concentration has been shown to occur over a fairly wide range, with maximum physiologic rises during sleep. It is hard to reconcile these findings with the current "substrate availability" concept of regulation of HGH release. Glucose, free fatty acid, and cortisol determinations are being done to gain more information about this nychthemeral HGH response. Sleep studies in disease and short stature are also being done.

225. Correlative Lipid Biochemical, Histochemical, and Ultrastructural Evidence for Chylomicron Triglyceride as the Major Source of Lipid in Diabetic Eruptive Xanthoma. FRANK PARKER,* GEORGE F. ODLAND,* AND JOHN D. BAGDADE,* Seattle, Wash. (introduced by Robert G. Petersdorf).

Xanthoma lipids are assumed to originate from plasma. It therefore seems paradoxical that cholesterol is alleged to be the principal component of all xanthomas, since diabetic eruptive lesions evolve in the presence of hypertriglyceridemia. Because studies are lacking on such xanthomas, biochemical and ultrastructural techniques were correlated to investigate the source of xanthoma lipids and thereby elucidate the pathogenesis of such lesions. Lipid distribution and fatty acid patterns were determined on xanthomas, fasting plasma, and chylomicron samples from seven diabetics on regular diets. These patients had moderately elevated plasma cholesterol (330–986 mg/100 ml) and greatly increased plasma triglyceride (1660–7790 mg/100 ml), of which 50–98% was found in chylomicrons. The major lipid of xanthomas was also triglyceride (46 $\pm$ 5%) with lesser amounts of cholesterol (24 $\pm$ 6%). The fatty acid patterns of xanthoma, plasma, and chylomicron triglycerides were identical, with 41 $\pm$ 4% 18:1, 24 $\pm$ 3% 16:0, and 12 $\pm$ 3% 18:2. Histochemically, lipid globules appeared within dermal capillary walls. Electron microscopy revealed accumulations of droplets 2000 $\pm$ 800 A in diameter within capillary basal lamina and in the perivascular space. Macrophage foam cells with lipid-containing cytoplasmic vacuoles congregated around capillaries. In three cases, when hyperlipidemia was reversed with dietary and insulin therapy, the prompt clearing of chylomicrons was closely followed by resolution of xanthomas. Electron microscopy of resolving xanthomas revealed collapsed and empty vacuoles in foam cells. These biochemical and ultrastructural findings support the theory that blood lipids contribute directly to xanthoma lipids, but they refute the generally held belief that all xanthomas are cholesterol rich. The major lipid in diabetic xanthomas is triglyceride derived primarily from chylomicrons, which apparently permeate the vascular wall and are temporarily sequestered by macrophages. Xanthomas serve as convenient models for study of lipid transport across blood vessel walls.

226. The Cardiotonic Effects of Glucagon in Man. WILLIAM W. PARMLEY,* GERALD GLICK,* AND EDMUND H. SONNENBLICK, Boston, Mass.

Glucagon has been shown recently to have substantial inotropic and chronotropic actions in isolated heart muscle preparations and in the intact dog heart, independent of catecholamines or beta adrenergic blockade. Accordingly, the effects of glucagon on hemodynamics in man were studied at the time of diagnostic cardiac catheterization. In eight patients, glucagon (3–5 mg) was administered intravenously over 1 min. Peak effects were observed after 5–7 min and lasted approximately 10–15 min. Cardiac index increased from an average of 2.4 to 2.8 liters/min per m^2 ($P < 0.025$), left ventricular (LV) dP/dt

from 1943 to 2435 mm Hg/sec ($P < 0.005$), arterial pressure from 123/72 ($\overline{93}$) to 137/79 ($\overline{105}$) ($P < 0.005$), and heart rate from 89 to 98 beats/min ($P < 0.025$), while LVED and systemic vascular resistance were unchanged. In three of the eight patients who were digitalized, the inotropic actions of glucagon were similarly evident. Blood glucose increased from 101 to 146 mg/100 ml ($P < 0.001$), while serum K^+ fell from 4.1 to 3.7 mEq/liter ($P < 0.025$). After administration of smaller doses of glucagon (1 mg i.v.) to six patients, cardiac index rose from an average of 1.9 to 2.3 liters/min per m^2 ($P < 0.05$), peripheral vasculr resistance fell from 2013 to 1612 dyne sec cm^{-5} ($P < 0.01$), and there was no significant change in arterial blood pressure, LVED, and heart rate. Thus, glucagon has been shown to be a potent cardiotonic agent in doses of 3–5 mg in man. Previous studies have indicated that this action is evident in the absence of catecholamines and in the presence of either adrenergic blockade or digitalization. Therefore, glucagon appears to be a useful new cardiotonic agent, which may be superimposed on other forms of therapy for the treatment of heart failure.

227. Light-Induced Free Radicals in Human Skin. M. A. PATHAK* AND K. STRATTON,* Boston, Mass. (introduced by T. B. Fitzpatrick**).

Resolution of the primary reactions, in relation to the molecular components in human skin, that take place during the process of light absorption may possibly be abetted by a study of free radicals generated. The effects of ultraviolet (UV) and visible light on "white" and pigmented human skin have been investigated by electron spin resonance (ESR) techniques. Unirradiated pigmented skin exhibited a stable ESR signal. A single absorption peak with $g = 2.003$ and line width of 5–6 gauss is associated with unpaired electrons in melanin. The concentration of unpaired spins in wet pigmented epidermis was about 2.6×10^{16}/g dry weight. The amplitude of this intrinsic melanin signal is related to the degree of cutaneous melanization. Irradiation with UV ($\lambda > 320$ mμ) induced two different types of free radicals. One type, designated as "melanin-free radicals," is generated in melanin of the melanosomes, and appears to be of semiquinonoid nature. The second type, designated as "other radicals," with a line width of approximately 24 gauss, is generated probably in the epidermal proteins. Irradiation of pigmented skin with wavelengths greater than 320 mμ at 77°K also produced a real increase of melanin signal, but irradiation of "white" skin with similar wavelengths produced no detectable free radicals. Radiation-induced free radicals were unstable at 300°K (half-life of the order of a few seconds). Radical yields measured in "white" and pigmented skin, as a function of UV dose, showed that fewer "other radicals" were generated in pigmented skin. This study emphasizes the close relationship between the solar-erythemal-action spectrum and the production of "other free radicals"; and also the photoprotective function of melanin.

228. The Use of Reaginic Antibody to Demonstrate Induced Autoantibodies against Insulin. ROY PATTERSON, GUILLERMO LUCENA,* ROBERT METZ,* AND MARY ROBERTS,* Chicago, Ill.

We have investigated the specificity of the reaction between a reaginic antibody to insulin and (1) extracted insulin from various species, including man, (2) circulating, unextracted human insulin, (3) synthetic "human" insulin, and (4) several fragments prepared from beef insulin. The antibody, with immunobiologic characteristics of reagin, including immunochemical characteristics consistent with IgE, was obtained from a diabetic patient who manifested systemic allergic reactions to insulin. Insulin-reaginic antibody reactions were studied by direct cutaneous testing and by passive cutaneous transfer of reactivity to human and monkey skin. The antibody did not react with isolated polypeptide chains of beef insulin, whereas enzymatic digests of insulin with less drastic alteration of primary structure did react. The antibody reacted with all extracted insulins tested, as well as with synthetic human insulin (supplied by Dr. P. G. Katsoyannis), but not with native, circulating human insulin. This last point, of basic interest, was established by sequential experiments using the same nonsensitive human recipient. Reactions occurred in passive transfer sites in normal human skin after intravenous administration of exogenous human insulin. In contrast, concentration of endogenous insulin, produced by stimulating the subject's own insulin secretion, did not induce a reaction in fresh passively sensitized sites. Insulin extracted from the patient's own serum was reactive on direct testing and on passive cutaneous transfer. These results demonstrate that in the process of extraction from the pancreas or from serum, insulin is altered so as to expose antigenic determinants which are normally inaccessible in the native molecule. Injections of extracted heterologous insulin can, therefore, cause the development of an antibody against potentially available antigenic determinants on homologous or even autologous molecules. Thus, a mechanism of inducing autoantibodies in man has been demonstrated.

229. Immobilization of Bacteria by Antibodies in Experimental Pyelonephritis. GEORGE J. PAZIN* AND A. I. BRAUDE,** Pittsburgh, Pa.

The predominance of motile bacteria in pyelonephritis suggests that motility may be involved in its pathogenesis and that resistance to kidney infection might be enhanced by bacterial immobilization. A search was made, therefore, for immobilizing antibodies in pyelonephritis. *Proteus mirabilis* was inoculated into bladders of 48 rats to produce pyelonephritis. Immobilization was demonstrated by suspending motile bacteria in serum or urine, and the titer was expressed as the highest dilution producing 90% immobilization. Immobilizing antibody first rose 3 to 5 days after infection, reached levels of 2000–16,000 at 7–10 days, and persisted until sacrifice at 19 days. 2-mercaptoethanol treatment of 6 day serum lowered the titer (8000 to 256), but did not affect 19 day serum. Upon sucrose gradient ultracentrifugation of 19 day serum, the bulk, if

not all, of immobilizing antibody was found in late-sedimenting (7S) fractions. Specificity of immobilization was demonstrated by failure of high-titer serum to immobilize a strain of *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, P. morganii,* and a different clinical isolate of *P. mirabilis.* All viable bacteria found in urine upon sacrifice at 19 days after infection were immobile. Normal urines characteristically had no immobilizing properties, but dialyzed urines from infected rats had titers ranging from 16 to 64. These results demonstrate that immobilizing antibodies appear in serum and urine during pyelonephritis produced by motile bacteria. Most of the early immobilizing antibody in the blood is 19S, and that appearing later is mainly 7S. It is possible that immobilizing antibody may not only limit the spread of bacteria in the urinary tract, but also impair their capacity to elude phagocytic cells. In addition, measurement of immobilizing antibody in the serum provides another approach for studying the humoral immune response in pyelonephritis.

230. Metabolic characteristics of Isolated Intact Parenchymal Cells from Rat Liver. LEROY A. PESCH,* JANICE A. PETERSON,* AND ROGER B. HOWARD,* Palo Alto, Calif. (introduced by Robert J. Glaser**).

Parenchymal cells have been isolated from rat liver by an enzymatic technique employing collagenase and hyaluronidase. The cells obtained by this method are structurally intact as determined by light and electron microscopy. Vital staining reveals that 80–95% of the cells remain viable. Suspensions of these cells are essentially free of other cell types and are not contaminated by intracellular elements. The suspensions maintain linear rates of respiration for 2 hr when incubated in Krebs-Ringer phosphate solution, and will maintain measurable oxygen uptake in medium 199 for as long as 3 days. These cells have been maintained in monolayer tissue cultures in small Leighton tubes for as long as 7 days. In culture the cells reassume a morphological appearance similar to that of hepatic parenchymal cells, with cordlike growth characteristics. Suspensions of these cells will synthesize glycogen from radioactive pyruvate. Incubation of the cells in the presence of crystalline insulin (0.5 unit/ml) results in a 30% stimulation of pyruvate-supported respiration. Insulin had no effect on glucose utilization. Studies utilizing other substrates indicate that the mode of action of insulin is on intracellular metabolic processes and that it has no effect on glucose transport across the plasma membrane.

231. Human Skeletal Muscle Mitochondria and Sarcotubular Vesicles in Hyper- and Hypothyroidism. J. B. PETER,* Los Angeles, Calif. (introduced by Carl M. Pearson**).

Clinically recognizable abnormalities of muscle frequently accompany severe hyper- or hypothyroidism. Little is known of the metabolism of skeletal muscle mitochondria in such patients or in normals, because previous techniques for isolation of skeletal muscle mitochondria were inefficient and mitochondria so isolated showed poor respiratory control ratios (RCR) and other evidence of damage. Using a CO_2-cooled mechanical shaker for homogenization, mitochondria with excellent, reproducible biochemical and morphological characteristics can be readily isolated from small samples of human muscle. Such mitochondria differ from those of rat skeletal muscle in the centrifugal force required for sedimentation, and from mouse skeletal muscle mitochondria in their capacity for α-glycerophosphate oxidation. Mitochondria from patients with thyrotoxic myopathy show normal Q_{O_2}, normal RCR, and near "theoretical" ADP/O ratios. The uncoupling of oxidative phosphorylation, decrease in RCR, and increased Q_{O_2} with α-glycerophosphate found in animals treated with large doses of thyroid hormones are not observed in muscle mitochondria of human thyrotoxic myopathy. Mitochondria from patients with hypothyroid myopathy generally show low Q_{O_2}, near "theoretical" ADP/O ratios, and diminished RCR, again contrasting with liver mitochondria from hypothyroid rats. Likewise, hypothyroidism does not greatly influence the slow rate of oxidation of α-glycerophosphate by human skeletal muscle mitochondria. The pseudomyotonia, delayed relaxation, and muscle spasms of hypothyroid myopathy could result from defective calcium accumulation by the sarcotubular system. Such a defect was demonstrated in sarcotubular vesicles isolated from one patient with hypothyroid myopathy.

232. Bone Responsiveness to Parathyroid Hormone in Fluorosis. JAMES M. PHANG,* THEODORE J. HAHN,* LEWIS R. CHASE,* AND CHARLES F. RAMBERG,* Bethesda, Md. (introduced by Donald P. Tschudy).

Although numerous reports have dealt with the effects of fluoride therapy on calcium metabolism in a variety of metabolic bone disorders, to date there has been no detailed study of the effects of long-term fluoride ingestion in an otherwise normal individual. A case of self-induced fluorosis (estimated mean intake approximately 500 mg/day of elemental fluoride for 14 yr) was studied utilizing ^{47}Ca kinetics and calcium balance techniques. The data were analyzed by use of a four-compartment computer model, and the results were compared with those obtained from similar studies in 14 normal and 18 hypo- or hyperparathyroid subjects. Calculated gastrointestinal and renal rate constants suggested increased parathyroid hormone (PTH) effect at these two sites. Since it has been shown that bone with a high fluoride content has a decreased ability to exchange ^{45}Ca in vitro, the above finding was compatible with the hypothesis that bone hyporesponsiveness to PTH results in a compensatory increase in PTH effect at kidney and gut. To test the hypothesis of decreased responsiveness of fluorotic bone to endogenous PTH, the effect of EDTA infusion was compared in three fluorotic and three age- and sex-matched control cows. Whereas initial falls in plasma calcium were similar in the fluorotic and the control groups, the response in plasma calcium to rising plasma PTH levels was markedly diminished in the fluorotic

animals. This suggests that the induction of fluorosis produced a selective target organ hyporesponsiveness to PTH.

233. Complement Studies in Hereditary Angioneurotic Edema. RICHARD J. PICKERING,* JOSEPH R. KELLY,* AND MALCOLM N. BLUMENTHAL,* Minneapolis, Minn. (introduced by Wesley W. Spink**).

Study of genetically determined abnormalities of the complement system promises to shed light on interactions of components and on the role of individual components and the entire complement system in the body economy. In this study of 50 members of a Negro family the diagnosis of hereditary angioneurotic edema was made on the basis of clinical symptoms and reduced C'4 hemolytic activity and confirmed by the absence of C'1-esterase inhibitor. Seven have symptoms and four have only serum abnormalities. Total hemolytic serum complement varied from normal to less than 15% of normal between and during attacks, respectively. Hemolytic C'1 activity and C'1q concentration determined by immunochemical methods were normal at all times in all patients studied. Hemolytic C'4 was markedly reduced (5% of normal) during episodes of edema and moderately reduced at all times. Hemolytic C'2 activity was as low as 25% of normal during attacks. As C'2 was occasionally near normal between attacks, this assay was less useful diagnostically than C'4 determination. Apparent reduction of C'2 activity was grossly exaggerated if the assay did not provide supplemental human C'4. Hemolytic C'3 and C'5 were normal at all times. Thus, C'4 and C'2 were reduced without detectable participation of the first component, C'1q, and later components, C'3 and C'5, suggesting activation of C'1-esterase by nonimmune mechanisms. Immune adherence complement activity was markedly reduced during attacks. This activity was restored by supplying as few as 10 (hemolytically) effective molecules of human C'4, but not by 50 molecules of C'2, per antigen-antibody particle (EA), suggesting that C'4 is a limiting component in immune adherence. Present methods of complement analysis provide aids to diagnosis, contribute to understanding of interactions within the complement system, and, possibly, will lead to recognition of the pathogenesis of this disease.

234. Effect of Aging of Human Red Cells in Vivo on Fatty Acid Composition. GERALD B. PHILLIPS, JAMES T. DODGE,* AND CALDERON HOWE,** New York, N. Y.

The recent development of improved methods of gas-liquid and thin-layer chromatography for the detailed analysis of the fatty acids of human red cells prompted an investigation of fatty acid changes of human red cells with aging in vivo. From the red cells of each of seven normal subjects, a young fraction and an old fraction were isolated by centrifugation and the fatty acids of each fraction were analyzed. 24 fatty acid peaks were quantified. Statistical analysis showed a significant increase in the relative amounts of 16:0 (palmitic), 18:1ω9 (oleic), and 18:2ω6 (linoleic) and a significant decrease in the relative amounts of 20:3ω6 + 22:0, 20:4ω6 (arachidonic), and 22:4ω6 + 24:1ω9 with aging. Analysis of the red cell fraction remaining after removal of the young and old fractions in one sample showed intermediate values, suggesting that the age differences were not confined to a small population of young (reticulocytes) or old cells. Estimation of the individual phospholipids in the young and old cells of two of the subjects indicated that the fatty acid differences were not entirely on the basis of a change in distribution of the major phospholipid groups. A striking difference in fatty acid pattern between young and old cells was that the mean value for every peak containing a fatty acid of C_{18} or shorter except 18:0 (stearic) was higher, and for every peak with a fatty acid of C_{20} or longer was lower, in the older fraction. Whereas the difference in every peak between young and old cells was not statistically significant, the consistency in the direction of the difference above C_{20} was highly significant. Although the basis of this difference in pattern is unexplained, it may be related to a change in red cell binding affinity for phospholipid fatty acids or in red cell acyltransferase activities with aging.

235. Diarrhea Following Ileal Resection: Pathogenesis and Treatment. J. RAINER POLEY* AND ALAN F. HOFMANN,* Rochester, Minn. (introduced by Hugh R. Butt**).

Diarrhea in iliectomized patients may be caused by increased passage of bile acid into the colon resulting from decreased ileal absorption. Therefore, we examined bile acid turnover, effective pool size, and response to cholestyramine, 16 g/day, in five iliectomized patients. Further, since two patients had steatorrhea and soaps might contribute to the diarrhea, we compared diets with normal (LCT) or medium-chain fat (MCT), because MCT is absorbed better and less soap reaches the colon. Four periods (4–10 days) were randomized: LCT, LCT + cholestyramine, MCT, and MCT + cholestyramine. All patients had greatly reduced bile acid absorption. However, three patients with mild steatorrhea (< 16 g/day) maintained their effective bile acid pool by increased synthesis, since micellar bile acid (and lipid) in jejunal content, after a test meal, was normal. Cholestyramine, with normal (LCT) diet, strikingly reduced fecal weight, fecal frequency, and fecal sodium in all three. By contrast, two patients with severe steatorrhea (> 30 g/day) could not maintain their bile acid pool, since micellar bile acid (and lipid), after a test meal, was reduced. Cholestyramine (with normal diet) caused no improvement in diarrhea, and steatorrhea worsened; cholestyramine probably bound the diminished jejunal bile acids completely, since its addition to a test meal greatly reduced micellar bile acid (and lipid). Elimination of LCT, i.e. MCT diet (without cholestyramine), abolished steatorrhea and partially reduced diarrhea, consistently with the postulated soap catharsis. Only the combination of MCT and cholestyramine resulted in normal fecal weight and frequency with minimal steatorrhea. Iliectomized patients

who maintain their bile acid pool have mild steatorrhea; diarrhea is probably caused by bile acid and responds to cholestyramine. Iliectomized patients who cannot maintain their bile acid pool have severe steatorrhea; diarrhea is probably caused by both bile acid and soap and responds to a low LCT fat diet (conveniently achieved with MCT) and cholestyramine.

236. Chemical Energetics of Cardiac Muscle in Hyperthyroidism. PETER E. POOL,* C. LYNN SKELTON,* SHIRLEY C. SEAGREN,* AND EUGENE BRAUNWALD, Bethesda, Md.

Certain features of clinical and experimental hyperthyroidism have been considered manifestations of changes in chemical energy generation. Alterations in efficiency of energy utilization may also be important in hyperthyroidism, but this efficiency has not been measured directly. Accordingly, conversion of chemical energy to mechanical work was studied in myocardium from hyperthyroid and normal cats. Hyperthyroidism was induced by intraperitoneal injection of L-thyroxine (1 mg/kg, 10–14 days). In each animal serum protein–bound iodine was greater than 20 μg per 100 ml (normal 5.2 ± 0.4 μg per 100 ml) and serum cholesterol was 57 ± 3 mg per 100 ml (normal 71 ± 5 mg per 100 ml). The utilization of chemical energy ($\Delta \sim P$), consisting of creatine phosphate and ATP, was determined in isolated right ventricular papillary muscles treated with iodoacetic acid and nitrogen. Under these conditions net production of $\sim P$ was inhibited. The basal rate of energy utilization in muscles resting without tension was higher in 39 muscles from hyperthyroid animals than in 76 normal muscles (0.99 vs. 0.77 μmole $\Delta \sim P$/g per min). The efficiency of energy utilization was determined in 34 muscles from hyperthyroid animals and 28 normal muscles. After inhibition of energy production, the muscles were stretched to the top of their previously determined length–active tension curves and stimulated to contract isometrically 25 times. Although the contractile element work performed was similar in each group (hyperthyroid, 370 ± 25 g-cm/g; normal, 371 ± 20 g-cm/g), energy utilization was significantly greater ($P < 0.0001$) in hyperthyroid muscles than in normals (5.56 ± 0.34 vs. 2.43 ± 0.31 μmoles $\Delta \sim P$/g per 25 contractions). Energy utilization per unit work was, therefore, greater in muscles from hyperthyroid animals than in those from normals (0.016 ± 0.001 vs. 0.007 ± 0.001 μmoles $\Delta \sim P$ per g-cm contractile element work). This implies a direct effect of thyroid hormone on mechanochemical coupling, leading to decreased efficiency in the conversion of chemical energy to mechanical work in the myocardium in hyperthyroidism.

237. Metabolism of Six Specifically Labeled Radiothyroxines in Normal Man and Animals. CONSTANCE S. PITTMAN* AND WILLIAM B. JONES,* Birmingham, Ala. (introduced by Ben Friedman).

Studies were undertaken to evaluate the metabolism of the phenolic ring and tyrosyl ring and the ultimate fate of the ether linkage during thyroxine (T_4) degradation. The following radiothyroxines were studied: (1) 3′,5′-^{131}I-L-T_4, (2) 3,5-^{125}I-L-T_4, (3) ^{14}C-D,L-T_4-alanine(β), (4) ^{3}H-D,L-T_4-alanine(α,β), (5) ^{14}C-D,L-T_4-tyrosyl ring (UL), (6) ^{14}C-D,L-T_4-phenolic ring(UL). Paired blood samples were obtained from the antecubital artery and hepatic vein of human volunteers after they were given intravenous injections daily for 7–10 days with one or a mixture of radiothyroxines in total dose < 50 μg/day. Liver extraction of thyroxine, liver blood flow, chromatography, and, later, biological half-life and radioactivity recovery were determined. The hepatic thyroxine clearance was 57–104 ml/min. The biological half-lives of compounds (1), (3), and (6) were 6.2–6.6 days. The differences in ^{3}H/^{14}C ratios between thyroxine and nonthyroxine fractions were not significant. In the rats, when compounds (1) and (2) were given together in dose < 15 μg/kg per day, no significant differences in biological half-lives (16.9–18.9 hr) or radioactivity recoveries were observed. Although trace amounts of partially deiodinated metabolites were recovered in the bile, only inorganic iodide was found in the urine. When compounds (3), (4), and (5) were given individually to rats, much longer biological half-lives (28.9–31.6 hr) were observed. Each of them gave rise to four completely deiodinated metabolites in the urine that were identical with the urinary metabolites of compound (6). The data suggest that during thyroxine degradation in living animals, iodines are removed completely. The remaining molecule retains an intact diphenyl ether structure while it undergoes alterations of its functional groups.

238. Dysfibrinogenemia (Fibrinogen Detroit). ANANDA S. PRASAD,* EBERHARD F. MAMMEN,* AND CHI-CHEONG AU,* Detroit, Mich. (introduced by Benjamin Lewis).

Congenital dysfibrinogenemia has been described in four families, but detailed studies were reported only in "fibrinogen Baltimore." Dysfibrinogenemia was encountered in a Detroit Negro family with an autosomal dominant pattern of heredity. Precipitation techniques gave normal values for fibrinogen, but conversion to fibrin by thrombin was defective. Patient's plasma clots were insoluble in 5 M urea. Other coagulation constituents were normal. No circulating anticoagulants or abnormal fibrinolytic activity was demonstrated. After purification, normal fibrinogen was 96.7% clottable, but patient's fibrinogen did not clot spontaneously after addition of thrombin. Incubation of patient's fibrinogen with thrombin at 37°C for 1 hr eventually resulted in a clot. Immuno- and polyacrylamide gel electrophoresis revealed homogeneity for both fibrinogens. In fresh samples, 3 moles of SH groups per mole of fibrinogen were titrated in both. $S_{20,w}$ in 0.1 M potassium phosphate buffer was 7.41 for both. Using methods of Yphantis, $\overline{M}_N$ averages for normal and abnormal fibrinogen were 292,034 and 279,496 respectively. $S_{20,w}$ in 5 M guanidine HCl was 6.45 for both samples. Cleavage with sodium sulfite

according to methods of Johnson and Mihalyi yielded $S_{20,w}$ values of 2.27 and 2.26, and $\overline{M}_N$ average of 68,608 and 58,766 for normal and abnormal fibrinogen subunits respectively. Determination of amino acid composition revealed a decreased content of lysine, valine, glucosamine, and galactosamine in abnormal fibrinogen. Carbohydrate contents in normal and abnormal samples were as follows: total, 4.3 vs. 2.9%; protein-bound hexoses, 2.82 vs. 1.77%; sialic acid, 0.84 vs. 0.65%; and hexosamine, 0.64 vs. 0.49%. "Fibrinogen Detroit" differs from "fibrinogen Baltimore" in its carbohydrate content and from "fibrinogen Cleveland" immunologically. Inasmuch as intact carbohydrate moiety seems to be essential for conversion of fibrinogen to fibrin, our studies indicate that the abnormal clotting properties of patient's fibrinogen may be related to its low carbohydrate content.

239. The Role of Oxidized Pyridine Nucleotides in Augmented Renal Production of Ammonia in Acidosis. H. G. PREUSS,* M. S. CAMPBELL,* D. P. PONDER,* AND H. V. MURDAUGH, Pittsburgh, Pa.

Recent reports indicate that decreased renal glutamate concentrations play an important role in increased renal ammoniogenesis in acidosis. This study is an inquiry into the association between alterations in renal glutamate and ammonia metabolism and the status of renal pyridine nucleotides. With the addition of malonate 20 mM to dog renal tubules incubating in vitro, more ammonia formation from glutamate occurred at pH 7.1 and pH 7.7. Although there was no change in gluconeogenesis in the presence of malonate, the increase in tubule ammonia formation was greater at the lower pH. Malonate administered to rats caused renal accumulation of succinate, decreased renal concentrations of glutamate, and a rise in the concentrations of ammonia, NAD, and NADP. Since increased gluconeogenesis was not present, these correlations suggested that changes in oxidized pyridine nucleotides altered renal glutamate metabolism. The changes in glutamate metabolism in response to malonate resemble changes seen during acidosis; therefore renal pyridine nucleotide concentrations in acidosis were investigated. 4 hr after an oral dose of NH_4Cl, renal tissue concentrations of glutamate decreased significantly. Renal cortical slices from these acutely acidotic rats failed to show increased gluconeogenesis from glutamate when compared with slices from nonacidotic rats. However, in these same rats, the kidney ratios NAD/NADH increased from mean 2.7 to 3.6 and NADP/NADPH from 0.63 to 0.89. These changes in ratio occurred mainly through a significant decrease in NADH and a rise in NADP. In chronic acidosis, the renal concentrations of NAD and NADP rose significantly. Further, nicotinamide injections which resulted in an elevation of renal NAD also were associated with a lowering of renal glutamate. These findings suggest that the ratio of oxidized to reduced pyridine nucleotides may play a role in decreased renal glutamate concentrations and increased renal ammoniogenesis seen in acidosis.

240. Evaluation of Myocardial Force-Velocity Relation in Close-Chest Man and Animals. PRITPAL S. PURI* AND RICHARD J. BING, Detroit, Mich.

Changes in instantaneous force-velocity relations of the left ventricle were investigated in close-chest human subjects and dogs by employing a newly devised strain gauge catheter assembly to register curves of fiber shortening on beat-to-beat basis. An inverse relation between force and velocity was demonstrated in dogs when primary changes in afterload were induced by inflating and deflating an intra-aortic balloon or by methoxamine. Norepinephrine and nicotine shifted the force-velocity (FV) curve upward and to the right; isoproterenol and epinephrine shifted it upward, and propranolol (β-blocker) shifted it downward. Acute experimental heart failure induced with pentobarbital shifted the FV curve downward and to the left; digitalis given subsequently reversed this effect. Regional ischemia induced by temporary coronary occlusion resulted in loss of fiber shortening and its replacement by paradoxical fiber lengthening. Drugs like norepinephrine and isoproterenol (used in treatment of cardiogenic shock) increased the amplitude of this paradoxical lengthening; at the same time they augmented the contractility of the nonischemic portions of the myocardium. On release of occlusion, and coinciding with hyperemia, systolic fiber shortening returned with an increase in its amplitude.

241. On the Lipoprotein Abnormality in Type III Hyperlipoproteinemia. STEVEN H. QUARFORDT,* ROBERT I. LEVY,* ARTHUR FRANK,* AND DONALD S. FREDRICKSON, Bethesda, Md.

Type III hyperlipoproteinemia is a familial disorder associated with accelerated arteriosclerosis and xanthomatosis. It is characterized by the presence in plasma of abnormal forms of very low density lipoproteins (VLDL, $D < 1.006$) having β-electrophoretic mobility. This abnormality has now been further defined. VLDL from normals and patients with other dyslipoproteinemias has α_2 (pre-beta) mobility on preparative starch block electrophoresis. In type III there are two distinct components of VLDL, one with the usual α_2 mobility, the other with slower β mobility (βVLDL). The lipid and protein composition of α_2VLDL is similar to that of conventional VLDL; immunochemical analysis after delipidation reveals alpha and beta lipoproteins. The slower βVLDL component has a different lipid and protein content. Delipidation reveals only β-lipoproteins, and the complexes resemble normal LDL (D 1.006–1.063) with 4–10 times the normal triglyceride content. In vivo, the turnover of ^{14}C-labeled triglycerides in α_2VLDL is rapid and comparable to that in normal VLDL. Triglyceride in βVLDL turns over slowly, comparably to normal LDL triglyceride. When plasma triglycerides are increased in type III by dietary carbohydrate induction, α_2VLDL increases. Acute depression of plasma triglyceride after starvation or intravenous heparin rapidly increases βVLDL at the expense of α_2VLDL. These observations suggest that a block in catabolism of α_2VLDL in type III hyperlipoproteinemia causes accumulation of β-lipoproteins

burdened with an unusual content of glycerides. The slow turnover of these complexes leads to gross hyperglyceridemia when flux of endogenous glyceride (VLDL) is high. Clofibrate or diets can quickly return plasma lipids to normal in type III. Some βVLDL persists, however, and remission is probably due to decreased triglyceride output from the liver rather than to correction of the inherent defect.

242. Phagocytosis in Endocarditis: Localization of the Primary Opsonic Site of γG to Fc Fragment. PAUL G. QUIE,* RONALD P. MESSNER,* AND RALPH C. WILLIAMS, JR., Minneapolis, Minn.

Specific interaction of immune 7S γ-globulin opsonin and bacteria isolated from 50 patients with subacute bacterial endocarditis was studied using the quantitative phagocytosis system of Hirsch. Primary opsonic activity was present in isolated γG fractions and could be blocked when 19S rheumatoid factors were added to phagocytic mixtures. In addition, heterologous rabbit antisera specific for the Fc portion of γG blocked phagocytosis when added to these systems. Thus, two separate reagents with primary affinity for Fc fragment—human rheumatoid factors and specific rabbit antisera—could block phagocytosis and subsequent killing of bacteria. Normal human 11S colostral γA was isolated by zone electrophoresis, DEAE cellulose chromatography, and gradient ultracentrifugation. Though possessing agglutinating antibacterial antibody activity for Gram-positive and -negative species, these antibodies which contain H-chain groups distinct from γG showed no opsonic activity. When isolated 7S γ-globulin opsonins were digested with pepsin, progressive enzymatic degradation of Fc, as measured by loss of Gm(a) or Gm(b), was accompanied by parallel loss of opsonic activity for various infecting bacteria. Though devoid of opsonic capacity, these pepsin-digested γ-globulins could still be shown to combine with their respective bacteria, using bacterial agglutination with Coombs reagents specific for 5S pepsin fragment. Reduction with 0.01 M and 0.1 M mercaptoethanol progressively abolished specific opsonic as well as complement-fixing properties of immune 7S opsonins, whereas agglutinating antibody titers and Gm(a) Fc antigens were unaffected by this treatment. Carbohydrate moieties reside mainly on the Fc fragment of γG. Mild periodate oxidation of γG opsonin (0.01 M for 5 hr) produced complete loss of specific opsonic activity. Thus, the opsonic site appeared to reside on Fc and to possess configurations stabilized by disulfide bonds and carbohydrate residues. It is postulated that γG opsonins may bind to polymorphonuclear leukocytes through specific attachment sites on Fc fragment.

243. Peripheral Subresponsiveness to Human Growth Hormone in a Proportionate Dwarf. DAVID RABINOWITZ,* THOMAS J. MERIMEE,* DAVID L. RIMOIN,* JUDITH G. HALL,* AND VICTOR A. MCKUSICK,** Baltimore, Md.

Sexual ateliotic dwarfs have normal body proportions, develop normally sexually, and have an isolated deficiency of human growth hormone (HGH). This is a report of F.S., a 36 yr old dwarf (120 cm in height), who, though clinically not distinguishable from sexual ateliotics, displayed sustained and nonsuppressible elevation in plasma HGH levels, and responded poorly to acute administration of exogenous HGH. Pertinent observations included the following: (1) basal HGH levels were high (4–26 mμg/ml); (2) high HGH levels were not suppressed by oral glucose (four separate tests); (3) provocative stimuli for HGH release (arginine monochloride and insulin-induced hypoglycemia) further increased HGH levels in plasma (from 4 to 30 and from 5 to 20 mμg/ml respectively); (4) there was an exaggerated and prolonged hypoglycemic response to exogenous insulin. The patient's response to exogenous HGH (2.5 mg b.i.d. intramuscularly for 4 days) was compared with that observed in four sexual ateliotics similarly treated. Results were as follows: (*a*) The sexual ateliotics showed an average fall in urinary nitrogen of 2.2 g, a fall in serum urea nitrogen of 9.8 mg/100 ml, a fall in urinary phosphorus of 231 mg, and a 2-fold rise in urinary calcium. F.S. did not exhibit a fall in SUN, or a decrease in urinary phosphorus, or hypercalciuria, and average urinary nitrogen decreased by only 0.8 g. (*b*) Sexual ateliotic subjects exhibited insulinopenia after glucose and arginine; after HGH treatment the plasma insulin response was greatly augmented. HGH treatment did not increase insulin levels after a glucose load in F.S. (*c*) When given an infusion of HGH (2.5 mg in 20 min), sexual ateliotics showed a decrease in plasma alpha amino nitrogen levels; alpha amino nitrogen levels were not changed in F.S. by a similar HGH infusion. Our studies do not differentiate between (*a*) tissue resistance to a normal HGH molecule and (*b*) the presence of an abnormal HGH molecule, antigenetically intact, but functionally impotent, and capable of competing with normal HGH for peripheral receptor sites. In either case, the net result is a state of peripheral subresponsiveness to HGH in a dwarfed individual.

244. Effect of Purified Staphylococcal Alpha Toxin on Active Sodium Transport and Mitochondrial Function. JAMES J. RAHAL, JR.,* MARTIN E. PLAUT,* AND LOUIS WEINSTEIN,** Boston, Mass.

Purified staphylococcal alpha toxin inhibits transepithelial short-circuit current in the isolated toad bladder and stimulates oxygen consumption by toad bladder tissue. Further studies have been made to determine whether the former effect represents an equivalent inhibition of active sodium transport and to explore the nature of the effect on oxygen consumption. ^{24}Na and ^{22}Na were added to the serosal and mucosal bathing media of the isolated toad bladder. Short-circuit current was measured by a voltage clamp apparatus, and bidirectional sodium flux determinations were calculated during four 30 min time periods. Purified staphylococcal alpha toxin (12,000 hemolytic units) was added to the serosal bath after the second period. In six experiments the mean net sodium flux was not significantly different from the short-circuit current. Both declined rapidly after the addition of

toxin. Alpha toxin (15,000 hemolytic units in borate buffer), 2,4-dinitrophenol (DNP; 0.33 mM), and borate buffer were added to comparable amounts of minced toad bladder in Warburg flasks. Alpha toxin and DNP produced 13.0% and 18.8% stimulation of oxygen consumption, respectively. When alpha toxin and DNP, a known uncoupler of oxidative phosphorylation, were added sequentially, similar increases resulted. DNP, however, blocked the stimulation of aerobic respiration caused by staphylococcal alpha toxin. Rabbit liver mitochondria treated with alpha toxin had a significantly increased respiratory rate and Mg^{++}-dependent ATPase activity. The optical density of mitochondrial suspensions exposed to toxin was decreased, indicating either swelling or lysis. These results suggest that purified staphylococcal alpha toxin inhibits active sodium transport by epithelial cell membranes and alters mitochondrial structure and function. The latter effect may result in a disturbance of ATP synthesis and utilization.

245. Characteristics of Phosphoribosyl Amidotransferase in Experimental Leukemia. GABRIELLE H. REEM* AND CHARLOTTE FRIEND,* New York, N. Y. (introduced by Eugene Y. Berger**).

Phosphoribosyl amidotransferase (PRPP amidotransferase), the rate-limiting enzyme in purine synthesis, catalyzes the first step of the pathway, the conversion of phosphoribosyl pyrophosphate (PRPP) to phosphoribosylamine (PRA). The properties of this enzyme obtained from livers of pigeons or chickens have been defined, and glutamine was found to be the preferred substrate. Infection of Swiss mice with Friend leukemia virus caused a marked increase in the activity of splenic PRPP amidotransferase. Enzyme activity was measured in the cell-free supernatant fraction of crude homogenate and in partially purified enzyme. It was found that the conversion of PRPP to PRA required ammonium or glutamine as substrate and the presence of magnesium ion. V_{max} with ammonium chloride as substrate was greater than V_{max} with glutamine. The activity of the enzyme was subject to feedback control in vitro by natural and synthetic purines. Among the natural purine nucleotides, 6-aminopurines were more potent inhibitors than 6-oxypurines. It was possible to inhibit PRPP amidotransferase in vivo by injecting adenine intraperitoneally into mice infected with Friend leukemia virus. Enzyme activity was assayed in the cell-free supernatant fraction of spleen. The inhibition caused by injection of adenine could be reversed and enzyme activity fully restored by dialyzing the supernatant fraction for 2 hr. The addition of adenine in vitro to the reactivated enzyme again inhibited its activity. These studies demonstrate that the activity of PRPP amidotransferase in experimental murine leukemia is sensitive to inhibition by natural and synthetic purines. The substrate requirements of the enzyme from leukemic tissues differ from those described for the enzyme of chicken and pigeon livers in that ammonium chloride as well as glutamine is a suitable substrate.

246. Regional Pulmonary Function in the Syndrome of Alveolar-Capillary Block. A. RENZETTI, JR.,* T. KOBAYASHI,* A. BIGLER,* AND M. MITCHELL,* Salt Lake City, Utah (introduced by Frank Tyler**).

This study is concerned with regional changes in ventilation and perfusion and their relationship to over-all pulmonary gas exchange in the diffuse diseases of the lung. 11 normal subjects and 11 patients selected on the basis of X-ray evidence of generalized densities and absence of spirographic evidence of ventilatory obstruction were studied by the radioactive xenon technique described by Ball and coworkers. Indices of ventilation during quiet breathing and after deep inspiration, as well as of perfusion, were calculated for each of three zones of each lung. Patients were divided into two groups on the basis of the absence (group A) or presence (group B) of the physiologic findings characteristic of the "syndrome of alveolar-capillary block." All six subjects in group A had nodular silicosis. The five subjects in group B had scleroderma, desquamative interstitial pneumonia, diffuse interstitial fibrosis, silicosis, and undiagnosed acute diffuse inflammatory disease. The principal finding of the study was a relatively uniform distribution of perfusion from apex to base, in contrast to the increasing perfusion indices found in normal subjects and those of group A. The distribution of ventilation was normal in both groups A and B. The 100% oxygen test did not show sufficient evidence of a right-to-left shunt to account for the hypoxemia found in group B. Since ventilation-perfusion relationships were probably already close to optimal, the findings of the study support the concept that the hypoxemia following exercise in the "syndrome of alveolar-capillary block" results in the main from the reduced diffusing capacity of the lung for oxygen.

247. Hemoglobin Philly: Increased Reactive Sulfhydryl Groups in an Unstable Hemoglobin. RONALD F. RIEDER* AND FRANK A. OSKI,* Brooklyn, N. Y., and Philadelphia, Pa. (introduced by L. W. Eichna**).

An 8 yr old Caucasian girl was found to have chronic compensated hemolysis and splenomegaly. The father and brother had increased reticulocyte counts. Erythrocyte morphology was normal and no enzyme defects were found. Exposure of the patient's erythrocytes to the redox dye brilliant cresyl blue for 2 hr at 37°C caused formation of multiple spheroidal inclusion bodies. Hemoglobin precipitation occurred when the patient's hemolysate was heated to 50°C. Starch gel electrophoresis at pH 8.6 revealed no abnormal hemoglobin. Upon titration with *p*-hydroxymercuribenzoate (PMB), hemolysates from the patient and her father showed an average of four reactive sulfhydryl groups per molecule of hemoglobin as compared with two reactive groups in normal hemolysates. When patient's denatured globin was reacted with 5,5′-dithiobis-(2-nitrobenzoic acid), a normal total of six sulfhydryl groups per hemoglobin molecule was found. After treatment of the patient's hemolysate for ½ hr with 4–5 moles of PMB per mole of hemoglobin in 0.05 M potassium phosphate, pH 7.0,

starch gel electrophoresis demonstrated prominent bands in the position of β-PMB, α-PMB, and free α chains. Starch block electrophoresis indicated that approximately 40% of the PMB-treated hemoglobin existed as separated α and β chains. Normal hemolysates revealed no splitting into α and β subunits after 24–48 hr of similar treatment. In native Hb A, two of the three pairs of sulfhydryls are in the interior of the molecule, inaccessible to PMB. The data indicate that our patient is heterozygous for an abnormal hemoglobin, Hb Philly, in which all six sulfhydryls readily react with PMB with cleavage of the tetramer into α and β subunits. Interaction of erythrocyte components with the exposed sulfhydryls of Hb Philly may result in hemoglobin precipitation and hemolysis.

248. Stimulation of Bone Marrow Colony Growth In Vitro by Human Urine. WILLIAM A. ROBINSON,* Melbourne, Australia, and Denver, Colo. (introduced by Gordon Meiklejohn**).

Using a recently devised method of bone marrow culture with which granulocytic-mononuclear cell colonies can be grown in vitro from single cells, urines from 50 humans have been tested for their ability to stimulate colony growth. 0.15 ml samples of dialyzed urine were placed in 35 mm plastic Falcon Petri dishes with a single cell suspension of 75,000 nucleated mouse bone marrow cells in 1 ml of a mixture of Eagle's Minimal Essential Medium and 0.6% agar. Plates were incubated at 37°C in 5% CO_2 in air and colony counts done at day 10 of incubation. The number of colonies counted was taken as an index of the level of colony-stimulating activity. Low levels of colony-stimulating activity have been found in the urine of normal human subjects with a definite diurnal variation. High levels have been found in urine from 8 out of 10 patients with acute myeloid leukemia and 2 out of 3 patients with multiple myeloma. The level of colony-stimulating activity in urine from 8 patients with severe anemia of various kinds did not differ from that found in the urine of normal subjects. The presence of colony-stimulating activity in urine could not be correlated with the peripheral white blood count or hemoglobin level. Colony-stimulating activity in urine is unaltered by heating to 65°C for 4 hr, treatment with ethyl ether, or prolonged dialysis. Activity is retained after lyophilization or storage at −20°C for 3 months. On the basis of zone sedimentation, the factor responsible for colony-stimulating activity appears to have a molecular weight of approximately 60,000. Colony-stimulating activity is recovered in the postalbumin region after starch Geon block electrophoresis of active urine.

249. "Big Insulin": A New Component of Plasma Insulin in Man. JESSE ROTH,* PHILLIP GORDEN,* AND IRA PASTAN, Bethesda, Md. (introduced by J. E. Rall**).

Although the concentration of insulin in the plasma of normals and diabetics has been studied extensively, the nature of circulating insulin is unclear. To characterize endogenous plasma insulin, heparinized plasma was filtered on G-50 Sephadex and the insulin in each fraction measured by double-antibody radioimmunoassay. Most of the plasma insulin was recovered as a discrete peak 0.45 of the distance between the protein and salt peaks ("little insulin"). Endogenous plasma insulin showed an additional peak 0.25 of the distance between the protein and salt peaks ("big insulin") which comprised from 0 to 40% of total plasma insulin. When "big insulin" and "little insulin" were separated, concentrated, and refiltered, each retained its characteristic migration pattern and remained free of the other. When highly purified crystalline pork insulin (pancreatic insulin) was added in increasing quantities to insulin-free plasma and to plasma containing 40% "big insulin," pancreatic insulin migrated only with "little insulin." In this immunoassay system, pork and human insulin react identically; "little insulin" over a 20-fold range reacted identically with pork insulin. However, "big insulin" over a 40-fold range was less reactive than pork but more reactive than beef. Appropriate control experiments excluded effects on gel filtration and on immunoassay due to variations in salt concentrations, buffer conditions, anticoagulant, damage to labeled hormone, interference with the second antibody step, and endogenous insulin antibody. Individual plasmas studied on different occasions and on different assays yielded reproducible patterns. 15 min after glucose ingestion, essentially all the insulin was "little insulin." By 60–120 min, up to 40% of the plasma insulin was "big insulin." As yet, we have not demonstrated the source of "big insulin" nor elucidated its biological activity or chemical relationship to pancreatic insulin. However, we conclude that plasma insulin by radioimmunoassay exists in two forms, one very similar to pancreatic insulin and the other a related but larger molecule.

250. In Vivo, Perfused Liver, and Microsomal Inhibition of Albumin Synthesis after a 24 Hour Fast. MARCUS A. ROTHSCHILD, MURRAY ORATZ,* SIDNEY S. SCHREIBER,* AND JOSEPH G. MONGELLI,* New York, N. Y.

The decrease in albumin production associated with malnutrition is thought to be due to a deficiency in available precursors; the acute effects of a short-term fast are not available. This question was studied by determining albumin synthesis in vivo, in the isolated perfused liver, and in subcellular systems derived from perfused and control livers studied before and after a 24 hr fast. Seven male rabbits were studied in vivo before and after a 24 hr fast. ^{14}C-carbonate was used to label the intracellular hepatic arginine pool and thus the carbon of urea and the guanido carbon of albumin, permitting a direct product-precursor relationship. Albumin synthesis averaged 343 ± 40 mg/kg per day before fasting and 206 ± 13 after the fast ($P < 0.001$). 15 rabbit livers were perfused for 2½ hr with a mixture of 2 parts fresh rabbit blood and 1 part Ringer's amino acid glucose solution at flow rates of 0.3–1.0 ml/min per g. Gluconeogenesis and lactate utilization were measured and indicated functioning preparations. ^{14}C-carbonate was used as the tracer. Even though the livers were perfused with the same

perfusion solution, those derived from fasted rabbits made 16.3 ± 1.8 mg albumin/2½ hr as compared with 31 ± 2.1 mg in "fed" livers ($P > 0.01$). Microsomes were isolated from fed control and fed perfused and fasted control and fasted perfused livers. Perfused and nonperfused "fed" microsomes were equally active in incorporating ^{3}H-leucine and ^{14}C-phenylalanine into TCA-precipitable material, but nonperfused fasted microsomes were only $67.5 \pm 3.6\%$ as active ($P < 0.05$), and perfused fasted only $54.8 \pm 8.8\%$ with ^{3}H-leucine as the indicator. The fasted microsomes were 68% as active with ^{14}C-phenylalanine. The present study demonstrates that a short-term fast rapidly inhibits albumin production and that even though the amino acids are resupplied, the inhibition persists at a subcellular level.

251. Nonidentical Peptide Chains in Human Plasma Alpha Lipoprotein. D. RUDMAN, L. A. GARCIA,* L. L. ABELL,* O. COOKE,* AND S. AKGUN,* New York, N. Y.

Human plasma high-density lipoprotein (αLP) was isolated by ultracentrifugation, and its purity was verified by electrophoresis and immunodiffusion. The lipoprotein was totally delipidated by lyophilization followed by extraction of lipids with ethanol-acetone. The delipidated protein (αP) moved as a single band on cellulose acetate electrophoresis, and gave a single precipitin arc with rabbit antiserum to human αLP, but was resolved into three fractions by gel filtration on Sephadex G-200, with K_d values corresponding to molecular weights 150,000 (fraction 1), 70,000 (fraction 2), and 25,000 (fraction 3). Analysis of these fractions by electrophoresis, immunodiffusion, quantitative amino acid composition, fingerprinting of tryptic digests, and rechromatography on G-200 after disaggregation by sodium dodecyl sulfate demonstrated that fraction 1 is a dissociable aggregate of approximately six molecules of a subunit (A) of molecular weight 20,000–25,000; fraction 3 is monomeric form of a different subunit (B) of similar molecular weight; fraction 2 is a dissociable aggregate with composition A_2B or A_3B. The antigenic site responsible for reaction of αP with rabbit anti-human αLP resides in subunit B. On zone electrophoresis at pH 8.6, A migrates 1.2 times more rapidly than B. Major compositional differences: Subunit A contains (per molecule) 12 arginine, 2 half-cystine, 4 histidine, 5 phenylalanine, and 9 serine; corresponding values for B are 3, 6, 1, 11, and 17. Tryptic fingerprints of A and B are different and together account for the fingerprint of αP. Amino acid composition of αP indicates that it contains A and B in 3/1 ratio.

252. Resistance to *Listeria* and *Salmonella* Induced by *Toxoplasma*. JOEL RUSKIN* AND JACK S. REMINGTON,* Palo Alto, Calif. (introduced by Herbert C. Schwartz).

Available data suggest that cellular mechanisms may be more important than antibodies in immunity to infections with intracellular bacteria (*Listeria, Salmonella*) and certain protozoa (*Toxoplasma*). To assess the possibility that a mechanism of immunity might be common to infections with these apparently unrelated intracellular organisms, mice infected with *Toxoplasma gondii* were challenged with *Listeria monocytogenes* or *Salmonella typhimurium*. Both intraperitoneal and subcutaneous routes of inoculation of *Toxoplasma* produced resistance to intravenous or intraperitoneal challenge with doses of *Listeria* which were lethal to normal mice. Protection against *Listeria,* as measured by survival or prolongation of time to death, was evident as early as 24 hr and for as long as 7 months after *Toxoplasma* infection. Protection against *Salmonella* was similarly evident in *Toxoplasma*-infected mice. In other experiments, resistance to a virulent strain of *Toxoplasma* could not be demonstrated in *Listeria*-immune animals. Intravenous passive transfer of serum obtained from *Toxoplasma*-immune mice (dye titer $> 1{:}8000$) did not confer protection on normal animals against challenge with *Listeria.* Since interferon production is induced by *Toxoplasma,* experiments were performed to determine whether high levels of interferon in normal animals would protect them against bacterial challenge. Such levels were induced with statalon, pyran copolymer, and Newcastle disease virus, but did not confer resistance on these animals. The clearance mechanism of the reticuloendothelial system was studied in *Toxoplasma*-infected animals and controls using intravenously administered carbon particles. No enhancement of the clearance mechanism was demonstrable in the protected animals. In preliminary experiments designed to determine whether similar immunity results from infection with other intracellular protozoa, *Besnoitia jellisoni* infection was found to produce comparable protection against *Listeria* and *Salmonella.* These observations suggest that similar immune mechanisms may be operative in apparently unrelated intracellular infections. Further studies to define these mechanisms are in progress.

253. (Abstract withdrawn)

254. Potent Vasodepressor Substance in Normal Lung. SAMI I. SAID, HERSCHEL L. ESTEP,* MARION E. WEBSTER,* AND HERMES A. KONTOS,* Richmond, Va., and Bethesda, Md.

When injected intravenously in anesthetized dogs, homogenates of normal lung from dogs, rabbits, or humans produce systemic hypotension, pulmonary hypertension, and contractile responses in certain extravascular smooth muscle. In earlier experiments we found that none of the vasoactive substances known to occur in lung tissue—histamine, 5-hydroxytryptamine, and prostaglandins—could account for these observations. We report here on further attempts to identify the responsible agent. Since the most pronounced and most constant effect in the whole animal was systemic hypotension, we examined the vasodepressor action of lung extracts in the isolated hind limb of dog, perfused

at constant flow. Perfusion pressure served as an index of dilatation or constriction of the limb vessels. As little as 0.01 ml of lung extract (5 mg lung tissue) caused a measurable vasodilatation. With larger doses of extract, the fall in mean perfusion pressure became increasingly greater and more prolonged, reaching a maximum of about 100 mm Hg for as long as 12 min. The vasodilator action was preserved after all particulate matter had been removed by centrifugation in the cold at 100,000 *g* for 45 min. After dialysis against water, the extract lost some potency, but boiling virtually destroyed its effectiveness. When the extract was filtered through Diaflo membrane ultrafilters, the vasodepressor material was largely in the fraction containing substances of mol wt $\leqslant 2000$, but partly in the fraction with mol wt $\geqslant 20{,}000$. Addition of Trasylol (kallikrein inhibitor, 1 mg/ml) reduced the magnitude and duration of the hypotensive effect by approximately 50%. These findings are consistent with the presence in lung tissue of at least two vasodepressor compounds, one of which might be a kallikrein or its activator. Release of these vasoactive compounds could play an important role in certain pathologic states, e.g., pulmonary embolism.

255. Effects of Sulfation Factor on Cartilage. WILLIAM D. SALMON, JR.,* MARGARET R. DUVALL,* AND EMMA Y. THOMPSON,* Nashville, Tenn. (introduced by R. Des Prez).

The action of growth hormone in stimulating sulfate incorporation by cartilage is mediated by a serum factor, the so-called sulfation factor (Salmon and Daughaday, 1957). On the basis of evidence implicating protein synthesis in the action of this factor, it has been suggested that the latter might increase formation of protein-polysaccharide complexes (noncollagenous protein complexed with chondroitin sulfate). In the present studies, serum extracts containing sulfation factor activity were tested for effects on in vitro metabolism of costal cartilage from hypophysectomized rats. Incorporation of both ^{35}S-sulfate and ^{14}C-leucine into protein-polysaccharide complexes was stimulated by the extracts, but increase in leucine incorporation preceded increase in sulfate incorporation. Together with earlier evidence, the findings suggest that impairment of sulfation in cartilage after hypophysectomy of the rat is the result of limited ability to synthesize protein. The extracts also stimulated incorporation by cartilage of ^{3}H-uridine into RNA and of ^{3}H-thymidine into DNA. The latter finding confirms the report (Daughaday and Reeder, 1966) of in vitro stimulation of DNA synthesis in cartilage by whole serum containing sulfation factor activity. Stimulation of uridine incorporation was an early effect, whereas stimulation of thymidine incorporation was delayed. Uridine incorporation into RNA, in the presence or absence of extract, was inhibited by actinomycin. Preliminary studies suggest that the extracts exert an initial stimulating effect on leucine incorporation into protein even when stimulation of uridine incorporation into RNA is inhibited.

256. In Vivo Synthesis and Turnover of Synovial Fluid Hyaluronateprotein. JOHN SANDSON, New York, N. Y.

Hyaluronate, a macromolecule isolated from human synovial fluid (SF), contains a small amount ($\pm 2\%$) of firmly bound noncollagenous protein and has been designated hyaluronateprotein (HP). By the use of three different isotopes, it has been possible to study both the synthesis and the turnover of HP in the "knee joints" of young calves: (1) HP isolated from bovine SF was labeled in vivo with ^{131}I and the ^{131}I-HP was then injected into the "knee joint." The half-life of ^{131}I-HP in SF was determined by serial measurements of specific activity of HP isolated from samples of SF obtained daily. The half-life of ^{131}I-HP varied between 20 and 30 hr. (2) ^{3}H-glucosamine was injected into the "knee joint." ^{3}H-glucosamine was rapidly incorporated into HP. It was possible to measure the half-life of ^{3}H-HP by serial measurements of specific activity. In three experiments, values of between 25 and 30 hr were obtained. (3) ^{14}C–amino acids injected into the "knee joint" were also incorporated into HP. The half-life of ^{14}C-HP was approximately 30 hr. Several points have been established by these studies. First, both glucosamine and amino acids can be rapidly incorporated into HP by tissues in the joint. Second, HP is not inert metabolically. Both the protein and the hyaluronate "turn over" rather rapidly. Third, the half-life of the protein (measured by either ^{131}I– or ^{14}C–amino acids) is approximately the same as the half-life of the hyaluronate (measured by ^{3}H-glucosamine). These in vivo experiments establish that the protein moiety of HP is synthesized in the joint and "turned over" at about the same rate as hyaluronate in SF. These results substantiate the theory that the small amount of protein firmly bound to hyaluronate is an integral part of this macromolecule.

257. Reentry of Nondividing Leukemic Cells into a Proliferative Phase in Acute Childhood Leukemia. E. F. SAUNDERS* AND A. M. MAUER, Cincinnati, Ohio.

In acute childhood leukemia the blast population consists of two kinetic compartments: one proliferative, the other nonproliferative (Go stage). Nonproliferative blasts, distinguishable morphologically and functionally from dividing blasts, are derived from the proliferative compartment. In steady-state kinetics the proliferative compartment is not self-maintaining. Nonproliferative blasts could recycle to maintain the proliferative compartment or could be end stage cells. A two-part label dilution experiment with tritiated thymidine and radioautography was done in a child with acute lymphoblastic leukemia. During the study the mitotic index and proportion of proliferative blasts remained constant, indicating a steady state. In part one, three intravenous injections of ^{3}H-T (100 μc/kg, q. 12 h) were given. During the subsequent 32 hr, labeling of proliferative blasts decreased from 75% to 20% and of mitotic figures from 93% to 13%. Labeled cells were diluted by un-

labeled blasts entering to maintain the proliferative compartment. Labeling of nonproliferative blasts increased from 10% to 20%. In part two, immediately following, nine additional injections of ^{3}H-T were given similarly. In contrast to part one, nonproliferative blasts were now 65% labeled, and labeling of proliferative blasts remained constant at about 85% during the subsequent 48 hr. Labeled mitotic figures decreased only from 93% to 75%. This lack of label dilution indicated that blasts now entering the proliferative compartment were themselves labeled. The only recognizable cell population virtually unlabeled in part one and heavily labeled in part two was the nonproliferative blasts. Therefore, the proliferative compartment must have been maintained by entry of nonproliferative blasts. The effectiveness of chemotherapy acting primarily on dividing cells may be explained by recycling of nonproliferative blasts. Nonproliferative blasts with prolonged recycling times, resistant to therapy and surviving into remission, could initiate relapse. As the total leukemic cell population is self-maintaining, continuing malignant transformation is unnecessary in acute leukemia.

258. Studies of Intracerebral Mechanisms of Encephalopathy in Thiamine Deficiency. STEVEN SCHENKER,* D. W. MCCANDLESS,* JAMES SHOREY,* AND MARTHA COOK,* Dallas, Texas (introduced by Robert L. Johnson, Jr.).

Thiamine deficiency induces encephalopathy in man and experimental animals. Histologic lesions occur primarily in brainstem (less extensively in cerebellum), but early neurologic signs reverse rapidly and fully with thiamine, indicating a metabolic disorder. Suggested mechanisms of thiamine-deficient encephalopathy involve two thiamine-dependent enzymes: (1) impairment of pyruvate decarboxylase with decreased cerebral energy (ATP) synthesis, and (2) impairment of transketolase with possible decrease in TPNH. TPNH may be important in maintenance of myelin and of reduced glutathione (GSH), the latter probably keeping enzymes in a reduced (active) conformation. This study examines these mechanisms in rats with encephalopathy induced by thiamine deprivation of 5 wk and in pair-fed and normally fed asymptomatic controls. In thiamine-deficient symptomatic rats as compared with controls: (1) blood transketolase and whole-brain thiamine decreased by 89% and 83% respectively; (2) cortical pyruvate decarboxylase activity (PDA) and lactate concentration were normal, whereas in brainstem and cerebellum PDA fell by 29% and 36% ($P < 0.03$) and lactate rose by 69% and 32% respectively ($P < 0.05$); (3) ATP concentrations in all three brain areas were normal; (4) cortical, brainstem, and cerebellar transketolase activity fell by 41%, 61%, and 57% respectively ($P < 0.03$); (5) cortical and cerebellar GSH concentrations were normal and brainstem concentrations fell by only 18% ($P < 0.05$). Thiamine, 10 μg, fully reversed the neurologic signs in 16–30 hr. In these "reversed" animals, as compared with controls, brain thiamine was decreased by 74%, brainstem lactate was higher by 30%, brainstem and cerebellar PDA were lower by 17% and 21%, and brainstem transketolase activity and GSH still lower by 53% and 12% respectively ($P < 0.05$). Thus, in rats with thiamine-deficient encephalopathy (1) brainstem and cerebellum are primarily affected, and (2) both cerebral PDA and to a larger extent transketolase are depressed. Since both enzymes rise on "reversal," their relative importance in causing encephalopathy is uncertain, although transketolase rises only slightly. The normal cerebral ATP concentration and small GSH fall during encephalopathy, with little GSH rise on "reversal," suggest that a depletion of neither substance is instrumental in inducing thiamine-deficient encephalopathy.

259. Comparative Kinetics of Active Transport of Bile Acid Analogues. EUGENE R. SCHIFF* AND JOHN M. DIETSCHY,* Dallas, Texas (introduced by Norman M. Kaplan).

Though maintenance of the enterohepatic circulation of conjugated bile acids depends primarily on active ileal absorption, precise definition of the kinetics of this active transport process has not been undertaken. In order to establish the molecular determinants for active transport, the active flux of various bile acid analogues was measured in vitro under conditions in which the mucosal bile acid concentration was varied systematically and the transmembrane electrochemical gradient was fixed at zero. V_{max} and K_m values for each bile acid were obtained by Lineweaver-Burke analysis. (1) V_{max} is directly related to the number of hydroxyl groups on the bile acid molecule, e.g., glycolithocholate (144 $\mu\mu$moles/min per cm), glycochenodeoxycholate (213), glycodeoxycholate (217), and glycocholate (1548). An identical relationship was found for taurine conjugates and unconjugated analogues of these bile acids. (2) Cholanic acid (possessing no hydroxyl group) is not actively transported. (3) The value of K_m is independent of the number of nuclear hydroxyl groups but is strikingly dependent upon conjugation at the C-24 position; K_m values for taurine and glycine conjugates of mono-, di-, and trihydroxy bile acids range between 110 and 220 mμmoles/ml, and those for the corresponding unconjugated acids equal 640–900. These studies establish the fact that a nuclear hydroxyl group is essential for active transport and, further, that the maximum rate of such transport is directly related to the number of hydroxyl groups on the bile acid molecule. Finally, for any particular bile acid, conjugation with taurine or glycine markedly enhances affinity for the transport carrier. From these data it is clear that under physiological conditions unconjugated bile acids cannot effectively compete for the active transport site in the ileum. Since fully one-third of bile acids in portal blood are unconjugated, it follows that a second transport mechanism, presumably passive nonionic diffusion, is also of major importance in maintenance of the enterohepatic circulation.

260. Treatment of Chronic Lymphocytic Leukemia with Extracorporeal Irradiation of the Blood (ECIB). L. M. SCHIFFER,* A. D. CHANANA,* E. P. CRONKITE,** M. L. GREENBERG,* S. OKUYAMA,* K. R. RAI,* P. A. STRYCKMANS,* AND P. C. VINCENT,* Upton, N. Y.

13 patients with chronic lymphocytic leukemia have been treated with repetitive ECIB. 8 patients had had chemotherapy and/or radiotherapy, but all 13 were ill and required treatment when seen initially. After 4 hr of ECIB, the peripheral blood lymphocyte count (LC) decreased to 20–95% (average 60%) of that measured before each ECIB session. Over a period of 2–6 wk the daily pre-ECIB LC declined exponentially until a stable level between 2% and 20% of the pretreatment value was reached. The LC remained at this plateau while ECIB was continued, but increased slowly when ECIB was stopped. The LC of one patient declined from 250,000/mm^3 to 4000/mm^3 in 25 days, remained at that level for 4 months while ECIB was given once or twice weekly, and then rose exponentially to 100,000/mm^3 during the subsequent 15 months. He has been successfully re-treated with ECIB. At the end of repetitive ECIB there was a decrease in the volume of lymph nodes and spleen in about half the patients, although shortly after the initial ECIB sessions these lymphoid organs frequently became significantly enlarged. After ECIB, erythropoietic elements in the bone marrow were relatively increased in 4 of 5 patients, as were myelopoietic cells in 2 of 5 patients. The quality of the clinical response was not proportional to the radiation dose received by the blood in a single transit past the gamma ray source. The average transit dose varied from patient to patient (236–498 rad). 8 patients had fever and/or chills after the initial few ECIB sessions. This reaction has not been noted during ECIB treatment of 30 patients with other diseases. ECIB offers the clinician an alternative treatment to chemotherapy in patients with chronic lymphocytic leukemia.

261. Isolation of "Native" Glycogen from Human Platelets. ROBERT B. SCOTT* AND LAVERNE W. COOPER,* Richmond, Va. (introduced by G. Watson James, III**).

A pure preparation of undegraded glycogen is a prerequisite for detailed in vitro study of its metabolism. Such a preparation should include the entire organelle ("glycosome"), i.e., the glycogen molecule and its attached enzyme protein. Platelets were studied because of relatively large glycogen stores, relative homogeneity of particle size, and little likelihood of nucleic acid contamination. Platelet-rich plasma was prepared from 400 ml of blood collected in EDTA, and the platelets were recovered by centrifugation at 10,000 *g* for 25 min. After washing in MEM spinner, the pellet was homogenized in 4.5 ml 0.01 M Tris, pH 8.0, by 20 strokes of a "no-clearance" homogenizer. Large fragments were removed at 1000 *g* for 10 min, after which the homogenate was layered on three 28 ml 10–29% (w/v) linear sucrose density gradients. These were centrifuged for 150 min at 24,000 rpm in an SW 25.1 rotor. The pellet contained granules and part of the glycogen; the remaining glycogen banded in the center of the gradient; smaller molecules remained at the top. Fractions (1 ml) were collected by puncturing the bottom of the tubes, fractions 6–20 were pooled, and the glycogen was sedimented at 29,500 rpm in a 30 rotor for 5 hr. The glassy yellowish pellet was washed once by centrifugation and suspended in Tris buffer. The isolated glycogen (3–5 mg/400 ml blood) sedimented as a single peak in the analytical ultracentrifuge and was degraded to smaller fragments by α-amylase. The material contained protein (0.15–0.37 $\mu g/\mu g$ glycogen) which was not removed by saponin treatment. The UV spectrum differed from that of chemically purified glycogen in a slight broad shoulder at 260–280 $m\mu$; this was unaffected by prior ribonuclease or saponin treatment. This product satisfies the general criteria for "native" glycogen and makes it feasible to study the nature of the glycogen-associated protein.

262. Translational Control of Protein Synthesis during the Immune Response. HELENA S. SELAWRY* AND JASON L. STARR,* Memphis, Tenn. (introduced by Gene H. Stollerman**).

The rate of enzyme synthesis in microorganisms is controlled by induction and repression, which are properties of specific regulatory genes. The Jacob-Monod hypothesis relegates the ultimate mechanism to the rate of transcription of labile messenger RNA. Mammalian RNA and protein metabolism differ from bacterial in the following respects: separation of nucleus and cytoplasm; confinement of majority of DNA-like RNA to the nucleus; relatively unstable ribosomes and stable mRNA. Cellular proliferation, furthermore, accompanies antibody induction. Recent reports suggest that mammalian protein synthesis is regulated by both the rate of RNA synthesis and the efficiency of translation of the message. This paper will offer evidence that the effects of immunization are associated with regulatory changes at the translational level. Primary immunization was achieved in male Sprague-Dawley rats with *Salmonella oranienburg* flagella. At specified intervals thereafter their spleens were removed for preparation of microsomes and ribosomes. Controls were obtained from unimmunized rats. The conditions for an amino acid incorporation system using liver pH 5 enzymes from another rat were determined. The efficiency of ribosomes and microsomes per unit RNA for incorporation of amino acids into protein was determined at each time interval. This included measurement of amino acid incorporation into native particles, incorporation after preincubation to remove native messenger, and after stimulation with synthetic polynucleotides. The rate of endogenous incorporation increased to 200% 48–72 hr after immunization, and remained slightly elevated at 10 days. Preincubation blocked incorporation and apparently eliminated the differences. The increased efficiency of immunized particles was again demonstrated by polynucleotide stimulation. A maximum of 2- to 3-fold was reached at 48–72 hr, with return to normal at 96 hr. The increased endogenous incorporation

is consistent with increased mRNA. The greater efficiency of preincubated immunized particles suggests participation of regulation at the translational level during the immune response.

263. The Effects of Thyroid Disease on Glucose Oxidative Metabolism: A Multicompartmental Analysis. DAVID SHAMES,* MONES BERMAN,* AND STANTON SEGAL, Bethesda, Md., and Philadelphia, Pa.

Knowledge of glucose metabolism in patients with thyroid disease has been limited to that obtained from standard glucose tolerance tests. An analysis of ^{14}C-glucose kinetics has been performed to further characterize derangements of sugar metabolism associated with thyroid malfunction. Glucose oxidation to CO_2 in man at the fasted, steady state has been investigated in normal, hypothyroid, and hyperthyroid patients by monitoring the specific activity of plasma glucose and expired CO_2 after the i.v. administration of 6-^{14}C-glucose, 1-^{14}C-glucose, and $NaH^{14}CO_3$ in tracer amounts. The data are incorporated into a multicompartmental model describing the kinetics of plasma glucose, plasma bicarbonate, and the conversion of glucose to CO_2 by the hexose monophosphate (HMP) and Embden-Myerhoff tricarboxylic acid (EMP) pathways. This formulation separates the kinetics of glucose and bicarbonate exchange from the kinetics of glucose oxidation to CO_2. It allows calculation of a minimal fraction of glucose loss from the plasma which is oxidized to CO_2 as observed over a 5 hr interval. Such a calculation is independent of the extrapolated values of the particular compartmental model chosen to fit the data. The analysis indicates that hypothyroidism is mainly associated with a decrease in the rate of glucose loss from the plasma. However, the fractions of glucose lost which are oxidized to CO_2 by both the HMP and the EMP are not significantly different from normal. In hyperthyroidism the rate of glucose loss from the plasma and the fraction of glucose loss oxidized to CO_2 by the EMP are within the normal range. The significant change in glucose metabolism accompanying hyperfunction is a decrease in the fraction of glucose lost which is oxidized to CO_2 by the hexose monophosphate shunt.

264. Hypertonic Sodium Chloride Infusion as a Test for Hyperparathyroidism. STANLEY R. SHANE,* EDMUND B. FLINK,** AND JOHN E. JONES,* Morgantown, W. Va.

Hypertonic saline infusion has been reported to increase markedly calcium excretion in hyperparathyroid as opposed to control subjects, and has been recommended as a possible diagnostic test. In order to evaluate further the diagnostic utility of this test, 33 infusions were carried out in 27 subjects: 5 with hyperparathyroidism (HP), 7 with nephrolithiasis and hypercalciuria (NH^+), 9 with nephrolithiasis without hypercalciuria (NH^-), and 6 normal subjects (NS). On the 3rd or 4th day of a low calcium (<250 mg) low phosphorus (<700 mg) diet, each received an infusion of 0.125 ml 5% NaCl per kg body weight per min for 45 min. 24 hr urine collections the day before (control day) and day of infusion (infusion day) were analyzed for calcium. The results, expressed as mean $\pm$ SE 23 hr urine calcium excretion in mg, were: HP control day 445 ± 85, infusion day 552 ± 108, mean increase 107 ± 36; NH^+ control day 251 ± 13, infusion day 335 ± 26, mean increase 85 ± 19; NH^- control day 126 ± 11, infusion day 166 ± 17, mean increase 40 ± 10; NS control day 126 ± 17, infusion day 176 ± 35, mean increase 50 ± 25. One patient, evaluated early in our study, with nephrolithiasis and hypercalciuria had a positive hypertonic saline infusion test by previously established criteria. Surgical exploration revealed normal parathyroids. Another patient with proved hyperparathyroidism had a negative response. Analysis of the data demonstrates the known calciuretic effect of saline but does not support use of hypertonic saline infusion as a diagnostic test for hyperparathyroidism.

265. Congenital Dysprothrombinemia: An Inherited Structural Disorder of Human Prothrombin. S. SHAPIRO* AND J. MARTINEZ,* Philadelphia, Pa. (introduced by A. J. Erslev).

Studies of prothrombin metabolism have been hampered by an inability to measure this zymogen except by the activity of its biologic product, thrombin. A specific immunoassay for human prothrombin, capable of detecting levels below 1 unit per milliliter (less than 0.5 microgram protein per milliliter), has been developed in this laboratory. Using this technique, we have studied a large family in which 13 of 21 members, in three generations, were found to have half normal levels of biological prothrombin activity. However, all affected individuals have been shown to possess normal quantities of immunologically reactive prothrombin. The pattern of inheritance is autosomal; both males and females are affected, and all affected individuals appear to be heterozygotes. The half-life of injected ^{131}I-prothrombin, measured in one affected individual, was 3.2 days (normal 2.25–3.25 days). Prothrombin was purified from one affected individual. Although a normal yield of protein was obtained, it possessed only half normal biological specific activity when tested in several activating systems. This material was subjected to acrylamide gel electrophoresis at several pH values, to equilibrium chromatography on IRC-50 columns, and to immunologic analysis, and was found to be indistinguishable from normal prothrombin by all these techniques. Thus a hereditary defect has been identified, in which a low level of biological prothrombin activity is due to the production of a structurally altered, and apparently biologically inactive, prothrombin molecule. Present evidence indicates that this structural defect is not accompanied by an alteration in molecular charge.

266. Similar Effects of Aldosterone and Adenosine-3′,5′-Monophosphate (Cyclic AMP) on Glucose Metabolism and Sodium Transport in Toad Bladder. GEOFFREY W. G. SHARP,* MADELEINE A. KIRCHBERGER,* DENIS G. MARTIN,* AND ALEXANDER LEAF,** Boston, Mass.

In the toad bladder, aldosterone causes a stimulation of sodium transport which is associated with an increase in

oxidative metabolism. This can be measured as an increase in oxygen consumption and in the amount of $^{14}CO_2$ evolved from 6-^{14}C-glucose. Both these effects are dependent upon the availability of sodium for increased transport. Aldosterone, however, even in the absence of sodium causes a decrease in the amount of $^{14}C_2$ evolved from 1-^{14}C-glucose. This is the single largest effect of aldosterone noted in this tissue and occurs with the same time course and response characteristics as the effect on sodium transport. It is abolished by prior administration of actinomycin D. Deoxycorticosterone and dexamethasone, which also stimulate sodium transport in this tissue, give this effect, whereas progesterone, which does not stimulate sodium transport, does not. This suggests that the change in 1-^{14}C-glucose metabolism stands in causal relationship to the effect on sodium transport, or both may result from a common precursor effect. Dibutyryl-adenosine-3′,5′-monophosphate and theophylline also stimulate sodium transport and inhibit $^{14}CO_2$ from 1-^{14}C-glucose. Since vasopressin acts via cyclic AMP, the possibility exists that this compound is an intermediate in the action of both hormones. Vasopressin alone, however, had no effect on 1-^{14}C-glucose metabolism, but in the presence of theophylline vasopressin produced a further significant decrease in $^{14}CO_2$ from 1-^{14}C-glucose. This indicates that endogenously produced cyclic AMP affects 1-^{14}C-glucose metabolism similarly to aldosterone. Since other evidence suggests two parallel independent pathways for sodium transport, one of which is vasopressin sensitive and the other aldosterone sensitive, the possibility exists that cyclic AMP serves as hormonal mediator in both pathways.

267. Internal Redistribution of Tissue Protein Synthesis in Experimental Uremia. LEROY SHEAR,* Cleveland, Ohio (introduced by George J. Gabuzda**).

Uremia may be associated with altered body protein metabolism as evidenced by skeletal wasting, negative nitrogen balance, and altered rates of synthesis of urea, hemoglobin, and antibodies. These observations prompted the present in vivo study, which demonstrates an internal redistribution of tissue protein synthesis in uremia. 200-g male rats were subjected to either bilateral nephrectomy or a sham operation. Immediately after surgery, all animals were fasted 48 hr, then injected intravenously with ^{14}C-leucine, and exsanguinated 2 hr later. Liver, heart, and muscle contents of protein, RNA, DNA, and amino acids, and ^{14}C activity in nucleic acid–free TCA precipitates were measured. Total DNA contents of heart (means 820 and 810 μg) and liver (means 10.4 and 9.6 mg) were unchanged by nephrectomy. This constancy justifies use of DNA as a reference standard in uremia. ^{14}C-leucine incorporation (^{14}C-cpm/DNA and ^{14}C-cpm/protein) was decreased in uremic muscle and increased in uremic heart and liver ($P < 0.01$). The decreased ^{14}C incorporation by muscle might represent artifact rather than a change in protein synthesis if the tissue leucine precursor pool were of low specific activity. This probably was not the case, because free leucine concentrations were unchanged in muscle and decreased in uremic plasma. Moreover, ^{14}C activity per ml plasma and ^{14}C-cpm/μmole leucine were increased in protein-free plasma. Changes in tissue composition further indicate that the leucine incorporations by muscle, heart, and liver reflect changes in net protein synthesis (anabolism minus catabolism): protein/mg tissue ($P < 0.005$) and protein/DNA ($P < 0.05$) were decreased in uremic muscle; protein/mg tissue, protein/DNA, RNA/DNA, and organ weights were increased in uremic heart and liver. These findings in uremic rats demonstrate that net protein synthesis is selectively altered in different organs (decreased in muscle, increased in heart and liver) by some metabolic consequence of nephrectomy.

268. Demonstration of Impedance to Glucose Transport in Man: Evidence for Its Role in Diabetes. SHIAO-WEI SHEN,* JOHN W. FARQUHAR, ABRAHAM SILVERS,* AND GERALD M. REAVEN, Palo Alto, Calif.

The causes of postprandial hyperglycemia in mild maturity-onset diabetes may include (1) decreased insulin secretion, (2) secretion of biologically defective insulin, and (3) impedance (either humoral or cellular) to peripheral glucose translocation. We have assessed the role of impedance in subjects classified as normal or diabetic on the basis of oral GTT. The mean glycemic response to oral glucose in diabetics was 1.68 times that of normal subjects. Also, the diabetics were slightly heavier, differing in ponderal index by a factor of 1.1. After conditioning with very high carbohydrate diets, patients received a continuous infusion (150 min) of glucose (7 mg/kg body weight per hr) and crystalline insulin (6 U/150 min). The variable of endogenous insulin was bypassed by simultaneous infusion of epinephrine and propanolol. (Under these conditions, endogenous insulin secretion is less than 10 μU/ml, and glycogenolysis and hepatic glucose output are considerably inhibited.) Immunoreactive insulin concentrations were virtually identical in the two groups (means of 98 and 101 μU/ml). The steady-state plasma glucose (PG) reached by diabetics (mean = 225 mg/100 ml, range 183–263 mg/100 ml) was considerably higher than that of normals (mean = 129 mg/100 ml, range 101–178 mg/100 ml). Thus PG of diabetics exceeded that of normals by a factor of 1.74. Under the conditions of these infusions, neither the secretion rate nor the biological effectiveness of endogenous insulin can play a role in glucose homeostasis, and the differences in PG must be due to impedance to glucose uptake. The glycemic response of diabetics exceeded that of normals by a similar degree after either oral (1.68) or intravenous (1.74) glucose. This similarity suggests that a major degree of the postprandial hyperglycemia of these diabetics also resulted from a greater impedance. Finally, the virtues of observed stability of responses and control of the insulin variable provide a unique model for study of glucose and insulin interaction in the intact organism.

269. Alpha Tocopherol, an Exchangeable Component of the Erythrocyte Membrane. R. SILBER, R. WINTER,* AND H. J. KAYDEN,* New York, N. Y.

In patients with abetalipoproteinemia, tocopheryl acetate administered in vivo or tocopheryl phosphate added in vitro corrects the abnormal autohemolysis or peroxide hemolysis tests. In human and rat red cells this vitamin also has a protective effect against the hemolytic action of hyperoxia and of several oxidant drugs. The present study investigates the transport and localization of tocopherol in the rat erythrocyte. When ^{14}C–tocopheryl acetate in corn oil was administered to rats by gastric intubation, the radioactivity in plasma and red cells reached a maximum at 6 and 20 hr respectively. The plasma:erythrocyte radioactivity ratio stabilized at about 2:1 after 24 hr. The radioactivity corresponded to authentic alpha tocopherol on thin layer chromatography and was localized almost entirely in the erythrocyte membrane. When erythrocytes which had incorporated ^{14}C-tocopherol in vivo were incubated in vitro at 37°C with nonradioactive serum, red cell radioactivity decreased exponentially with a $t_{\frac{1}{2}}$ of 1.9 hr. The transport rate from red cells to plasma was almost twice as great as that from plasma to red cells. The radioactivity was transferred from erythrocytes to serum lipoproteins, but albumin and other nonlipid components did not serve as acceptors. Temperature dependence was demonstrated by inhibition of transport when the experiments were performed at 4°C. The addition of iodoacetate or sodium fluoride had no effect on influx or efflux of tocopherol, indicating that there was no dependence on glycolysis. Tocopherol transport was not dependent on the presence of divalent cations. These studies demonstrate that alpha tocopherol is another component of the erythrocyte membrane found in equilibrium with the surrounding plasma. The membrane localization of tocopherol may account for the protective effect of this vitamin against peroxidation of red cell lipids and subsequent hemolysis in the presence of oxidants.

270. Regulation of Renal Glutamine Metabolism by Bicarbonate Ion and pH. DAVID P. SIMPSON* AND DONALD J. SHERRARD,* Seattle, Wash. (introduced by Belding H. Scribner**).

In metabolic acidosis, renal glutamine utilization increases, producing more NH_3 for buffering H^+ in the urine. In the present study two factors have been defined by which systemic acid-base changes regulate the metabolism of glutamine in dog renal cortex. When slices of cortex were incubated in media of decreasing pH and $[HCO_3^-]$ (40 to 10 mM), the oxidation of ^{14}C-glutamine rose progressively. Glutamine oxidation averaged 57% greater at 10 than at 30 mM HCO_3^-. When pH and $[HCO_3^-]$ were varied independently by appropriate adjustment of the P_{CO_2}, a decrease in either of these variables was accompanied by an increase in glutamine oxidation. In isolated mitochondria a similar increase in glutamine oxidation was present when pH and $[HCO_3^-]$ decreased. In this case, however, varying the pH without change in $[HCO_3^-]$ did not significantly alter glutamine oxidation; decreasing the $[HCO_3^-]$ with fixed pH stimulated glutamine oxidation. Thus, intracellular $[HCO_3^-]$ regulates the rate of glutamine metabolism in renal cortex. The rate of glutamine oxidation also was compared in slices and mitochondria from dogs with chronic metabolic acidosis and alkalosis. Pairs of littermates were fed ammonium chloride or sodium bicarbonate for 7–10 days before sacrifice. Slices, but not mitochondria, from acidotic animals oxidized glutamine 25–75% faster than tissue from alkalotic dogs at bicarbonate concentrations of 10 or 30 mM in the media. Therefore, metabolic acidosis acts in two ways to increase glutamine breakdown and ammonia production. As plasma pH and $[HCO_3^-]$ fall, glutamine oxidation increases owing to the effect of intracellular HCO_3^- on mitochondrial glutamine metabolism. When acidosis persists, an adaptive cytoplasmic effect occurs, increasing still further the rate of glutamine oxidation.

271. Differential Inhibition of Neonate and Adult Leukocyte Migration by Endotoxin. KURT P. SLIGAR,* Baltimore, Md. (introduced by Sheldon E. Greisman).

The mechanisms underlying bacterial endotoxin toxicity are unknown. Hypersensitivity acquired by prolonged contact with Gram-negative bacteria, and primary toxicity appear the most likely possibilities. To examine these alternatives, the effects of endotoxin on in vitro migration of buffy-coat leukocytes were quantitated, employing healthy adult and neonate New Zealand rabbits. Washed buffy-coats suspended in autologous serum containing *E. coli* endotoxin (Boivin extracted) were drawn into capillary tubes, centrifuged (1600 *g*), and incubated vertically (37°C). Each assay was performed with 10 capillary tubes, and migration distances were measured at 4, 8, and 12 hr. In 10 adult rabbits, consistent and complete inhibition of leukocyte migration occurred with endotoxin concentrations of 100 μg/ml; partial but significant ($P < 0.001$) inhibition at 10 μg/ml, and no inhibition at 1 μg/ml. In contrast, in seven neonate rabbits (24–48 hr old), leukocyte migration remained consistently uninhibited by 100 μg/ml endotoxin regardless of whether suspended in adult or neonate serum. Adult leukocyte migration was inhibited equally by endotoxin in either adult or neonate serum. Neonate leukocytes responded as adult cells to a nonspecific toxic stimulus. Old tuberculin equally inhibited adult and neonate leukocyte migration at concentrations of 1:10 and 1:25 and failed to inhibit either at 1:100. Inability of endotoxin to inhibit neonate leukocyte migration in the manner of adult cells appeared attributable to failure to release an inhibitory factor into the suspension rather than to intrinsic differences in migratory activity. In the absence of endotoxin, neonate leukocytes migrated at rates comparable to those of adult cells, and addition of adult leukocytes (1:1 mixtures) resulted in inhibition of all migration by 100 μg/ml endotoxin. These observations demonstrate that adult leukocytes are significantly more sensitive than those of the neonate to *E. coli* endotoxin and support the hypothesis that certain injurious effects of bacterial endotoxins may be mediated by acquired cellular hypersensitivity.

272. The Relationship between Iron-Induced Hemolysis and Lipid Peroxidation. KENDALL A. SMITH* AND CHARLES E. MENGEL,* Columbus, Ohio (introduced by James V. Warren**).

The unique enhancement of hemolysis by Fe given to patients with paroxysmal nocturnal hemoglobinuria (PNH) prompted the present study. Mice given an acute Fe load (1.5 mg/g as Imferon) developed hemolysis (fall of Hct from 48% to 42%, reticulocytosis, and hemoglobinuria). No evidence of hemolysis or mortality occurred in mice given appropriate amounts of dextran or phenol, other constituents of Imferon. Iron is a well known catalyst of lipid peroxidation, and the following results imply that Fe-induced hemolysis is related to peroxidation of RBC lipid: (1) Fe-loaded mice deficient in tocopherol, a lipid antioxidant, have a greater incidence of hemolysis (90%) as compared with normal (30%), a higher mortality rate (90% as compared with 50%), and a shortened survival (21 hr as compared with 43 hr). (2) Supplementation with tocopherol prior to Fe completely prevents hemolysis. (3) Erythrocytes from Fe-loaded mice have significantly ($P < 0.02$) higher levels of lipid peroxides (17 mμmoles/ml RBC) than do normals (10 mμmoles/ml RBC) and are significantly ($P < 0.005$) more susceptible to lysis by H_2O_2 (55% vs. 30%). (4) Fe loading (1.0 mg/g) also increased susceptibility of mice to hemolysis by hyperoxia, a reaction proved to result from peroxidation of RBC lipid. These studies show that excess iron administration can result in in vivo hemolysis, and suggest that such hemolysis is related to peroxidation of erythrocyte lipid. They also imply that the unique effect of Fe in PNH could reflect an unusual peroxidatic behavior of the PNH red cell lipid.

273. Correlative Study of ^{47}Ca Absorption in Man. J. SODE,* J. SABOL,* E. SCROM,* AND J. CANARY, Bethesda, Md., and Washington, D. C.

Contradictory reports have appeared concerning the utility of early serum radioactivity (SRA) peaks or specific activity peaks (SA) after oral calcium-47 (^{47}Ca) as an index of Ca absorption. SRA after oral ^{47}Ca reflects intestinal transit, absorption, excretion, and the miscible Ca pool (CaP). Conversion of SRA to SA corrects for variations in serum Ca level, but not for alterations in CaP or Ca excretion. We found poor correlation between SA and ^{47}Ca absorption (Ab ^{47}Ca) in several patients. To evaluate this problem, the following studies were performed. Ab ^{47}Ca was measured with a multiple-crystal geometry-independent whole-body counter in 31 patients on an estimated 800–1200 mg calcium diet; serum SA was measured at 2 hr. Good correlation between ^{47}Ca absorption and serum SA was found in 18 normal absorbers, whose calcium absorption ranged from 30 to 55%, $r = 0.88$, $P < 0.001$. No correlation was found in 6 hypo or 7 hyper absorbers. Mean serum SA of the groups was not significantly different. When CaP size was estimated from calculation of the theoretical serum SA peaks, using percentage of ^{47}Ca absorbed, the discrepant results could be resolved in patients with disordered calcium metabolism. Retention of infused ^{40}Ca also permitted resolution of apparent discrepant values in 5 patients with disordered calcium metabolism. Evaluation of calcium absorption from early serum SA peaks with oral ^{47}Ca in patients with disorders of calcium metabolism may be misleading unless CaP size and urinary calcium excretion are considered. The size of the CaP may be approximated if simultaneous serum SA peaks and quantitation of Ab ^{47}Ca are performed. Without quantitation of Ab ^{47}Ca, serum SA peaks may be made more useful if the results of ^{40}Ca infusion are analyzed.

274. Elevated Plasma Free Fatty Acids (FFA) and Impaired Glucose Tolerance (GT) during Nicotinic Acid (NA) Administration. THOMAS A. STOCKERT,* ANN M. LAWRENCE,* HENRI FRISCHER,* JAMES V. MCNAMARA,* KARL H. RIECKMANN,* ROBIN D. POWELL,* AND PAUL E. CARSON, Chicago, Ill.

A 4- to 6-fold increase in postabsorptive (6:30 a.m.) plasma FFA was observed in four inpatient volunteers given NA, 1 g q.i.d., for 17 days. FFA rose progressively for 3–5 days after the start of NA. From day 5 through day 17 the ratio of the mean FFA value to the mean pretreatment value for each subject and a control was 4.1, 6.6, 3.6, 4.0, and 0.92, respectively. FFA decreased promptly after 1 g NA doses but returned to elevated levels within several hours. The nitroprusside test for ketones on fasting urines was intermittently positive after development of maximal FFA values. Despite prompt, increased, and prolonged insulin release, oral GT decreased in three subjects. In a fourth subject an initially abnormal GT was further impaired. Serum cholesterol and triglycerides decreased in three subjects; serum urate increased in all. An increase in erythrocyte glutathione reductase activity, as seen in G-6-PD deficients and some diabetics, occurred during NA administration, confirming previous results. GT and FFA gradually returned to pretreatment levels 3–7 days after NA was stopped. NA is known to acutely inhibit FFA mobilization from adipose tissue. Our data show that during chronic NA administration a decrease in serum cholesterol and triglycerides was accompanied by an elevation of postabsorptive FFA, persisting several days after NA was stopped and only transiently interrupted by the drug's acute effect. FFA elevation was associated with increased urinary ketone excretion, decreased GT, and evidence for impaired insulin effectiveness, and suggests occurrence of a net increase in FFA mobilization. These findings cast doubt on the hypothesis that the plasma neutral fat–lowering effect of NA during chronic administration results from its acute action impeding FFA flux to the liver, and suggest caution in the chronic use of large doses of NA.

275. "Inappropriate" Growth Hormone Elevations in Prediabetic Males. P. H. SONKSEN,* G. BODEN,* R. E. GLEASON,* AND J. S. SOELDNER,* Boston, Mass. (introduced by A. Marble**).

Although growth hormone (GH) has been implicated in the pathogenesis of the diabetic syndrome with acro-

megaly, its role in "idiopathic" diabetes remains controversial. A potentially important abnormality in GH secretion was observed after oral glucose tolerance tests (OGTT) in prediabetic males (nonobese offspring of two diabetic parents, age 15–35 yr, with no blood glucose abnormality seen during this and an intravenous and a cortisone-primed OGTT). In addition to the late rise in GH that occurred 240 and 300 min after glucose (peak values 7.51 and 8.21 mμg/ml in prediabetics and controls matched for age, sex, and ethnic group), an early peak occurred in 7 of 18 prediabetics and 3 of 21 controls. Fasting GH was <0.25 mμg/ml in 16 of 18 prediabetics and 20 of 21 controls. In those with an early GH rise, the peak occurred within 15–45 min (prediabetics, mean 10.9, range 1.6–19.5 mμg/ml; controls, mean 1.9, range 0.76–3.8 mμg/ml, $P < 0.05$), and reached a nadir at 90 min (prediabetics, 0.31 mμg/ml; controls, <0.25 mμg/ml). Significantly higher mean GH levels occurred in the prediabetics at 15, 30, 45, and 60 min. In addition, the prediabetics also exhibited a lower serum insulin (IRI)/blood glucose (BG) relationship during the tests (slope of linear regression of IRI upon BG lower than controls; $P < 0.01$). The three controls with an early peak of GH secretion also showed an IRI/BG relationship significantly lower than that of the remaining controls ($P < 0.02$) but not different from that of the prediabetics. These findings extend and confirm previous studies from this laboratory that have shown significantly different GH response in prediabetic males after i.v. tolbutamide and i.v. GTT, and suggest reevaluation of the role of GH in diabetes mellitus.

276. Production of Septic Survival and Enhancement of Immunogenesis by In Vivo Phytohemagglutinin. HENRY D. SOLTYS* AND JEROME I. BRODY, Philadelphia, Pa.

The purpose of this investigation was to determine whether phytohemagglutinin (PHA), administered in vivo, is an anti-infectious agent and is able to protect rabbits against lethal pneumococcal sepsis. The present study extends concordant work in this laboratory which shows that intracellular protein, derived from human lymphocytes provoked in vitro by PHA, behaves as avid, polyvalent agglutinating antibody against several bacterial antigens. The experimental protocol consisted of giving one series of rabbits five daily intravenous injections of PHA, 50 mg/kg, and a second group the same dose of bovine serum protein (BSPr) as a control stimulant of the lymphoid-immune system. A third category of rabbits remained unprepared. All animals were challenged intravenously with 0.4 ml of a virulent broth culture of pneumococci. To define the in vivo cellular response to phytohemagglutinin, peripheral blood lymphocytes were grown in suspension tissue culture with a ^{14}C-amino acid hydrolysate both before and after PHA had been given to their donors. Intralymphocyte antibody, extracted by cellular disruption, was quantitated by scintillation spectrometry after agglutination of pneumococcal antigen by this radioactive protein. Within 72 hr, all untreated animals, and those receiving BSPr, developed lethargy, purulent nasal discharge containing, as did their blood, cultured viable pneumococci, and, finally, death. In striking contrast, rabbits premedicated with PHA demonstrated no signs of intoxication and survived the bacterial stress undisturbed. Also of immediate relevance was the 10-fold increase in intralymphocyte pneumococcal antibody after in vivo PHA administration, a phenomenon duplicating human lymphocyte antibody synthesis when exposure to the same substance was initiated in vitro. These observations indicate that the in vitro and in vivo biochemical effects of phytohemagglutinin are parallel and, more importantly, suggest that this material may play a significant in vivo role in mammalian host defense in a manner which differs completely from that of conventional antibiotic therapy.

277. The Effect of Acute Metabolic Acidosis on Proximal Tubular Sodium Reabsorption in the Rat. J. H. STEIN,* F. C. RECTOR, JR., AND D. W. SELDIN,** Dallas, Texas.

To determine the influence of acute metabolic acidosis on proximal tubular reabsorption, six studies were performed in which tubular fluid samples were collected from the end of the proximal tubule before and after the administration of NH_4Cl. Mean per cent proximal reabsorption (% PR), calculated from tubular fluid/plasma inulin ratio, was 60% in the control period. After administration of NH_4Cl, % PR fell to 32%, as mean blood pH fell to 7.08 and plasma HCO_3 decreased to 8.7 mmoles/liter. Isotonic saline, in an amount estimated to expand extracellular volume to the same extent as the NH_4Cl, decreased % PR only slightly, from 50 to 43%. The intrinsic reabsorptive capacity (K), calculated from the tubular fluid/plasma inulin ratio and transit time, was inhibited by 48% after NH_4Cl. K was then measured directly by the shrinking-drop technique of Gertz. In the control period, K was 0.064 sec^{-1} and fell to 0.036 sec^{-1} after NH_4Cl, indicating 43% inhibition. In another group of studies acidosis was initially induced with NH_4Cl and then corrected with $NaHCO_3$. K was 0.038 sec^{-1} during acidosis and rose to 0.066 sec^{-1} after correction with $NaHCO_3$. In studies in which a comparable acidosis was induced with 15% CO_2, there was no change in K. It is concluded, therefore, that NH_4Cl acidosis suppresses proximal reabsorption by inhibiting K. Since respiratory acidosis had no effect on K, the inhibition after NH_4Cl is related to a lowering of plasma HCO_3, rather than pH.

278. Activation of Aldosterone Secretion in Primary Aldosteronism. RICHARD F. SPARK,* SIDNEY L. DALE,* PAUL C. KAHN,* AND JAMES C. MELBY, Boston, Mass.

Angiotensin infusion evokes marked increases in aldosterone secretion in patients with primary aldosteronism, and little change in secondary aldosteronism. The low plasma renin activity (PRA) of primary aldosteronism and the elevated PRA of secondary aldosteronism are thought to account for this differential response. The effect of angiotensin was studied during adrenal vein catheterization in six patients with primary aldosteronism

(PRA elevated after spironolactone therapy), two patients with secondary aldosteronism (PRA elevated), and one anephric patient (O-PRA). Adrenal venous aldosterone and 18-hydroxycorticosterone (18-OH-B) were measured before and after a 10 min subpressor infusion of angiotensin. Effluents from both adrenals in secondary aldosteronism, from nontumorous glands in primary aldosteronism, and from the adrenal of the anephric patient had similar base-line values, both for aldosterone (1.0 μg/100 ml) and for 18-OH-B (1.3 μg/100 ml). Angiotensin evoked trivial increases in steroid efflux from all nontumorous adrenals (0.6 μg/100 ml increase for aldosterone and 1.5 μg/100 ml for 18-OH-B). Base-line levels from tumor-bearing glands were elevated to a mean of 5.2 μg/100 ml for aldosterone and 7.5 μg/100 ml for 18-OH-B. After angiotensin, there was a marked increase, averaging 6.4 μg/100 ml for aldosterone and 13.4 μg/100 ml for 18-OH-B. High base-line values of aldosterone and 18-OH-B and increased sensitivity to infused angiotensin were observed, regardless of the size of the aldosterone-producing adenoma (APA). In terms of aldosterone secretion, low circulating PRA does not predispose to increased sensitivity of the adrenal to exogenous angiotensin, as evidenced by the response in the anephric patient, nor does the presence of high PRA blunt the sensitivity of the APA to exogenous angiotensin, as demonstrated by the response in primary aldosteronism after treatment with spironolactone. This unusual sensitivity of the cells of the APA to angiotensin may be etiologically important in the initiation and perpetuation of the syndrome of primary aldosteronism.

279. The Role of Hemoglobin H as a Reducing Agent and Its Effects on Cation Flux. THOMAS B. STOSSEL,* ROBERT B. GUNN,* MITSUKO T. LAFORET,* AND DAVID G. NATHAN, Boston, Mass. (introduced by Louis K. Diamond**).

Precipitation of hemoglobin H and depletion of glutathione occur in the old cells of patients with hemoglobin H disease. Advantage was taken of these age-dependent abnormalities to investigate their effects on hexose monophosphate shunt activity (HMPS) and cation flux. Young and old cell populations of five such patients were separated by centrifugation. Three patients had been splenectomized. Glycolysis, HMPS measured with 1-^{14}C-glucose, potassium flux, G-6-PD activity, and glutathione concentration were assessed in the separated cell fractions. Eight normal subjects and five patients with beta thalassemia provided control young and old cells. In these control subjects, the potassium fluxes of young cells exceeded those of old cells and were particularly excessive in the inclusion-bearing young cells of splenectomized beta thalassemia subjects. Glutathione levels did not change with age, and HMPS was higher in young cells. Normal old and young cells had equal HMPS per unit of G-6-PD activity. Young hemoglobin H cells had two-thirds the absolute HMPS of young normal cells and only one-third their HMPS per unit of G-6-PD activity. The HMPS of old hemoglobin H cells increased absolutely and became equal to that of normal old and young cells per unit of G-6-PD activity. This increase in HMPS was associated with a fall in glutathione and in soluble hemoglobin H. In splenectomized hemoglobin H cells, potassium flux was higher in old inclusion-bearing cells than in young reticulocyte-rich cells. These results are consistent with two conclusions: (1) Hemoglobin inclusion formation is associated with increased membrane cation flux. (2) Soluble hemoglobin H can serve as a reducing agent for the erythrocyte in hemoglobin H disease, sparing glutathione and therefore glutathione reductase activity. Hence HMPS is lower than normal when soluble hemoglobin H is present. Hemoglobin H becomes irreversibly oxidized and forms a precipitate in older cells. With its loss, the glutathione reduction mechanism must be employed, and therefore HMPS increases to normal. In contrast to hemoglobin H, hemoglobin A does not serve as a primary reducing agent for the normal red cell.

280. 17-Hydroxyprogesterone (17OHP) and Progesterone (P) Blood Levels and Production Rates in Normal Adults and in Patients with Congenital Adrenal Hyperplasia (CAH). C. A. STROTT,* T. YOSHIMI,* AND M. B. LIPSETT,** Bethesda, Md.

Using competitive-protein binding methods, 17OHP and P can be measured in plasma at levels above 0.005 μg/100 ml. With a 3 ml plasma sample, the blank of the methods is indistinguishable from zero. The plasma levels of 17OHP in normal adults were (μg/100 ml): men, 0.097 (0.060–0.140, n = 11); women, follicular phase, 0.044 (0.010–0.070, n = 14); women, luteal phase, 0.150 (0.100–0.230, n = 7). In three normal men, the average 17OHP metabolic clearance rate (MCR) was 2000 liters/24 hr, and the mean blood production rate was 2.0 mg/24 hr. Plasma levels of P were (μg/100 ml): men, 0.021 (0.010–0.041, n = 14); women, follicular phase, 0.034 (0.011–0.11, n = 10); women, luteal phase, 0.69 (0.12–1.75, n = 10). Since the MCR averaged 2000 liters/24 hr in three normal men, the P blood production rate was 400 μg/24 hr. When 40 U of ACTH were given i.v. for 8 hr to nine women in the follicular phase, plasma 17OHP, P, and cortisol increased 400%. The proportional increase of plasma P and 17OHP suggests that in vivo, an important pathway in the biosynthesis of 17OHP from pregnenolone in the adrenal cortex is via progesterone. In five patients (ages 5–16 yr) with simple C-21-hydroxylase deficiency CAH, plasma 17OHP ranged from 4.0 to 22.0 μg/100 ml, and plasma P from 0.2 to 1.1 μg/100 ml. The MCR for 17OHP and P in two patients with CAH did not differ from those of normal men when corrected for body surface area. The blood production rates in these patients were: 17OHP, 113 and 55 mg/24 hr, and P, 6.54 and 3.34 mg/24 hr, respectively. Four patients with CAH received ACTH intravenously for 8 hr. Plasma P and 17OHP were unchanged in two subjects. The responses of the other two subjects were (μg/100 ml): 17OHP, 12 → 27, 5 → 43; P, 0.6 → 1.1, 0.2 → 0.95, respectively. From these data we conclude that the testis and luteal-phase ovary, as well as the adrenal cortex, are important sources of plasma 17OHP; ACTH produces proportional increases in plasma levels of 17OHP and P. In CAH,

the plasma levels and production rates of 17OHP may be 100–200 times those of normal men, and P levels and production rates 10–20 times those of normal men.

281. Alcohol-Induced Thrombocytopenia in Man. LOUIS W. SULLIVAN,* YONG KIA LIU,* LILIA TALARICO,* AND CHARLES P. EMERSON,** Boston, Mass.

These investigations were to ascertain the etiology of acute thrombocytopenia in alcoholics without splenomegaly. Four alcoholics with thrombocytopenic purpura (platelets, 17,000–43,000/mm^3) had normal coagulation studies and liver function, serum folates 1–3.8 ng/ml, normal B_{12} levels, hematocrits 41%, 39%, 34%, and 23%, and normal leucocytes. Marrows were megaloblastic with reduced megakaryocytes, young forms predominating. Platelets increased 1–5 days after hospitalization on normal or folate-deficient diets. One patient with 348,000 platelets/mm^3 on discharge resumed heavy alcohol ingestion; 12 and 20 days later platelets were 167,000/mm^3 and 31,000/mm^3. Megakaryocytes were markedly reduced; erythroid and myeloid cellularity were normal. On a folate-deficient diet, platelets were 16,000–22,500/mm^3 for 96 hr. At 120 hr, platelets were 19,000/mm^3 (megakaryocytes markedly increased, young forms predominating); at 144 hr, 110,000/mm^3; and at 12 days, 450,000/mm^3 (many mature megakaryocytes and platelet clusters in marrow). Ingestion of 193 g of alcohol daily (19th–30th day) was associated with disappearance of megakaryocytes, and a linear fall in platelets (460,000/mm^3 to 47,000/mm^3). Autologous ^{51}Cr-labeled platelets disappeared at a similar rate, without evidence of splenic or hepatic sequestration. Tests for "defibrination" were negative. After alcohol was discontinued, platelets were 23,000–28,000/mm^3 for 72 hr, 34,000/mm^3 at 96 hr (numerous megakaryocytes, predominantly young), 67,000/mm^3 at 144 hr, and 92,000/mm^3 at 168 hr (increased mature megakaryocytes, platelet clusters in marrow). In a second patient, after recovery from thrombocytopenia, 75 μg of folic acid daily did not prevent subsequent fall in platelets or diminution in adult megakaryocytes with alcohol. These studies suggest that thrombocytopenic purpura in alcoholics is a direct effect of alcohol upon megakaryocyte maturation and is not due to increased platelet destruction. The fall in platelets within 48 hr after commencement of alcohol, rise in platelets 120 hr after its discontinuance, and morphologic alterations of megakaryocytes suggest that alcohol impairs thrombopoiesis at early and later stages.

282. Formation of Iodoprotein during the Peripheral Metabolism of ^{125}I-Triiodothyronine in the Euthyroid Man and Rat. MARTIN I. SURKS* AND JACK H. OPPENHEIMER, Bronx, N. Y.

T3 metabolism was studied in seven normal human subjects on blocking doses of stable iodide after the intravenous injection of ^{125}I-triiodothyronine (^{125}I-T3). The fractional disappearance rate of plasma radioactivity did not achieve the expected constant value, but progressively slowed with time. Analysis of each plasma sample by dialysis, extraction, and electrophoretic techniques revealed three radioactive components: ^{125}I-T3, ^{125}I-iodide, and an unidentified material which was nonextractable (NE) in acid butanol (^{125}I-NE). ^{125}I-NE rose to a maximum 24 hr after injection of the tracer and then declined, with a $t_{\frac{1}{2}}$ appreciably greater than that of T3. The plasma ^{125}I-T3 concentration obtained by subtraction of ^{125}I and ^{125}I-NE from the total plasma radioactivity declined with time as a single exponential function with a $t_{\frac{1}{2}}$ of approximately 1.5 days. Qualitatively similar plasma disappearance curves were observed in rats. After 72 hr, 50–60% of the plasma and 40% of the liver radioactivity was ^{125}I-NE. Chromatographic purification of ^{125}I-T3 before injection did not alter these results. The peripheral origin of ^{125}I-NE was further demonstrated by similar results in thyroidectomized animals maintained on thyroxine. ^{125}I-NE from human sera separated from the other radioiodinated substances by ion exchange column chromatography was 85% insoluble in acid butanol and $CHCl_3$:CH_3OH, precipitated in trichloroacetic acid, was not dialyzable, and migrated in the albumin zone on starch gel electrophoresis. On the basis of these properties it was tentatively identified as an iodoprotein. Observations in rats equilibrated for 70 days with ^{125}I, as well as nonradioactive iodine determinations in human sera before and after acid butanol extraction, suggest that 5–10% of the serum PBI may be in the form of iodoprotein. Our studies indicate that this moiety may be derived from the peripheral metabolism of the thyroid hormones.

283. ACTH Activation of Adenyl Cyclase in Purified Adrenal Membranes. O. DAVID TAUNTON,* JESSE ROTH,* AND IRA PASTAN, Bethesda, Md.

ACTH regulates steroid production in the adrenal by increasing intracellular cyclic 3′,5′-AMP. We recently reported that this ACTH effect is due to activation of adenyl cyclase. In the present study, using purified adrenal cell membrane preparations we have characterized the ACTH-responsive adenyl cyclase. A homogenous population of ACTH-responsive adrenal tumor cells were grown in tissue culture and in mice. After homogenization and fractionation, adenyl cyclase was assayed by measuring the accumulation of cyclic 3′,5′-AM^{32}P formed from α-labeled AT^{32}P. Optimal conditions for ACTH activation of cyclase included 2.5 mM ATP, 2.5 mM $MgCl_2$, and pH 7.7. Adenyl cyclase is wholly particulate and probably located in the plasma membrane, since it (1) largely sedimented at 600 *g* and was thereby separated from microsomes and mitochondria, and (2) was separated from nuclei by equilibrium centrifugation in a sucrose gradient, where it banded at a density of 1.19, similarly to liver cell plasma membranes. Increased adenyl cyclase activity was detected within 3 min after ACTH addition and remained increased for 15–30 min. Adenyl cyclase activity increased in proportion to ACTH concentration, from 0.1 to 3 μg/ml with half of the maximum response at 0.3 μg/ml. At 3 μg/ml the activity was increased 40-fold. Adrenal adenyl cyclase was specifically activated by ACTH, since other polypeptide hormones and epinephrine were inactive. The ACTH

effect on adenyl cyclase was inhibited by 3–6 mM propranolol; basal activity was affected negligibly. Calcium, 5 mM, decreased both basal and ACTH-responsive adenyl cyclase activity equally. We previously showed that the first step in ACTH action is equilibration of hormone in the medium with sites on the external surface of the cell. Our present study suggests that adenyl cyclase activation is one of the earliest steps in ACTH action and that adenyl cyclase is geographically very close to or identical with the ACTH-binding site.

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

A study was undertaken to determine the requirements for lysis of clots formed by the addition of 1 U of thrombin to whole blood diluted 1:10 in phosphate buffer (μ 0.082, pH 7.4, 4°C). Removal of platelets inhibited normal clot retraction and lysis. Addition of antisera specifically directed against purified γM globulin (19S cold agglutinin), C'4, C'3, and plasminogen inhibited normal clot retraction and lysis. Antisera to γG globulin and albumin did not inhibit. Washed platelets were preferentially agglutinated by antisera to the above proteins. Photomicrographs of clots formed in the presence of γM antisera had fewer platelets and less platelet aggregation than control samples. These findings suggest that (1) platelets, a γM globulin, C'4, C'3, and plasminogen may participate in a series of reactions leading to lysis of the clot under these conditions; (2) the γM globulin, C'4, and C'3 are preferentially adsorbed onto the platelet surface under the conditions of the assay; and (3) these platelets with their attached proteins in turn are adsorbed onto the fibrin network. The resulting physical-chemical milieu is postulated to be favorable for cold agglutinin activation of complement, which in turn activates plasminogen either directly at the platelet-fibrin-serum interface or indirectly by aggregation and lysis of platelets and release of plasminogen-activating substance. Clots from patients with Bruton's hypogammaglobulinemia, low C'3, low platelet counts, and Glanzman's thrombesthenia retracted poorly and lysed slowly; whereas clots from patients with high platelet counts or hepatitis (↑19S cold agglutinin) retracted and lysed very rapidly.

285. Hypercatabolism of IgG and Albumin: A New Familial Disorder. WILLIAM D. TERRY,* EDWARD J. MILLER,* AND THOMAS A. WALDMANN, Bethesda, Md.

Two siblings (W.J., a female, age 34, and D.W., a male, age 17) were studied because of marked reduction of serum IgG concentration (1.3 and 4.4 mg/ml respectively; normal, 12 ± 2.6) associated with essentially normal levels of IgA and IgM. Hypoalbuminemia (19 and 21 mg/ml; normal, 42 ± 3) was also noted. Metabolism of iodinated IgG and albumin was studied in the two patients and 15 controls. The patients' total body pool of IgG was less than 15% of normal, and their IgG fractional catabolic rates were increased 5-fold to 34% and 35% of the IV pool/day (normal, 6.7 ± 2%/day). IgG synthetic rates were within normal range for both patients. Studies of albumin metabolism similarly showed reduced total body pool, increased fractional catabolic rate, and normal synthetic rate. Both patients had normal fecal ^{51}Cr albumin clearance tests, thus excluding excessive gastrointestinal protein loss. There was no proteinuria or abnormality of thyroid, adrenal, renal, or liver function tests. Despite low serum IgG concentration, there was no increased incidence of infections. Both patients had diabetic glucose tolerance tests (with necrobiosis lipoidica diabeticorum in W.J.) and bilateral bowing of the radius. These patients have a previously unrecognized familial disorder characterized by reduced levels of serum IgG and albumin due to increased catabolism of these proteins, associated in this family with chemical diabetes and skeletal deformity.

286. Effects of Autonomic Nervous System Blocking (ANSB) Agents on Angiotensin Responses. GURDARSHAN S. THIND AND LYSLE H. PETERSON,** Philadelphia, Pa.

Recently, investigators have postulated a peripheral mechanism for the cardioaccelerator action of angiotensin which can be abolished by alpha or beta adrenergic receptor blocking agents. This suggestion is important with regard to the question whether or not angiotensin combines with the receptors, or depletes the catecholamine stores, or both. We have investigated these possible alternatives by in vitro studies of the effects of ANSB agents on the isometric responses of rabbit thoracic aortic strips to angiotensin. In our preparations, alpha receptors are completely blocked by phentolamine mesylate (Regitine) 100.0–125.0 μg/ml, phenoxybenzyamine (Dibenzyline) 10.0 μg/ml, tolazoline hydrochloride (Priscoline) 625.0 μg/ml, and almost completely by propranolol (Inderal) 100.0 μg/ml. Beta receptors are completely blocked by Regitine 1.0 μg/ml and Inderal 37.5–50.0 μg/ml. Angiotensin responses were not significantly altered by ANSB agents (Regitine, Priscoline, Dibenzyline, Inderal, Ansolysen, and atropine) at dose levels which were sufficient to block either the alpha receptors, the beta receptors, the parasympathic system, or the ganglia. However, angiotensin responses were significantly decreased by Regitine 125.0–250.0 μg/ml (no difference between reserpinized and nonreserpinized rabbits), Priscoline 2.5 mg/ml, and Inderal 100.0–200.0 μg/ml. The blockade of angiotensin responses by the ANSB agents was a dose-dependent phenomenon. We conclude: (1) Small doses of Priscoline, Dibenzyline, and Inderal are selective alpha or beta receptor blockers; however, large doses of these agents block all adrenergic receptors. In contrast, Regitine (1.0 μg/ml) blocks the beta receptors whereas the alpha receptor blockade is still incomplete, indicating that Regitine cannot be used to differentiate adrenergic receptors. (2) Angiotensin does not act through the autonomic nervous system, but has specific receptors in the rabbit aorta. However, angiotensin receptors can be nonspecifically blocked by large doses of

alpha or beta adrenergic blocking agents. Also our findings fail to support the postulate mentioned at the beginning of this abstract.

287. Cellular and Humoral Control of Myxovirus Infection. LAWRENCE TREMONTI,* JUEY-SHIN LIN,* AND GEORGE GEE JACKSON,** Chicago, Ill.

Guinea pigs were hyperimmunized subcutaneously with a suspension of complete Freund's adjuvant and two myxoviruses, parainfluenza type 2 and mumps. Monkey kidney tissue culture was infected with parainfluenza type 2 or mumps virus and exposed to either fresh serum, heat-inactivated serum, peritoneal exudate cells, or spleen cells from the immunized animals. Washed peritoneal exudate cells from parainfluenza-immunized animals consistently diminished the cytopathic effect of the virus. A similar effect to less degree was noted for spleen cells. Fresh serum applied to the infected tissue culture did not inhibit and may have enhanced the viral cytopathic effect, but good protection was produced by serum after heat inactivation. No toxicity from cells or serum was noted for uninfected tissue culture. Using fluorescein-labeled antibody, γG globulin coating was demonstrated on a high percentage of peritoneal exudate cells, but it was not demonstrated by gel diffusion of the supernatant from a 3-day culture of these cells. Studies are currently being completed to demonstrate the viral specificity of the immunoglobulin. Mumps virus caused no cytopathic effect in infected tissue culture, therefore no protective effect of cells was demonstrable. However, when fresh serum from specifically immunized animals was applied to the mumps-infected tissue monolayer, almost complete cytolysis occurred. On uninfected tissue culture, serum was without effect. Decomplementation of the serum by heat inactivation or by antigen-antibody complexes removed the cytolytic effect of fresh serum. The maximum destructive effect was noted in serum obtained from specifically immunized animals but was present in nonimmunized animals with low antibody titers to other myxoviruses and with human serum. The suggestion is made that in host defense against viral infections, infected cells can be destroyed by complement lysis and uninfected cells are protected from infection by circulating and cell-bound antibody.

288. DNA, RNA, and Protein Synthesis in Phytohemagglutinin (PHA)-Stimulated Lymphocytes: Effect of Chloramphenicol (CAP). JOHN E. ULTMANN,* New York, N. Y. (introduced by Elliott F. Osserman).

Because of the current controversy regarding the inhibitory effects of CAP on the metabolism of mammalian cells, CAP, as well as four of its analogues which are known to have little effect on bacterial growth, was studied in PHA-stimulated 3-day human lymphocyte cultures. The effect on the synthesis of DNA, RNA, and protein after 4-hr incubation with CAP (D-threo-1-*p*-nitrophenyl-2-dichloroacetamido-1,3-propanediol), the 2-amino- analogue (analogue 1), the L-erythro- isomer (analogue 2), the -1-hydroxypropane analogue (analogue 3), or the -1-phenylureidophenyl- analogue (analogue 4) was examined employing ^{3}H-thymidine, ^{3}H-uridine, and ^{14}C-amino acids as precursors. CAP, at bacteriostatic concentration (3×10^{-5} M), had no effect on DNA, RNA, or protein synthesis. At higher concentrations ($3\text{–}8 \times 10^{-4}$ M), CAP partially inhibited DNA (37–56%) and RNA (42–60%) synthesis, but inhibited protein synthesis only slightly (18–34%); analogues 1 and 2 inhibited protein synthesis more than CAP (62%, 49%, respectively) but had less effect on DNA and RNA synthesis; and analogue 3 significantly inhibited DNA, RNA, and protein synthesis (70–75%). Analogue 4 proved to be a potent inhibitor of DNA (80–94%), RNA (89–91%), and protein (81–84%) synthesis at concentrations of $2\text{–}6 \times 10^{-4}$ M. Short-term incubation studies (15–60 min) with analogue 4 at 6×10^{-4} M showed marked inhibition of DNA and RNA with little effect on protein synthesis. These results indicate that CAP levels (3×10^{-5} M) which effectively inhibit protein synthesis in bacteria have no such effect in human lymphocytes, and that high concentrations (8×10^{-4} M) effect a nonspecific inhibition of macromolecular synthesis. Analogue 4 inhibits the synthesis of DNA and RNA with a lesser, probably secondary, effect on protein synthesis. It is possible that analogue 4 may be a useful antimetabolite for inhibiting human lymphocyte function.

289. TSH Secretion in Newborn Infants and Children. ROBERT D. UTIGER, JOHN F. WILBER,* MARVIN CORNBLATH,* JOHN P. HARM,* AND ROBERT E. MACK,* St. Louis, Mo., Chicago, Ill., and Detroit, Mich.

Plasma TSH concentrations were measured by radioimmunoassay in children from birth through adolescence. Plasma TSH concentrations in 102 euthyroid children from 1 wk to 14 yr were similar and ranged from 0.6 to 2.8 mμg/ml (2.4–11.2 μU/ml), values within the range found in euthyroid adults. Striking changes were found in newborn infants. TSH in cord plasma from 53 babies ranged from 0.6 to 15.0 mμg/ml (mean, 2.6), whereas in 47 mothers at delivery TSH was 0.6–2.8 mμg/ml (mean, 1.0). Of the 46 pairs of maternal and cord samples simultaneously assayed, in 39 the TSH level in cord plasma exceeded that in maternal plasma. This suggests that there is active fetal TSH secretion in utero. During the first hours of life there was a marked increase in plasma TSH (to as much as 20 mμg/ml), which returned to normal 24–48 hr after birth. Multiple determinations were done in some infants, and the following mean values were found (number of infants studied): 1 hr, 12.9 mμg/ml (5); 2 hr, 9.3 (6); 4 hr, 6.3 (5); 6 hr, 4.3 (18); 12 hr, 3.3 (10); 24 hr, 2.6 (11). Thereafter, all values were within the normal child or adult range. These changes precede and thus undoubtedly produce the transient increases in thyroidal ^{131}I uptake, PBI, and free T_4 levels observed the first few days after birth. Because PBI and free T_4 concentrations (measured in some of these babies and by others) are not low during the first hours of life, the increased TSH secretion observed must be due to another stimulus, per-

haps evoked by exposure to the cooler extrauterine environment. Since plasma TSH returns quickly to normal, this stimulation must subside rapidly.

290. Hemodynamic and Humoral Factors in Renal Electrolyte Cycles. A. H. VAGNUCCI,* A. P. SHAPIRO,** AND R. H. MCDONALD, JR.,* Pittsburgh, Pa.

In view of increasing evidence that multiple factors affect sodium excretion, simultaneous investigation of renal hemodynamics and aldosterone in electrolyte balance is of particular interest. An important aspect of electrolyte balance is its circadian variation. The circadian rhythm of Na, K, Cl excretion under constant electrolyte intake (Na, 130–140 mEq; K, 90–100 mEq) was investigated in five normal subjects and two patients with primary aldosteronism. Each subject was studied for periods of 1–2 wk. The protocol was divided into two equal phases; in each phase either (I) continuous 24 hr recumbency or (II) 7 a.m.–7 p.m. quiet standing or (III) 4 p.m.–4 a.m. quiet standing was maintained. 3 hr urines were collected throughout the study; electrolytes, creatinine, catecholamines, and aldosterone excretions were measured. In each phase, GFR and RPF were measured by 3 hr clearances of inulin and PAH over 24 hr. Quiet standing (II, III) depressed Na excretion, modifying the circadian pattern of recumbency previously reported. The K cycle was less sensitive to the postural stimulus, and dissociation from the Na cycle frequently was observed. Standing had minimal effect on the GFR cycle in either II or III. Correlation of GFR and Na cycles was good during recumbency; it became poor on standing (II, III). RPF response was more variable; its correlation with electrolyte cycle was poor. Urinary norepinephrine increased on standing even in primary aldosteronism; epinephrine showed no correlation with posture. Urinary aldosterone was low and showed no changes during I; it increased on standing in normal subjects. Its contribution to decreased sodium excretion on standing, however, is doubtful because its increase was small and no increase occurred in patients with primary aldosteronism. It is concluded that additional factors must play a role in postural regulation of electrolyte balance.

291. Transfer of Cellular Hypersensitivity In Vitro. FRED T. VALENTINE* AND H. S. LAWRENCE,** New York, N. Y.

In humans the transfer of delayed hypersensitivity has been accomplished with cell-free supernatants prepared from sensitive leukocytes incubated with specific antigen. Also, a percentage of lymphocytes from tuberculin-positive individuals respond in culture with that antigen by transformation to lymphoblasts, synthesis of DNA, and proliferation. We report on a factor, released into the culture medium by leukocytes from PPD-sensitive individuals upon exposure to the specific antigen, which is capable of transferring in vitro to nonsensitive lymphocytes the ability to transform and proliferate in the presence of antigen. The response of blood lymphocytes is quantitated by morphological enumeration of transformed cells and by ^{14}C-thymidine incorporation. This factor is produced only by sensitive cells in the presence of antigen. Activity is present before cell division occurs. Plasma from a sensitive person is not required for the production of this factor, nor does it allow its production by nonsensitive cells. Studies on properties of this factor have revealed the following: Removal of supernatants from stimulated sensitive cells depresses the lymphocyte response despite addition of fresh media and antigen. This factor when added to nonsensitive cells plus PPD permits a response 4–15 times the background response to antigen alone or to antigen plus media from unstimulated sensitive cells. The magnitude of the transferred response increases with increasing amounts of added antigen. Nonsensitive cells so stimulated in turn elaborate a material which renders other autologous cultures capable of transforming in the presence of antigen. The factor is not sedimented at 100,000 *g*, is nondialyzable, and is inactivated by 56°C for 1 hr. These observations indicate that the activity is not due to transplantation antigens or to antigen-antibody complexes. The similarities between this in vitro transfer of altered cellular responsiveness and the in vivo transfer of delayed hypersensitivity with such preparations are apparent.

292. Micropuncture Studies on the Effect of ADH on Renal Tubular Reabsorption of Sodium and Water. H. VALTIN, J. SCHNERMANN,* AND K. THURAU,* Hanover, N. Y., and Munich, Germany.

Tubular reabsorption of sodium and water was studied in rats with hereditary hypothalamic diabetes insipidus, before and after giving ADH. These rats almost certainly have an absolute deficiency of endogenous ADH. Animals were given cortisone acetate and oral water loads before being anesthetized. Water diuresis was maintained through the intravenous infusion of 2% glucose at 0.2 ml/min. During water diuresis (U/P_{osm} 0.41; U/P_{In} 14), mean GFR was 1.11 ml/g kidney per min, and the following average values were obtained in the late proximal tubule: passage time, 11.5 sec; TF/P_{osm}, 1.02; TF/P_{Na}, 1.03; TF/P_{In}, 2.53. After intravenous infusion of 50 μU ADH/100 g rat per min, average U/P_{osm} and U/P_{In} had risen significantly to 2.04 and 130, respectively, but there were no significant changes in GFR or in the variables within the proximal tubules: passage time, 10.6 sec; TF/P_{osm}, 0.99; TF/P_{Na}, 0.97; TF/P_{In}, 2.27. Mean values in early distal samples in the same experiments during water diuresis were as follows: passage time, 32 sec; TF/P_{osm}, 0.45; TF/P_{Na}, 0.31; TF/P_{In}, 6.46. During antidiuresis in the same experiments, early distal samples were as follows: passage time, 26.9 sec; TF/P_{osm}, 0.42; TF/P_{Na}, 0.28; TF/P_{In}, 4.75. Only the decrease in distal TF/P_{In} during antidiuresis was significant statistically. Resumption of water diuresis in the same animals by stopping the infusion of ADH was accompanied by return of all values toward or to those seen in the initial water diuresis. The results suggest the following conclusions: that ADH does not influence the reabsorption of Na or water from the proximal tubules; that ADH does

not enhance the reabsorption of Na from superficial loops of Henle; that there is significantly more water at the end of superficial loops of Henle during acute antidiuresis than during water diuresis.

293. Thyroxine-Mediated Stimulation of In Vitro Transport of Magnesium. STANLEY WALLACH AND PHOEBE J. GAMPONIA,* Brooklyn, N. Y.

Studies of radiomagnesium turnover in hyper- and hypothyroid humans have indicated a positive relation between thyroidal activity and the influx of magnesium into cellular tissues. In the rabbit, Aikawa has reported that thyroxine, dinitrophenol, and salicylate all increase tissue uptake of radiomagnesium in vivo. To investigate this phenomenon further, the bidirectional transport of ^{28}Mg in rat liver was studied in vitro, by a previously reported method. In this system, with an extracellular concentration of 2.5 mM Mg^{++}, the fractional transport rates for the influx and efflux of ^{28}Mg in liver slices from adult male CD rats were 0.074 ± 0.0031 and 0.0186 ± 0.00080 (SEM), respectively. A single subcutaneous injection of 500 μg thyroxine in acutely thyroparathyroidectomized rats, 72 hr before study, had no effect on ^{28}Mg transport. However, administration of 750–1000 μg of thyroxine over a 1 wk period caused a 50% stimulation of the cellular influx of ^{28}Mg. Continuation of thyroxine for 3–6 wk resulted in a comparable but not greater degree of stimulation of the influx of ^{28}Mg. A lesser, irregular stimulation of the efflux of ^{28}Mg was also observed. These data indicate that the stimulation of the cellular transport of magnesium by thyroxine is detectable in vitro and is a delayed effect of the hormone. This delay does not necessarily imply that the stimulation of magnesium transport is a nonspecific response to a general stimulation of metabolism or of protein synthesis by thyroxine, since a direct effect of thyroid hormone on magnesium transport requiring de novo synthetic activity by the liver cells might be involved. Further study of in vitro transport systems which are responsive to thyroxine may assist in elucidating the mechanisms involved in the transport of magnesium by cellular tissues.

294. Calcium Transport by the Toad Bladder. MACKENZIE WALSER, Baltimore, Md.

Interdependence between calcium and sodium transport across epithelia has often been observed; yet some independent regulation must exist, for example in the distal nephron. To clarify this problem, simultaneous transport of calcium and sodium in the toad bladder was studied. In earlier experiments, the proportions of these cations were measured in the serosal fluid which accumulates within bladders everted empty and incubated for several hours. In the present work, bidirectional calcium fluxes ($S \rightarrow M$ and $M \rightarrow S$), potential difference (PD), and short-circuit current (SCC) were measured simultaneously in 60 bladders mounted in plastic chambers. ^{47}Ca and ^{45}Ca were used, added to serosal and mucosal baths, respectively, or vice versa. In normal Ringer's, buffered with bicarbonate or Tris, and maintained open-circuited except for brief intervals, calcium backflux ($S \rightarrow M$) averaged 0.15 μEq/cm^2 per hr, corresponding to a permeability coefficient of 10^{-5} cm/sec. In one-third of the bladders, $M \rightarrow S$ flux exceeded $S \rightarrow M$ despite the electrical gradient. In all, flux ratio ($M \rightarrow S$/$S \rightarrow M$) exceeded the value predicted from the potential. Continuous short circuiting augmented flux ratio as predicted for a monovalent, rather than divalent, cation. Net calcium transport was a small fraction of SCC (50 ± 6 μa/cm^2). In high-calcium Ringer's ([Ca] = 50 mM, [Na] = 15 mM), with and without vasopressin, calcium permeability was the same as in normal Ringer's, as was resistance (550 ohm cm^2). Again, one-third of the bladders exhibited net $M \rightarrow S$ transport despite a mean PD of 14 mv. In several, net calcium flux exceeded SCC, indicating either calcium-sodium exchange or an electrically neutral calcium pump. Aldosterone and ouabain both appeared to augment calcium transport; dinitrophenol was inhibitory. Thus calcium transport by this tissue is comparable in maximal rate to sodium transport. Both mechanisms appear to compete for ATP stores, but they are otherwise distinct.

295. The Foreign-Body Granuloma as a Response to Chemical Mediators of Inflammation. KENNETH S WARREN AND ROBERT W. KELLERMEYER,* Cleveland, Ohio.

Granulomas are classically divided into two types: foreign body and infectious. Although the etiology of foreign-body granulomas remains unknown, recent studies suggest that the infectious type, as exemplified by the schistosome egg granuloma, is a form of delayed hypersensitivity. In order to elicit foreign-body granulomas, divinyl-benzene copolymer beads (mean diameter 56 μ) of a size similar to schistosome eggs were injected intravenously into the lungs of mice by von Lichtenberg's technique. Bead granulomas, in contrast to egg granulomas, developed more rapidly (hours vs. days), reached peak size earlier (2 vs. 16 days), had somewhat different cellular compositions, and were not characterized by accelerated reactions (anamnestic) after previous injections of beads. Egg granulomas but not bead granulomas were suppressed by both neonatal thymectomy and antilymphocyte serum. Thus it appeared that the bead reaction did not have an immunological basis; further experiments have suggested that the bead granuloma is initiated by chemical mediators of inflammation. We have demonstrated that the beads activate Hageman factor and permeability-enhancing activity and deplete complement in human serum, thereby having the potential to initiate vascular permeability and chemotactic responses. Attempts to inhibit bead granulomas in mice, in vivo, with an antihistamine (triprolidine) were unsuccessful. However, ellagic acid, a Hageman factor activator which could deplete substrates necessary for kinin formation, virtually eliminated granuloma formation. Polyanetholsulfonate (Liquoid®), an inhibitor of some components of complement as well as Hageman factor–dependent permeability reactions, markedly suppressed granuloma formation around beads, but had relatively little effect on schisto-

some egg granulomas. These data suggest that the plastic bead-induced foreign-body granuloma, in contrast to the schistosome egg-induced infectious granuloma, is not an immunological reaction, and is dependent on certain chemical mediators of inflammation.

296. Protein Synthesis by Mucosa of the Human Small Intestine In Vitro: Effect of Disease and an Inhibitory Effect of Gliadin in Gluten-Sensitive Enteropathy. ANDREW L. WARSHAW* AND LEONARD LASTER, Bethesda, Md.

In the first part of this study we examined the effect of disease on intestinal protein synthesis. Synthesis by intact biopsy specimens from mucosa of the small intestine was measured by determining incorporation of uniformly labeled ^{14}C-L-leucine into trichloroacetic acid-precipitable material. Incorporation of isotope into protein (mean $\pm$ 1 standard deviation) in 70 biopsies was: (1) 15 normal volunteers, 1489 $\pm$ 404 dpm per mg of wet tissue per 30 min; (2) 8 sprue syndrome patients with flat intestinal mucosa (6 were gluten sensitive), 3056 $\pm$ 1402; (3) 2 patients with abetalipoproteinemia, 535 $\pm$ 67; and (4) 5 patients with treated Whipple's disease in remission, 1449 $\pm$ 171. The mean values for groups (2) and (3) differed significantly ($P < 0.001$) from normal. These results indicate that mucosal protein synthesis can be altered by intestinal disease. In the second part of this study we explored the in vitro effect of gliadin on mucosal protein synthesis. Incorporation of ^{14}C-L-leucine was determined for paired tissue specimens obtained simultaneously from the same subject. Ethanol was added to the control incubation mixture, and ethanol containing gliadin was added to the experimental mixture. In 13 studies of 11 normal volunteers, gliadin stimulated protein synthesis 24% (range, -1 – $+61$) ($P < 0.001$). In 8 studies of 5 gluten-sensitive patients, gliadin inhibited protein synthesis 28% (range, -5 – -52) ($P < 0.002$). The difference between the stimulatory effect on normal tissue and the inhibitory effect on tissue from gluten-sensitive subjects was significant ($P < 0.001$). Gliadin stimulated protein synthesis by flat mucosa from a patient not sensitive to gluten, and by mucosa from 1 patient with tropical sprue in remission, 2 patients with treated Whipple's disease, and 2 patients with granulomatous enteritis. These studies demonstrate a biochemical effect of gliadin on mucosal protein synthesis apparently specific for gluten-sensitive enteropathy.

297. Defective DNA Synthesis in Human Megaloblastic Bone Marrow: Accentuation by Methionine, Improvement by Homocysteine. SAMUEL WAXMAN,* JACK METZ,* AND VICTOR HERBERT, New York, N. Y.

Synthesis of thymine DNA from deoxyuridine (dU) was studied in megaloblastic and normal human bone marrow cultures by measuring the ability of 1 hr preincubation with dU to block incorporation of subsequently added ^{3}H-thymidine (^{3}H-TDR) into DNA. In this system, abnormal DNA synthesis in megaloblastic anemia was demonstrable by reduced ability of preincubation with dU to block incorporation of subsequently added ^{3}H-TDR. This defect was corrected by in vitro addition of B_{12} or pteroylglutamic acid (PGA). In three B_{12}-deficient marrows, methionine prevented B_{12} correction of the defective dU blockade of ^{3}H-TDR incorporation into DNA. Conversely, homocysteine not only enhanced B_{12} correction, but also had some corrective effect when given alone. Similar results occurred in folate-deficient marrows (three nutritional and two methotrexate induced), although homocysteine potentiated PGA less than it potentiated B_{12}. Addition of homocysteine or methionine to four normal marrows produced a slight effect, similar to that produced in megaloblastic marrow cultures. These results support the concepts that (1) inadequate DNA synthesis in B_{12} deficiency is due in large measure to blockade of tetrahydrofolic acid (THFA) regeneration from 5-methyl THFA (i.e., the "methyl-folate trap") brought about by lack of B_{12}; (2) in man there is an additional, B_{12}-independent, pathway for regeneration of THFA by methylation of homocysteine to form methionine (i.e., an "escape hatch"). Among possible explanations for the methionine effect is repression of homocysteine transmethylase, resulting in further trapping of 5-methyl THFA, as demonstrated by others in chickens. We are now studying in vivo whether methionine potentiates antifol action and whether homocysteine serves as a partial antidote to antifol toxicity.

298. Corticosterone-Secreting Tumor: A Defect in 17-Hydroxylation. RICHARD WEINSTEIN,* JOSEPH NEEMAN,* AND BERNARD KLIMAN,* Boston, Mass. (introduced by Jacob Lerman**).

A unique biosynthetic defect of steroid hydroxylation has been demonstrated in an adrenal tumor of a 9 month old infant with hemihypertrophy. This defect was manifested by increased production of corticosterone with limited synthesis of 17-hydroxylated products. Adrenal content and in vitro production rates of cortisol (F), 11-deoxycortisol (S), corticosterone (B), deoxycorticosterone (DOC), and aldosterone (A) were measured by double-isotope derivative dilution and compared with normal adrenal tissue. Normal adrenal content was 4.4 (F), 1.1 (B) μg/100 mg; tumor content was 0.10 (F) and 0.49 (B) μg/100 mg. The predominant incubation products of normal control tissue were (F) 1.15 and (S) 0.60 μg/100 mg per hr, and the major tumor product was (B) 0.75 μg/100 mg per hr. Addition of pregnenolone (Δ^5P) or progesterone (P) to tumor tissue primarily stimulated formation of B to 7.5 μg/100 mg per hr, with a lesser increment in DOC production, 3.3 μg/100 mg per hr. Addition of P to normal tissue stimulated synthesis of 17-OH steroids (F, S) with a minor increment in B, DOC, and A. A defect in 11,21 hydroxylation of 17-OH precursors in the tumor was ruled out by addition of 17-OH-P with resultant (S) 7.3 and (F) 12.1 μg/100 mg per hr production rates now comparable to those of normal tissue. Plasma levels of F and B were 23 μg/100 ml and 6.3 μg/100 ml, respectively, before surgery. Postoperatively, plasma B fell to less than 1 μg/100 ml, while plasma F remained normal. This study is the first dem-

onstration of defective 17-hydroxylation in an adrenal tumor.

299. Absorption of Hemoglobin Iron: The Release of Iron from Heme by Intestinal Xanthine Oxidase. LEWIS R. WEINTRAUB,* R. BEN DAWSON, JR.,* AND SHEILA RAFAL,* Boston, Mass. (introduced by William H. Crosby**).

We have recently reported that heme from ingested ^{59}Fe hemoglobin was taken into the mucosal cell of the dog's small intestine, but subsequently the ^{59}Fe appeared in plasma bound to transferrin. A substance was demonstrated in homogenates of the intestinal mucosa which released iron from a hemoglobin substrate in vitro. The kinetics of the reaction suggests that an enzyme may be involved. The present studies were performed to define the heme-splitting reaction. (1) Sodium azide, a catalase inhibitor, added to a 50,000 *g* supernatant of the mucosal homogenate, increased from 25% to 70% the release of ^{59}Fe from a hemoglobin substrate. This suggests that a peroxide-generating system is present, since heme splitting requires an oxidative reaction. (2) Allopurinol was added to the system in varying concentrations to inhibit xanthine oxidase, a ubiquitous enzyme which produces H_2O_2 by oxidation of its substrates. A concentration of allopurinol of 10^{-5} M achieved a maximal decrease (from 35% to 14%) of the ^{59}Fe released from a hemoglobin substrate. The inhibitory constant in this heme-splitting reaction was similar to the K_i of allopurinol for the oxidation of xanthine by xanthine oxidase. (3) Fractionation of the 50,000 *g* mucosal supernatant on a G-200 Sephadex column showed the heme-splitting substance to have the same elution volume as commercially obtained xanthine oxidase, indicating a similar molecular weight. (4) The addition of xanthine to the 50,000 *g* supernatant resulted in the production of uric acid, as measured by an increase in OD of the solution at 292 mμ. Allopurinol inhibited this reaction. These data suggest that xanthine oxidase in the intestinal epithelial cell is important in the release of iron from absorbed heme. The enzyme may mediate the heme-splitting reaction by the generation of peroxides which result in the oxidation of the heme ring and release of iron.

300. Differences between the Effects of Aspirin (Acetylsalicylic Acid) and Sodium Salicylate on the Reactions of Platelets to Connective Tissue, Epinephrine, and Adenosine Diphosphate. HARVEY J. WEISS,* New York, N. Y. (introduced by Kurt Hirschhorn).

Recent studies indicate that the prolonged bleeding time after aspirin ingestion is associated with impaired platelet aggregation by connective tissue due to inhibition in the release of platelet adenosine diphosphate (ADP). Since sodium salicylate does not prolong the bleeding time, this study was initiated to compare the effects on platelet function produced by ingesting 1.5 g of either aspirin or sodium salicylate with that of a placebo (lactose). Six normal subjects received each drug once during the study. 2 hr after aspirin ingestion the bleeding time increased by 1.75 ± 0.4 SE min as compared with a change of -0.17 ± 0.46 SE in the control group ($P < 0.01$). Ingestion of sodium salicylate had no significant effect (-0.6 ± 0.3 SE min). A similar difference was observed in the effect of the two drugs on platelets. Two hours after aspirin ingestion, release of platelet ADP by connective tissue, studied in citrated platelet-rich plasma, showed a decrease of -10.8 ± 2.5 SE mμmoles per 10^9 platelets (control group, $+1.67 \pm 2.24$ SE; $P < 0.01$) and platelet aggregation showed a decrease of $-18.5 \pm 8.2\%$ SE (control group $+8.5 \pm 2.3$ SE; $P = 0.01$). Total platelet ADP and ATP were unchanged. No significant changes were observed after ingestion of sodium salicylate (ADP release, $+1.0 \pm 2.4$ SE; aggregation $+3.0 \pm 4.3$ SE). In vitro, aspirin, in pharmacologic concentration ($0.25–0.50 \times 10^{-3}$ M), inhibited platelet ADP release by connective tissue and secondary platelet aggregation by adrenaline (5 μM) and ADP (1.5×10^{-6} M); sodium salicylate had no effect. Further in vitro studies showed that aspirin, in contrast to sodium salicylate, inhibited the binding of platelets to connective tissue without altering platelet surface charge, as indicated by measurements of electrophoretic mobility. These studies lend further support to the hypothesis that ingestion of aspirin, in contrast to sodium salicylate, prolongs the bleeding time by inhibiting platelet ADP release, possibly reflecting a more general property of cellular inhibition, as indicated by recent findings that it also inhibits release of leukocyte pyrogen.

301. A Mechanism for Widespread Gene Activation of Mammalian Cells. GERALD WEISSMANN, ROCHELLE HIRSCHHORN,* WALTER TROLL,* DANIEL WEISSBERG,* AND KATHRIN KRAKAUER,* New York, N. Y.

We have previously shown that transformation of lymphocytes by phytohemagglutinin (PHA) is accompanied by redistribution of acid hydrolases (labilization of lysosomes) and increased nuclear template activity for exogenous RNA polymerase (from *M. lysodeikticus*). Since such gene activation and subsequent mitosis might be mediated by hydrolases which stripped DNA of repressor materials, we studied the action of hydrolytic enzymes upon purified nuclei. Nuclei from rabbit and mouse liver and from human lymphocytes were tested for their capacity to act as template for RNA polymerase-mediated, actinomycin D–sensitive incorporation of ^{3}H-GTP into RNA. As shown by Frenster and coworkers, trypsin increased the DNA template capacity of nuclei to endogenous polymerase. In the present studies, trypsin (0.02–0.2 μg/μg DNA) also increased nuclear template activity to exogenous, bacterial RNA polymerase 5- to 10-fold, but had no effect on protein-free DNA. To find intracellular hydrolases capable of trypsin-like effects, rabbit and human leucocytes (A. Janoff) were fractionated in 0.34 M sucrose. Lysosome-rich fractions were found to contain neutral protease and tosyl-L-arginine methyl esterase (TAME) activity. With nuclease activity reduced to minimum, lysates of rabbit or human lysosomes (1.73 μg protein/μg DNA) produced 2- to 10-fold increases in

template activity of mouse or rabbit liver nuclei. These increases could no longer be augmented by trypsin. Similarly, trypsin failed to augment the already increased template capacity of lymphocytes stimulated for 2 hr by PHA, suggesting a preemptive action of endogenous protease. Therefore TAME and ϵ-aminocaproic acid (5–10 mM) were added as competitive protease inhibitors; PHA-induced transformation was markedly inhibited. These studies demonstrate mammalian enzymes (at least partly associated with granules) which have trypsin-like actions on template activity of nuclear DNA. Access of these to nuclei in living cells may initiate the kind of gene activation seen in blast transformation.

302. The Importance of Conjugation in the Excretion of Phenoltetrabromphthalein Disulfonate (BSP) in Adult and Neonatal Guinea Pigs as Revealed by Studies with Phenol-3,6-dibromphthalein Disulfonate (diBSP). GREGORY WHELAN,* STEVEN SCHENKER,* JANE HOCH,* AND BURTON COMBES, Dallas, Texas.

BSP taken up by liver undergoes conjugation with glutathione, and both conjugated and unconjugated dye are excreted into bile. The related compound diBSP (BSP minus two bromines) is excreted unconjugated. Since BSP and diBSP presumably share common hepatic transport processes, comparison of their relative maximal rates of biliary excretion (Tm) should permit an assessment of the importance of conjugation of BSP for its excretion. BSP Tm and diBSP Tm are reported to be the same in rats, rabbits, and dogs. This either (1) suggests that diBSP possesses some property more favorable for biliary excretion than unconjugated BSP, since conjugation is considered to be an important determinant of BSP Tm, or (2) casts doubt on the importance of BSP conjugation. In the present studies, the maximal rate of biliary excretion of diBSP was much greater than that of BSP in adult guinea pigs, 7.56 vs. 1.09 μmoles/100 g body weight per 10 min. Equilibrium dialysis with liver homogenates revealed that diBSP and conjugated BSP are bound much less avidly by liver proteins than is unconjugated BSP. These findings suggest that conjugation renders BSP less tightly bound to intracellular proteins and thus more readily available to the biliary transport mechanism. DiBSP, by contrast, does not require conjugation to facilitate access to the transport process. This probably accounts for its greater rate of biliary excretion than that of BSP in the guinea pig, a species known to conjugate BSP relatively poorly. In further studies, neonatal guinea pigs on the 2nd, 6th, and 9th days of life excreted diBSP at 51, 70, and 84%, whereas BSP was excreted at 19, 34, and 70% of respective maximal adult values. Diminished diBSP Tm in neonates implies impairment of the biliary transport system concerned with dye excretion. The relatively greater decrease in BSP Tm, however, suggests that conjugation is the major rate-limiting step for maximal BSP transport during the first 9 days of life.

303. The Relationship of Natriuretic Hormone (NH) to Sodium Excretion in Chronic Renal Disease (CRD). M. G. WHITE,* N. A. KURTZMAN,* A. R. HULL,* F. C. RECTOR, JR., AND D. W. SELDIN,** Dallas, Texas.

Patients with CRD may maintain NaCl balance during wide variations in NaCl intake despite a markedly reduced GFR. This must be mediated by reduction in Na reabsorption per residual nephron, and has been attributed to tubular damage, altered nephron perfusion, osmotic diuresis, and stimulation of normal physiologic regulatory mechanisms. We have identified a hormonal inhibitor (NH) of tubular reabsorption which appears to be an important regulator of Na excretion. To explore the role of NH in the regulation of Na excretion, six edema-free patients with CRD (GFR 2–10 ml/min) were studied while in Na balance on a 100 mEq Na diet. Na excretion varied from 4 to 17% of filtered Na. Plasma was assayed for NH by the shrinking-drop technique of Gertz. NH was not present in plasma of normal subjects maintained on a 100 mEq Na diet. Five of six patients with CRD had plasma NH at a titer of 1:4. The patient with no NH had the highest GFR (10 ml/min) and lowest percentage of filtered Na excreted (4%). When this patient was infused with 2000 ml saline, Na excretion increased to 10% of filtered Na, and NH appeared in plasma at titer of 1:4. Normal subjects given this amount of saline increased Na excretion from 0.5 to 1.5% filtered Na, but still did not have detectable NH. One of the patients with NH who was excreting 12% filtered Na was salt depleted with diuretics; Na excretion fell to 4% of filtered Na, and NH was no longer detectable. We conclude that in patients with CRD, increased levels of NH account in part for diminished Na reabsorption per residual nephron. The high NH may be due to chronic, but slight, overexpansion of extracellular volume (ECV). The level of NH is not fixed, but responds to variations in ECV.

304. Production of Carbon Monoxide by Bone Marrow and Reticulocytes In Vitro. PETER WHITE,* BRENDA C. SHAFER,* MARY L. ROTHER,* AND WILLIAM J. WILLIAMS, Philadelphia, Pa.

Previously reported studies using 2-^{14}C-glycine have shown increased "early labeled" peaks of both carbon monoxide (CO) and stercobilin in patients with ineffective erythropoiesis. These findings have been thought to reflect rapid turnover of heme within the marrow, but direct evidence for such a degradative process has been lacking. We therefore incubated marrow aspirates with 2-^{14}C-glycine in vitro and determined ^{14}C activity in heme and in CO, liberated by ferricyanide and trapped as CO_2 after oxidation by Hopcalite. To calculate degradation of newly synthesized heme, we assumed that recovered ^{14}CO activity had arisen from ^{14}C-heme in a molar ratio of 1 dpm:8 dpm. After 12–16 hr incubation at pH 7.4, mean per cent degradation of heme to CO was 1.7 $\pm$ (SD) 1.0 in marrows from eight control subjects, and 13.3 $\pm$ (SD) 9.6 in five patients with sideroblastic anemia and

ineffective erythropoiesis. Similar incubations of reticulocyte-rich peripheral blood showed a mean per cent heme degradation of 0.56 ± (SD) 0.67 in 10 control subjects with hemolytic anemia, and 9.53 ± (SD) 7.5 in six patients with sideroblastic anemia, erythroleukemia, or thalassemia. Increased degradation of heme (3.6–11.6%) was also found in reticulocytes from three patients with congenital Heinz body anemias. Addition of unlabeled hemoglobin (1.5×10^{-5} M) or hemin (7.6×10^{-5} M) to the reticulocyte incubations had no effect on ^{14}CO activity, suggesting that heme degradation was an intracellular process and not a reflection of extracellular liberation of heme. Altering the number of leukocytes in the incubations was also without effect. These findings demonstrate that heme degradation can take place both in marrow and in circulating erythrocytes and that the degradative process is increased in states of ineffective erythropoiesis.

305. The Influence of Cardiac Beta Adrenergic Receptor Stimulation and Blockade on End Diastolic Pressure-Volume Relations in Dog Left Ventricle. KERN WILDENTHAL,* CHARLES B. MULLINS,* M. DEAN HARRIS,* AND JERE H. MITCHELL, Dallas, Texas.

End diastolic pressure has been used as an index of end diastolic volume and, hence, "fiber length" of the left ventricle. The validity of such usage depends on a constant end diastolic pressure-volume (P-V) relation. Recent studies, however, have suggested that end diastolic P-V relations may change during inotropic interventions. In the present study the volume of an assumed nonprolate ellipsoidal shell of muscle near the left ventricular endocardium of open-chest dogs was calculated from biplane cinefluorographic exposures, taken at 1/60 second intervals, of six small lead beads previously implanted to define the major and two minor axes of the ellipsoid. With heart rate and aortic pressure held constant, venous return was systematically varied during control periods and while norepinephrine or propranolol was being infused intravenously. No significant change in the end diastolic P-V relation could be detected during norepinephrine infusion which caused a marked positive inotropic effect. During propranolol infusion three of eight dogs showed no change in end diastolic P-V relation, but five displayed an increased diastolic compliance. All dogs receiving propranolol had adequate beta adrenergic blockade and showed a negative inotropic response. The dogs with altered left ventricular diastolic compliance were characterized by the development of especially severe cardiac depression, reflected by a minimal or absent stroke volume increase with rising end diastolic volumes. In all dogs, changes in the relation of end diastolic pressure to area and to linear dimensions of the left ventricle were similar qualitatively to those in the P-V relation. The data are consistent with the hypothesis that cardiac beta receptor stimulation or blockade, of itself, does not cause significant changes in the relation of end diastolic pressure to left ventricular "fiber length," but that increased diastolic compliance may occur under conditions of acute, severe left ventricular depression or failure.

306. Factors Affecting Calcium Absorption: The Crucial Role of Hyperabsorption in "Idiopathic" Hypercalciuria. MICHAEL R. WILLS,* CHARLES Y. C. PAK,* AND FREDERIC C. BARTTER,** Bethesda, Md.

Factors which affect gastrointestinal calcium (Ca) absorption have been studied with oral ^{47}Ca as tracer and stable Ca loads. The rate of absorption was followed by the hourly measurement of forearm radioactivity for 4 hr in a large-sample scintillation counter after oral administration of the isotope label and Ca load. Since this measurement includes chiefly accretion by bone, which represents more than 90% of absorbed radioactivity, counting efficiency is 10 times that obtained from counting blood alone; it is not appreciably affected by variations in blood and urinary radioactivity. The results were expressed as "% dose trapped in arm." To calculate the "absolute" amount of Ca absorbed from the oral load, each patient was also given an intravenous dose of ^{47}Ca and the forearm was counted over the same period of time. The ratio of "% dose trapped after oral load" to "% dose trapped after intravenous load" represents fractional Ca absorption. The product of the fractional Ca absorption by the stable oral Ca load gave the "absolute" amount of Ca absorbed. Gastrointestinal absorption was low in untreated hypoparathyroidism, and rapidly increased with vitamin D. In normal subjects, Ca absorption was increased with parathyroid extract. The effect of varying oral Ca loads on gastrointestinal absorption of Ca was studied in patients with nephrolithiasis, both with and without hypercalciuria. Ca loads were varied over the range 20–1000 mg Ca given as $CaCl_2$. In both groups Ca absorption increased with oral load over the range studied. In the patients with idiopathic hypercalciuria, the absolute amount of Ca absorbed at any one oral load was significantly greater than that in normal subjects. Thus excessive gastrointestinal absorption may explain the hypercalciuria in many patients with nephrolithiasis.

307. Incorporation of Iodopyracet (Diodrast) into RBC of Azotemic and Nonazotemic Patients: In Vitro Studies. D. M. WILSON* AND F. D. SCHWARTZ,* Chicago, Ill. (introduced by R. M. Kark**).

Active transport of cations (Na^+) has been shown to be impaired in red cells incubated in plasma of azotemic patients. In a previous study we have shown a defect in the incorporation of the anion Diodrast into red cells of azotemic persons in vivo. These studies suggested bilateral flux of Diodrast against an electrochemical gradient. Red cell incorporation was measured from 10 min to 24 hr after incubating with ^{131}I-Diodrast (5 mg/100 ml) and glucose (150 mg/100 ml) in a Dubnoff shaker (O_2, 95%; CO_2, 5%). (1) The change in incorporation after increasing temperature (Q_{10}) was large, e.g. at 120′ incorporation of Diodrast was 15, 30, and 65% at 25°, 37°, and 45°C respectively in "nonazotemic" blood. Incorporation was significantly higher in blood from "azotemics" at 37° and 45°C. (2) Incorporation

into both nonazotemic and azotemic RBC was higher in azotemic plasma (90%) than in nonazotemic plasma (65%). (3) Inhibition of glycolysis (iodoacetate 10^{-4} M) did not affect incorporation, which was also independent of glucose utilization up to 24°C. (4) Protein binding of Diodrast was 30% as determined by plasma ultrafiltration. The differences between Diodrast incorporation when incubated in azotemic and nonazotemic plasma were not abolished by removing protein (incorporation was increased by 20%). (5) Dialysis of both azotemic and nonazotemic plasma (4 hr) increased Diodrast incorporation, suggesting multiple factors affecting incorporation. The data suggest that Diodrast flux in RBC is altered by some factor(s) in azotemic plasma rather than by an intrinsic RBC alteration. This effect is independent of glycolysis or protein binding.

308. Intramucosal Gastric Acid Concentration Determined by Glass Microelectrode Technique. DANIEL H. WINSHIP* AND CARLTON R. CAFLISCH,* Milwaukee, Wis. (introduced by William W. Engstrom**).

All theories of gastric HCl production center on the acidity of fluid elaborated from the intact mucosa, but no direct measurements from within the mucosa have been reported. We measured pH of rat gastric mucosa using micro-pH-electrodes. Insulated glass microelectrodes, tip diameters 0.5 μ, were constructed from Pyrex and Corning 0150 capillaries. Pyrex electrodes measured potential difference (PD) only; 0150 electrodes registered PD and were H^+ sensitive, with tip potentials of 20 to 40 mv per pH unit limited to the distal 1–2 μ of the tip. Rats were anesthetized. The stomach was opened and clamped in a Lucite chamber, mucosa facing up, serosa contacting Ringer's agar in the chamber, blood supply intact. Electrodes were driven into the secreting mucosa (total thickness 700 μ) in 10 μ increments, and PD between electrode and serosa (ES) or mucosal surface (EM) were recorded. Tip localization was accomplished by iontophoresis of lithium carmine from the tip; the dye was subsequently identified in unstained microscopic sections. 104 mucosal penetrations with Pyrex electrodes revealed that (*a*) the electrode was electrically negative (−4 to −80 mv) whether ES or EM was recorded; (*b*) the greatest PD occurred in the middle third of the mucosa, suggesting that the transmucosal PD is generated in that region; (*c*) lithium carmine localization confirmed that measured penetration was actual tip depth. In contrast, penetrations with 0150 electrodes in 25 rats disclosed areas of 10 μ to 30 μ in dimension where sudden high positive PD deflections occurred, indicating the presence of acid. Calculation of pH from PD revealed pH of 0.70 at depths between 175 μ and 630 μ from the mucosal surface in eight rats. The pH in the remaining rats was 0.75 to 2.50. We conclude that H^+ activity within the secreting rat gastric gland is 170 mEq/liter. This supports the two-component theory of gastric HCl formation, rather than other theories.

309. Prevention of Hypercholesterolemia and Atherosclerosis by 3β-, 5α-, 6β-Cholestane-Triol. DONALD T. WITIAK,* WILLIAM E. CONNOR,** DAMODAR M. BRAHMANKAR,* ANNA WARTMAN,* AND ROGER PARKER,* Iowa City, Iowa.

Cholestane-triol, an analogue of cholesterol, has been found in body tissues and has been synthesized. This drug (0.5%) was incorporated in a basal diet which contained 0.5% cholesterol in 2.5% peanut oil and which invariably caused hypercholesterolemia and atherosclerosis in rabbits. 11 animals received the triol addition and seven control animals received only the cholesterol-oil diet, all for 3 months' time. The usual hypercholesterolemia was blocked in triol-treated rabbits. The terminal values (± SE) for triol (T) and control (C) rabbits were: serum, 118 ± 31 (T), 1733 ± 248 (C) mg/100 ml; liver, 16.96 ± 4.35 (T), 130 ± 12 (C) mg/g; intestine, 19.09 ± 1.32 (T), 21.44 ± 2.38 (C) mg/g; and aorta, 3.32 ± 0.05 (T), 69.14 ± 16.38 (C) mg/g. Aortic atherosclerosis was virtually absent in triol rabbits (grade 0.08) and severe (grade 2.48) in controls. In other studies hypercholesterolemia was produced in six rabbits by the control diet for 3 wk and then triol was added. Prompt regression of the hypercholesterolemia occurred (from 1030 ± 149 to 43 ± 10 mg/100 ml). When triol was withdrawn from six other animals, the serum cholesterol promptly rose in 4 wk, from 54 ± 23 to 759 mg/100 ml. The rabbits gained weight and were in good health throughout these experiments. Cholestane-triol acted to prevent the absorption of cholesterol through the intestinal mucosa. This was documented by the oral administration of 4-^{14}C-cholesterol and fecal sterol analyses. Most of the administered sterol was found in the stool. 4-^{14}C-cholestane-triol was prepared, and this compound was likewise found poorly absorbed. It was recovered (by both isotopic and chromatographic analysis) in intestinal mucosal scrapings and in the stool. Only small quantities of triol were found in other tissues. The three hydroxyl groups of cholestane-triol appeared biologically important for its activity. The esterification of these groups as triacetate prevented its hypocholesterolemic effect.

310. The Metabolism of Intravenously Injected ^{3}H-Norepinephrine in Normotensive and Hypertensive Subjects. ROBERT L. WOLF,* MILTON MENDLOWITZ,** AND JULIA ROBOZ,* New York, N. Y.

7-^{3}H-DL-norepinephrine (^{3}H-NE) was rapidly injected intravenously in equal amounts to four normotensive subjects and to four untreated patients with essential hypertension. 3 hr sequential urine collections were obtained and assayed for radioactive normetanephrine (^{3}H-NM) and total normetanephrine (NM). Although there were no statistically significant differences between the sequential, 3 hr urine total NM excretions over eight collection periods in the normotensive and hypertensive subjects, the hypertensive subjects excreted significantly more

^{3}H-NM than the normotensive subjects, especially during certain collection periods. In the normotensive subjects the amount of ^{3}H-NM in the first 3 hr urine collection was greater than the amounts in the subsequent seven collection periods. The amounts of ^{3}H-NM in the second through the fifth 3 hr urine collection periods were constant and then subsequently declined slowly. This ^{3}H-NM urinary excretion curve could approximately be fitted to a logarithmic function of time. In the hypertensive patients the amount of urinary ^{3}H-NM increased from the first 3 hr collection period to a maximum at the fourth 3 hr collection period and then decreased. This urinary ^{3}H-NM excretion curve was bell-shaped. A theoretical two-pool model could be constructed to fit these data. It is concluded that after the intravenous injection of ^{3}H-NE, patients wth essential hypertension excrete more ^{3}H-NM than normotensive subjects in accordance with previously reported data, and that they excrete this greater amount of ^{3}H-NM at a later time after the injection of ^{3}H-NE than normotensive subjects.

311. Effects of Hydration on Sweating in Man. S. M. Wolfe,* R. H. Thompson,* and R. S. Gordon, Jr., Bethesda, Md.

Previous work has indicated that thermal sweating increases rapidly after ingestion of fluids. Our study attempts to quantify this relationship and to determine its mechanism. Normal volunteers were exposed daily for 90 min to 110°F, 55% relative humidity. Total sweat output was determined by serial weighing, and instantaneous changes in forehead sweat rate by measurement of the humidity of an air stream flowing over a defined skin area. Local sweat rate and chemical composition of forehead sweat were also measured by collecting samples on filter paper. In 90 min a typical acclimatized subject sweated 1.25 ± 0.13 liters (mean $\pm$ SD) without additional water, and 2.06 ± 0.27 liters while drinking 2 liters of water. Unacclimatized subjects sweated less, but responded similarly to water. After a subject had been in the environmental chamber without water for 1 hr, the ingestion of water at body temperature always resulted in a significant increase in sweat rate within 3 min. Rapid i.v. administration of 2–3 liters of isotonic fluid also resulted in increased sweat output, though the effect was less pronounced. Sodium chloride in the drinking water also reduced the response. The relation of sodium, potassium, chloride, and lactate concentrations to sweat rate was the same with or without hydration, suggesting that increased stimulation of active glands rather than recruitment of new sweat glands was occurring. Patients with diabetes insipidus showed the increase in sweat rate after drinking water; this and the rapidity of the response indicate that ADH is not involved. Our present hypothesis is that ingested water, like fluid given by vein, is rapidly effective in altering plasma volume and/or osmolality, and that these changes affect sweat rate through a neural reflex.

312. A Novel Form of Experimental Diabetes due to Selective Insulin Inhibition. Frederick Wolff,* Alison Grant,* John Wales,* Stephen Krees,* and Josef Viktora,* Washington, D. C. (introduced by Hyman Zimmerman).

During the screening of analogues of the antidiuretic hypotensive diazoxide, we have found a high biological correlation between antidiuresis and hyperglycemia in most of the compounds tested. Many of them have characteristics similar to those of diazoxide, a compound which when given orally to man has profound hyperglycemic and anti-insulin activity, useful for the treatment of refractory hypoglycemic states. There were, however, a number of exceptions. One, AO25, 7-chloro-1,2,4-benzothiadiazine-1,1-dioxide, was shown in the rat to have prolonged hyperglycemic activity at a dose of 200–500 mg/kg i.p., and 200 mg to 1 g/kg orally, without renal action and with a far lower degree of toxicity, expressed as the hyperglycemic/toxic ratio, than diazoxide, our standard compound. At 1 g/kg orally hyperglycemia lasts for up to 24 hr in the rat. In the monkey, AO25 given at a dose of 50 mg/kg i.v. caused marked hyperglycemia for at least 3.5 hr, with no change of blood pressure. There was evidence of depression of immunoreactive insulin, as shown by a reduction of the insulinogenic index. Oral administration of AO25 at a dose of 1 g/kg by stomach tube led again to marked hyperglycemia, lasting for at least 8 hr. There was no evidence of permanent diabetes being produced in either rats or monkeys during the present series of experiments. A close derivative of diazoxide has been shown to have prolonged and marked hyperglycemic and anti-insulin activity in rats and monkeys, without hypotensive action or marked renal activity. The compound appears to have advantages over diazoxide, and may provide an important tool for diabetes research, as well as better treatment for intractable hypoglycemia.

313. In Vivo Antioxidant Effect of Thyroxine. James Wynn, Little Rock, Ark.

Thyroxine has been shown to function as an efficient antioxidant in certain lipid peroxidations. Since these have been in vitro demonstrations, studies were undertaken to demonstrate whether thyroxine may have a similar effect in vivo. Male albino rats, pretreated orally with vitamin E, were divided into control and experimental groups. Experimental groups received 1 mg of sodium thyroxine pentahydrate subcutaneously in a 1 ml saline suspension. All animals were killed 24 hr after the administration of thyroxine or of control saline without thyroxine. Epididymal fat pads were removed and immediately extracted into benzene. The contents of weighed lipid, acyl fatty acid ester, and lipid peroxide were assayed. Esters were determined by the method of Rapport and Alonzo. Lipid peroxide was estimated by a modification of the method of Siddiqi and Tappel. Estimation of relative increase or decrease of peroxide content was made by comparing the peroxide/acyl ester ratio in treated and control extracts of epididymal fat

pads 24 hr after the administration of thyroxine. Animals treated with thyroxine showed a 13–63% decrease in peroxide content of the fat. In light of the in vitro work demonstrating an antioxidant effect of thyroxine at very low concentrations (2.5×10^{-8} M), it seems likely that thyroxine may decrease peroxide content in vivo by a similar mechanism.

314. Variations in the Response of Normal Human Subjects to Vitamin K_1. PHILIP D. ZIEVE* AND HARVEY M. SOLOMON,* Baltimore, Md. (introduced by Louis Lasagna).

The present studies measured the effect of vitamin K_1 on the anticoagulant response of 10 human volunteers to warfarin. A single oral dose of 40 mg of warfarin was given to these subjects, after which daily prothrombin times as well as serum warfarin levels were determined. The rate of metabolism of the drug was the same in all individuals studied, but there was considerable variation in the anticoagulant response. In subsequent weeks single doses of vitamin K_1 (1–20 mg) were administered intravenously either simultaneously with ingestion of warfarin or at various times thereafter. Although any one individual reacted consistently from week to week, there was a marked variation in the effect of vitamin K_1 from one individual to another. For example, in some subjects 1 mg of vitamin K_1 completely abolished the anticoagulant response; in others this dose diminished but did not abolish the response; in still others it had no effect. These variations could be due to individual differences in rates of metabolism of the vitamin and/or differences in receptor site sensitivity for the vitamin. The consistency of response in a given subject suggests that these differences may be inherited. In other studies the doses of vitamin K_1 and of warfarin were raised progressively, maintaining a constant ratio of one to the other. The drugs were given simultaneously by mouth. Invariably the anticoagulant effect was greater at the higher dosages. These experiments may indicate that at relatively high doses of warfarin, the anticoagulant binds to an additional site or sites important in the synthesis of vitamin K_1–dependent clotting factors. Alternatively, absorption of the vitamin may be less efficient as its dose is increased, or affinity of the receptor site(s) for warfarin may be increased at higher doses of anticoagulant.

315. Ultrastructural Analysis of Transport and Storage by a Platelet Membrane System. DOROTHEA ZUCKER-FRANKLIN,* New York, N. Y. (introduced by Edward C. Franklin).

Electron microscope studies were conducted to elucidate the pathway by which substances are taken up and retained by human platelets against high concentration gradients. Heparinized platelets show few vesicles and saccules surrounded by a membrane resembling the surface membrane. In platelets treated with EDTA this system of saccules or canaliculi is more prominent and occasionally appears continuous with the extracellular space. When platelets are incubated with latex particles, Thorotrast, or mycoplasmas, these substances come to occupy plasma membrane invaginations and canaliculi. Uptake of particulates does not increase oxygen consumption. Inhibition of either glycolysis or oxidative phosphorylation alone does not interfere with particle uptake, whereas inhibition of both metabolic pathways prevents it. Under these conditions, crescent- and hook-shaped cellular processes approach the main body of the platelet but fail to fuse with it, so that most canaliculi remain open to the exterior. Ordinarily, the canalicular system appears to be continuously formed by invagination of the plasma membrane, and it is likely that substances adsorbed to the membrane would be interiorized nonspecifically. Within unaltered platelets the continuity of this system as a labyrinth of connected sinusoids was delineated with horseradish peroxidase (Karnovsky). Further evidence suggesting similarity of the canalicular and surface membranes was obtained by allowing platelets to undergo osmotic swelling. Lowered cytoplasmic density revealed a layer of fibrils and 80–100 A particles attached to the outside of the canalicular membrane as well as to the inner aspect of the surface membrane. These fibrils are similar to those seen elsewhere in the platelet cytoplasm and to those found in the contractile protein extracted from the cells. The canalicular system may represent a mechanism whereby platelets take up, store, and transport physiologic substances like serotonin, catecholamines, fibrinogen, and other coagulation factors.