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Abstracts

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ABSTRACTS

*Explanation of symbols: No symbol = Member; * = Nonmember; ** = Emeritus or senior member*

1. The Effect of Estrogens on the Serum Level of Thyrotropic Hormone in Humans. LEVI ADAMS* AND FARAHE MALOOF,** Boston, Mass.

Attempts to evaluate the capacity of the normal human pituitary to release thyrotropic hormone (TSH), while circulating thyroxine levels remained unaltered, have been relatively unsuccessful. Using a sensitive radioimmunoassay for human TSH, results have been obtained which demonstrate that ethinyl estradiol (0.5–1.0 mg) when administered to normal males can cause a 3- to 10-fold increase in serum TSH, without a demonstrable change in the level of circulating thyroxine. Comparable changes occur in hypogonadal females. Preliminary observations, involving the administration of other synthetic and natural estrogens or contraceptive steroids, reveal that the dosage and molecular structure of the estrogen plus the magnitude of receptor sites available to the hormone may be critical for a positive TSH response in females. Specificity of the TSH elevation has been confirmed by dilution studies, bioassay, and immunological procedures involvng other pituitary hormones. The elevation reaches a peak at 24–36 hr and regresses with a half-life apparently similar to that of normal human TSH. Preliminary studies suggest that it results from the release of TSH from the pituitary, since it is suppressible by the administration of triiodothyronine, and does not occur in patients with hypopituitarism. Estrogens are known to accumulate in the anterior pituitary, to stimulate the synthesis of pituitary protein, and to alter the release of gonadotropins and growth hormone from this tissue. These data indicate that estrogens also produce alterations in the release of TSH. A sequential study of how and under what metabolic conditions estrogens modulate the secretion of TSH from the human pituitary is in progress and should help in understanding the basic mechanisms involved in the secretion of TSH and in assigning a potential role for estrogens and TSH in thyroid disease. (Research supported by grant AM-3274 from the NIH.)

2. Pulmonary Iron and Ferritin Transport. JAMES S. ADAMSON* AND EUGENE D. ROBIN,** Pittsburgh, Pa.

The unavailability of iron released by pulmonary hemorrhage for hemoglobin synthesis suggested studies of iron and ferritin transport in isolated alveolar macrophages (AM) and of ferritin transport across distal pulmonary exchange sites. The rates of uptake and loss of ^{59}Fe and ^{3}H-ferritin were determined in isolated rabbit AM obtained by pulmonary lavage using standard flux techniques. ^{59}Fe uptake is rapid. Within 5 min, intracellular/extracellular concentration ratios (R) reach 5.3 ± 3.1 (SD), and they continue to rise progressively, averaging 73.8 ± 25 ($n = 3$) at 3 hr. ^{59}Fe efflux is exceedingly slow, the $T_{0.5}$ being greater than 40 hr, and 3 hr R values average 1894 ± 275 ($n = 4$). This selective trapping of Fe results from rapid conversion of Fe to ferritin as noted under electron microscopy. Ferritin uptake is rapid, so that within 3 min R values are greater than 1 and they continue to rise progressively, averaging 11.6 ± 3.8 ($n = 4$) in 3 hr. Efflux is slow, with $T_{0.5}$ averaging 40 ± 10.4 hr and 3 hr R values averaging 251 ± 196 ($n = 4$). Electron microscope observation shows that uptake involves two phases: (a) diffuse cytoplasmic deposition; (b) sequestration into membrane-bounded vesicles. Both phases are active, since ferritin is excluded from nonviable macrophages. Relative permeability coefficients (P′) of ferritin/glucose were measured in liquid-filled segments of lung. Ferritin is transported bidirectionally; the ratio of P′ glucose to P′ ferritin averaged 3.7 ± 1.39 ($n = 6$) (predicted ratio on the basis of simple diffusion would be 17). Iron released by pulmonary bleeding is taken up by AM and converted to ferritin. Ferritin is sequestered in the AM. Ferritin appears to cross plasma cell membranes of both alveolar epithelial cells and macrophages at a relatively rapid rate, suggesting that diffusion is not the transport modality. Lung iron unavailability for hemoglobin synthesis is chiefly related to macrophage sequestration rather than to pulmonary alveolar-capillary impermeability.

3. Suppression of Delayed Hypersensitivity Granulomas by Pyridoxine and Thiamine Analogues. CYRIL A. AKPOM* AND KENNETH S. WARREN, Cleveland, Ohio.

Granuloma formation around *Schistosoma mansoni* eggs is a form of delayed hypersensitivity which provides a quantitative assay for immunosuppressive measures. Since severe malnutrition has an antagonistic effect on murine hepatosplenic schistosomiasis, decreasing worm egg output and diminishing host granulomatous response, granuloma suppression by calorie, protein, and vitamin deficiencies was investigated. The most striking results were achieved with antivitamins. Schistosome eggs isolated from infected mice were injected i.v. into lungs of unsensitized mice or mice sensitized by previous i.p. egg injection. Lungs were removed at different time periods, were processed, and the diameters of 50–100 egg lesions were measured. Mice placed on thiamine- or pyridoxine-deficient diets 2 wk before egg injection had moderate granuloma suppression only with the former diet. Neopyrithiamine hydrochloride (NPT) 50 μg daily subcutaneously, or deoxypyridoxine hydrochloride (DPD) 50 μg daily in drinking water, markedly suppressed inflammation. NPT was highly toxic, all animals dying by 10 days. In DPD-treated mice, peak lesion volume (at 16 days) was 1.31 ± 12 (SE) $mm^3 \times 10^{-4}$ (egg = 0.95) as compared with a control value of 30.54 ± 1.53. In sensitized mice, granuloma formation was both accelerated and augmented: at 8 days the respective figures for DPD-treated and control mice were 3.10 ± 0.55 and 46.44 ± 4.04. Fivefold reduction of DPD dosage still provided marked granuloma suppression. As

with all other immunosuppressive measures previously investigated, DPD had relatively little effect on granuloma formation in infected mice, but egg output by the worms was reduced to one-third that in control animals. Comparison of the degree of schistosome egg granuloma suppression achieved by drugs, antilymphocyte and antimacrophage sera, neonatal thymectomy, and X-irradiation with that produced by deoxypyridoxine reveals that this antivitamin is the most powerful immunosuppressive thus far examined. (Research supported by grants from the NIH.)

4. Response of Human Ileal Mucosa to Choleragen and Theophylline. Q. AL-AWQATI,* J. L. CAMERON,* M. FIELD,* AND W. B. GREENOUGH III,* Baltimore, Md., and Boston, Mass. (introduced by C. C. J. Carpenter).

Purified exotoxin of *Vibrio cholerae* (choleragen), theophylline, and cyclic adenosine-3',5'-monophosphate (cAMP) have been shown to inhibit sodium absorption and to stimulate chloride secretion in rabbit ileal mucosa in vitro. Evidence that these agents produce similar responses in human ileal mucosa is presented here. Normal specimens of ileum obtained at laparotomy from five patients were stripped of muscularis and mounted as flat sheets in chambers. Four tissues were mounted from each patient. 1 μg/ml choleragen was added to the luminal surface of two. 2 hr later, unidirectional lumen-to-serosa (L→S) and serosa-to-lumen (S→L) fluxes of sodium-22 and chloride-36 were measured simultaneously in paired tissues under short-circuit conditions. Flux data and short-circuit current (SCC) are in μEq/cm^2 per hr $\pm$ 1 SEM; net = L→S flux minus S→L flux. † indicates $P < 0.05$ from zero; § indicates $P < 0.05$ from control value. In controls, net fluxes were Na + 2.1 $\pm$ 0.9†, Cl − 0.4 $\pm$ 0.6, and SCC was 3.4 $\pm$ 0.2†. In choleragen-treated tissues, net fluxes were Na − 0.2 $\pm$ 0.2§, Cl − 2.2 $\pm$ 0.5†§, and SCC was 3.8 $\pm$ 0.2†. Serosal addition of 10 mM theophylline to controls immediately produced changes similar to those in choleragen-treated tissues (net fluxes Na −0.5 $\pm$ 0.4§, Cl − 3.4 $\pm$ 0.9†§; SCC 4.4 $\pm$ 0.4†). Mucosal addition of glucose to choleragen-treated tissues increased net sodium flux to + 4.2 $\pm$ 1†§. In the absence of net chloride movement, the unidirectional L→S chloride flux may be taken to represent passive ionic flow and permeability. The unidirectional L→S chloride flux was 9.5 $\pm$ 1 in controls, with a membrane resistance (R) of 52 $\pm$ 4 ohms; in choleragen-treated tissues, the L→S chloride flux was 5.7 $\pm$ 0.7§ and R was 64 $\pm$ 0 ohms§. These data indicate that choleragen decreases mucosal permeability, inhibits sodium absorption, and stimulates chloride secretion by human *ileal* mucosal strips. The similarity of the effects of choleragen and theophylline suggests that these changes may occur via a mechanism involving cAMP. (Supported by USPHS research grant AI-07625.)

5. Effect of Prostaglandin E_1 on Electrolyte Transport in Rabbit Ileal Mucosa. Q. AL-AWQATI,* M. FIELD,* N. F. PIERCE,* AND W. B. GREENOUGH III,* Baltimore, Md., and Boston, Mass. (introduced by Douglas Carroll**).

Small intestinal fluid loss occurs after infusion of prostaglandin E_1 (PGE_1) of theophylline into the canine superior mesenteric artery. Application of exotoxin of *Vibrio cholerae* to the small bowel mucosa produces similar results in the same preparation. Using flat sheets of rabbit ileal mucosa in vitro, theophylline and cholera exotoxin (CE) were shown to inhibit sodium (Na) absorption and to stimulate chloride (Cl) secretion. Unidirectional lumen-to-serosa (L→S) and serosa-to-lumen (S→L) fluxes were measured across short-circuited rabbit ileal mucosa using ^{22}Na and ^{36}Cl. Theophylline 10 mM or PGE_1 0.1 mM was added to the serosal surface. Tissues exposed for 2–3 hr to the effects of CE in vivo were then mounted in vitro, and fluxes were determined as above. Data are expressed in mEq/cm^2 per hr $\pm$ 1 SEM; net flux = L→S flux minus S→L flux. † indicates $P < 0.05$ from zero; § indicates $P < 0.05$ from control value. In controls (n = 13), net fluxes were Na + 1.7 $\pm$ 0.4†, Cl + 1.2 $\pm$ 0.6†, and short-circuit current (SCC) was 2.6 $\pm$ 0.2†. After PGE_1 (n = 8), net fluxes were Na − 0.5 $\pm$ 0.7§, Cl − 1.3 $\pm$ 0.6§, and SCC was 2.3 $\pm$ 0.2†. With theophylline (n = 10), net fluxes were Na + 0.3 $\pm$ 1, Cl − 2.6 $\pm$ 1§†, and SCC was 3.5 $\pm$ 0.2†§. In CE-treated tissues (n = 8), net fluxes were Na 0.2 $\pm$ 0.5§, Cl − 3.0 $\pm$ 0.5§†, and SCC was 3.2 $\pm$ 0.3†§. PGE_1 produces the same directional changes in ion movement in vitro as do cholera exotoxin and theophylline. This suggests that all three agents may produce these alterations by a common mechanism, possibly involving cyclic adenosine-3',5'-monophosphate. (Supported by USPHS research grant AI-07625.)

6. Increased Turnover of Mitochondrial Constituents in Cardiac Hypertrophy and Acute Hypoxia in the Rat. R. ALBIN,* V. ASCHENBRENNER,* AND M. RABINOWITZ, Chicago, Ill.

The effects of acute cardiac hypertrophy and severe hypoxia on mitochondrial synthesis and turnover has been investigated in the rat. Heart weight increased 20–50%, 48 hr after banding of the ascending aorta. Mitochondrial functional mass increased proportionally more than total myocardial proteins 1 day after aortic banding, as indicated by an increase in homogenate specific activities of cytochrome oxidase, succinate-cytochrome *c* reductase, and succinic dehydrogenase. Increments in incorporation of ^{3}H-leucine into mitochondrial proteins 1 day after aortic banding were also greater than into other cell protein fractions. Preferential synthesis of mitochondrial proteins was less apparent 3 days after banding. To gain insight into the dynamics of synthesis and degradation of mitochondrial proteins, we studied the effect of hypertrophy and hypoxia on turnover of cytochrome *c* labeled with heme group by ^{3}H-δ-aminolevulinic acid (ALA). Cytochrome *c* and heme *a* turn over with half-time of 5–6 days in normal rat heart. Cytochrome hemes were labeled with ^{3}H-ALA 3 days before aortic banding, and specific activity and total cytochrome *c* content and radioactivity were measured in banded and sham-operated controls. There was a significant 20–35% dilution of cytochrome specific activities 1 and 3 days after banding, which was greater than the 10% increase in cytochrome *c* content. Calculations show that cytochrome *c* degradation rate increased 2.5 times but synthetic rate increased 3.5 times, 1 day after banding. At 3 days synthetic and degradation rates increased 2 times. Similarly, in animals exposed for 6 hr to 4% O_2,

after return to a normal atmosphere the specific activities of cytochrome *c* and heme *a* decreased 20–30%. Cytochrome *c* content did not change significantly. Hypoxia therefore leads to increased cytochrome destruction with subsequent resynthesis. (Supported by the USPHS, the Illinois Heart Association, and MIRU.)

7. Thromboembolism and Oral Contraceptive Medication. NORMA ALKJAERSIG,* ANTHONY P. FLETCHER,** AND ROBERT BURSTEIN,* St. Louis, Mo.

Women receiving oral contraceptive medication are significantly predisposed to the development of thromboembolic disease complications, but no method now exists for detecting individual subject susceptibility to thromboembolism or for assessing the thrombogenic potency of the various medications. A new method for detecting blood hypercoagulability and/or occult thrombosis, dependent upon the detection of high molecular weight fibrinogen complexes (either fibrinogen-fibrin monomer or fibrinogen-fibrin proteolysis product complexes) in plasma, has been validated by trial in various clinical disease situations, in particular, in patients suffering from postoperative venous thromboembolism. The presence of high molecular weight fibrinogen complexes is detected by gel filtering plasma on Bio-Gel 5 M, assaying the effluents for fibrinogen, and determining fibrinogen molecular weight distribution curves. Plasma samples containing high molecular weight fibrinogen complexes are easily distinguished from normals (a single molecular species of fibrinogen) by this method. During a clinical trial of conventional combined oral contraceptive medication vs. mini-pill progestin contraceptive therapy, we have detected 14 subjects (13 of 14 on combined therapy, one on progestin therapy, data not statistically significant for medication differences) who, while demonstrating essentially normal conventional blood coagulation assays, developed blood hypercoagulability as documented by the new technique; no control subjects did so. The subjects ranged from the wholly asymptomatic to those admitted to the hospital with either suspected or proved pulmonary embolism. The etiological significance of oral contraceptive medication was established by discontinuing medication and demonstrating reversion to normal fibrinogen molecular weight distribution patterns in 1–4 wk. Our new methods provide a means by which both individual susceptibility to the thrombogenic actions of oral contraceptive agents may be determined, and the thrombogenic potency of various preparations may be tested. (Supported by USPHS grant HE-03745 and NIH grant 69-2263.)

8. A Voltammetric Study of Metabolizing Erythrocytes. MILTON J. ALLEN,* Washington, D. C. (introduced by Lawrence S. Lilienfield).

Investigations were undertaken to determine changes in the metabolic viability of stored erythrocytes (RBC) with age. It was found that using the techniques of tastpolarography on a suspension of washed RBC in isotonic pH 7.4 phosphate buffer gave a wave $E_{\frac{1}{4}} = -0.230$ v *vs.* SCE[1] and an n value ≈ 1 e. This wave, which is obtained only with the dropping Hg electrode, suggests detection of —SH groups. The presence of sulfhydryl groups in reduced form is essential for the integrity of RBC. Washed RBC incubated aerobically (18 hr at 37°C) in pH 7.4 buffer showed a decrease in diffusion current (Id) which is related to concentration of —SH. Incubation in a buffered glucose solution resulted in an increased Id. The system devised for evaluation of metabolic viability vs. age of stored RBC involved determination of ΔId after 18 hr incubation of the washed RBC in buffered glucose. Samples of RBC were evaluated in this manner within the first 3 days of storage after receipt from donor and at intervals thereafter. Results suggest that metabolic viability of packed RBC stored at 4°C in ACD (solution A) falls off rapidly within approximately the first 8 days and more slowly thereafter. A preliminary study on RBC from three folate-deficient patients demonstrated a significantly smaller ΔId than in seven "normal" controls. (Supported by a NSF grant and assisted by a GRS grant from the USPHS.)

[1] Saturated calomel electrode.

9. Heterogeneity of Genetic Variants in Hereditary Angioneurotic Edema. CHESTER A. ALPER, FRED S. ROSEN, JACK PENSKY,* MARTIN R. KLEMPERER,* AND VIRGINIA H. DONALDSON, Boston, Mass., Cincinnati, Ohio, and Cleveland, Ohio.

Hereditary angioneurotic edema is inherited as an autosomal dominant trait and is associated with a deficiency of serum inhibitory capacity for an esterase of the first component of complement, $C1\bar{s}$. The inhibitor is an $alpha_2$ glycoprotein (C1EI) against which monospecific antiserum has been prepared. All affected persons in 44 kindred had deficient inhibitory capacity. In 36 kindred, the concentration of C1EI, estimated immunochemically, averaged 17.5% of normal in the sera of 75 patients. In the remaining 8 kindred, C1EI serum concentrations were normal or elevated. Studies were carried out with the sera of patients with normal or increased C1EI concentrations to define further the nature of the functionally abnormal proteins. On incubation of these sera with purified, ^{125}I-labeled C1s and subsequent radioimmunofixation C1EI binding of C1s varied from kindred to kindred from normal to none detectable. On immunofixation electrophoresis, differences in electrophoretic mobility of C1EI from kindred to kindred were striking. In only one kindred was mobility normal; in four kindred C1EI mobility was modestly increased in affeced sera, and in two kindred it was markedly increased. In patients from a kindred with markedly increased C1EI concentration, the protein was double-banded and consisted of an $alpha_1$ and a modestly fast component. The $alpha_1$ band consisted of a complex between C1EI and albumin. Except for the one kindred mentioned above, none of the affected sera showed normal mobility C1EI. Thus, these studies have demonstrated that at least four different abnormal genes are associated with hereditary angioneurotic edema; the presence of one such gene can cause either markedly diminished serum concentration of C1EI or an abnormal and nonfunctional protein. (Supported by USPHS and American Heart Association grants.)

10. Pathogenesis of Malabsorption in Intestinal Stasis Syndrome. MARVIN E. AMENT,* HENRIK P. PORTER,* STANLEY S. SHIMODA,* DAVID R. SAUNDERS,* AND CYRUS E. RUBIN,** Seattle, Wash.

On the basis of fasting studies, the steatorrhea of intestinal stasis syndrome has been attributed to a luminal defect in micelle formation caused by bacterial deconjugation of bile salts. Intestinal mucosal structure has been considered normal. We studied a patient with diffuse small intestinal dilatation, duodenojejunal bacterial overgrowth (profuse *E. coli* and *Aerobacter klebsiella,* very scanty *Bacteroides* species), and steatorrhea (coefficient of absorption [CA] 40–56%). Hypoalbuminemia (2.6 g), enteric protein loss (569 ml plasma per day), and low serum folate (1–2 ng/100 ml) were also demonstrated. Surprisingly, this patient had normal micellar FFA (13 mM) after a fatty meal despite deconjugation of 15% of total bile salts. Moreover, patchy duodenojejunal mucosal abnormality was documented on "blind" review (22 of 36 biopsies). We investigated the roles of folate deficiency and of bacterial overgrowth in this patient's steatorrhea. Neither a folate-deficient diet nor folate repletion altered mucosal morphology (21 of 29 biopsies abnormal); however, folate repletion lessened steatorrhea (CA 56–88%) and caused some weight gain (6 lb. in 49 days). Institution of tetracycline eliminated Gram-negative bacteria from the proximal, but not the distal, small intestine, and produced immediate clinical improvement, weight gain (18 lb. in 12 days), and reduction of enteric protein loss (116 ml plasma per day). Some steatorrhea remained (CA 60–92%) although the morphological pattern of jejunal fat absorption as observed by electron microscopy appeared less abnormal. Thus mucosal malfunction, bacterial overgrowth, and folic acid deficiency contributed to malabsorption. Claims of impaired micelle formation were not confirmed by postprandial luminal studies; it is much more likelv that the previously unreported mucosal injury was of prime importance in this patient with stasis syndrome. (Research supported by grant CA-04320 from the NIH.)

11. Two Pathways for Bile Acid Synthesis in Man. KARL ANDERSON,* ENGELINE KOK,* AND NORMAN B. JAVITT, New York, N. Y.

The metabolism of cholesterol to primary bile acids (cholate and chenodeoxycholate) is considered to begin by either steroid ring (C-7) or side chain (C-26) hydroxylation. Studies of the metabolism of 7α-hydroxycholesterol and 26-hydroxycholesterol in rodents have shown that only 7α-hydroxycholesterol is metabolized to cholate in amounts approximating that normally present in rodent bile. To evaluate these pathways in man, equivalent tracer amounts of 7α-hydroxycholesterol-4-^{14}C (sp. act. 1.33 μc/μmole) and 26-hydroxycholesterol-16-22-^{3}H (sp. act. 13.3 μc/μmole) dissolved in human serum albumin were given simultaneously to four individuals requiring T-tube biliary drainage. Collected bile was analyzed quantitatively for radioactivity by liquid scintillation spectrometry. After hydrolysis, bile acids were separated by thin-layer chromatography and analyzed quantitatively as the methyl ester trifluoroacetates by gas-liquid chromatography using internal standardization. Radioactivity in each bile acid was determined by (1) a stream-splitting gas-liquid chromatography technique and (2) crystallization of derivatives of cholate and chenodeoxycholate to constant specific activity and constant ^{3}H/^{14}C ratios. It was found that radioactivity appeared in bile during the 2 hr infusion period, and 55–88% of the administered dose was recovered within 24 hr. 88–92% of the recovered radioactivity was metabolized to cholate and chenodeoxycholate. 34–62% of 26-hydroxycholesterol and 40–76% of 7α-hydroxycholesterol were metabolized to cholate, which represented 59–79% of the total bile acids in the bile of these individuals. Thus significant amounts of cholate can be derived from either pathway in man. A pathway from cholesterol to bile acid beginning with side-chain oxidation implies hepatic synthesis of monohydroxy bile acids capable of causing cholestasis and chronic inflammatory liver disease. (Research supported by grant AM-13094 from the NIH.)

12. Serum Immunoreactive Parathyroid Hormone in Normal and Hyperparathyroid Man. C. D. ARNAUD,* H. S. TSAO,* AND T. LITTLEDIKE,* Rochester, Minn., and Ames, Iowa (introduced by A. Albert**).

Study of human parathyroid gland function has been severely restricted by the general unavailability of a radioimmunoassay which can measure the low levels of parathyroid hormone (PTH) circulating in normal serum. Because of the limited cross-reaction between bovine and human PTH, we developed a radioimmunoassay for human PTH which uses a guinea pig antiserum directed against porcine PTH. Its sensitivity limit is in the range of 50–100 picograms of human PTH, and serum immunoreactive human PTH (SIhPTH) can be measured in 94% of normal sera tested (100). Values for the serum calcium (SCa) in this normal group, which vary over the entire normal range (8.9–10.2 mg/100 ml), correlate negatively with values for SIhPTH ($P < 0.001$); and only sera with values of SCa >10.0 mg/100 ml have values for SIhPTH which are undetectable. There is a diurnal rhythm in normal subjects, with SIhPTH rising in the late afternoon and evening. Rapid suppression of normal levels of SIhPTH to undetectable occurs with calcium infusion–induced increases in SCa above 11.0 mg/100 ml, and prompt and sustained increases in SIhPTH occur in response to hypocalcemia produced in Paget's disease patients given intravenous homogeneous porcine calcitonin. Patients with surgically proved primary hyperparathyroidism (HPT) with SCa >11.0 mg/100 ml (58) can be uniformly distinguished from normal on the basis of SIhPTH alone. Those with SCa <11.0 mg/100 ml (12) are clearly separated from normal by multivariate function discriminate analysis of both SCa and SIhPTH. Parathyroid tumor weight and degree of hypercalcemia correlate positively with SIhPTH ($P < 0.05$). SIhPTH is undetectable in surgical hypoparathyroidism (10) and is always increased in patients with hypocalcemia due to other causes. These results demonstrate the usefulness of an extremely sensitive radioimmunoassay for human PTH, based on the cross-reaction of human and porcine PTH, in the study of human parathyroid function in health and disease. (Supported by a grant from the NIH.)

13. Norepinephrine Metabolism and Receptor Response to Norepinephrine in Recurrent Intrahepatic Obstructive Jaundice. NUZHET O. ATUK,* THOMAS C. WESTFALL,* AND VIRGINIA WESTFALL,* Charlottesville, Va. (introduced by J. Edwin Wood III).

The simultaneous occurrence of hypertension, increased excretion of urinary norepinephrine (NE), and jaundice in a young woman with recurrent intrahepatic obstructive jaundice (RIOJ) prompted the following investigation. (1) In the patient and in normal subjects the responsiveness of adrenergic α-receptor was tested by infusion of NE (0.011, 0.023, 0.046 μg/kg per min). The results indicate that blood pressure response to NE infusion is greater in the patient than in normals. (2) In six normal subjects and nonicteric and icteric phases of the patient's disease, i.v. injections of ^{14}C-NE were given. Plasma was collected at 2, 5, 15, 25, and 30 min and urine at 1, 2, 3, 4, 8, 12, 24, 48, and 72 hr and analyzed for ^{14}C-NE, normetanephrine, and vanilmandelic acid. The results indicate that in RIOJ NE metabolism is altered. In the icteric phase of the disease there is a decrease in formation of the O-methylated metabolites throughout the collection period as compared with normal subjects. The O-methylated, deaminated metabolites are decreased in the first hour after injection of ^{14}C-NE, but thereafter they are increased. We conclude that in a patient with RIOJ the mechanism of increased NE production is not clear. However, our studies demonstrate a relation between the impairment of liver function and an initial slower inactivation of circulating NE. (This study was supported by grant 1-MO1-FR-00304 from the NIH.)

14. Nephron Filtration and Proximal Reabsorption during Saline Infusion, Arterial Clamping, and Hemorrhage in the Dog. R. B. AULD,* EDWARD A. ALEXANDER,* AND NORMAN G. LEVINSKY, Boston, Mass.

Nephron function was studied by re-collection micropuncture techniques. After infusion of 1.5–2.5 liters of saline in seven dogs, nephron GFR rose 57% from 113 ± 5.2 (SE) to 177 ± 9.2 nl/min, whereas whole-kidney GFR increased 11% from 41 ± 3.3 to 46 ± 4 ml/min. TF/P inulin fell from 1.52 ± 0.03 to 1.27 ± 0.03, indicating a 38% reduction in fractional reabsorption. However, absolute reabsorption did not change significantly (40 ± 2.7 to 36 ± 5.0 nl/min). When kidney GFR was reduced 24% by clamping in four of these saline-loaded dogs, nephron GFR fell 46% from 180 ± 11.2 nl/min to 97 ± 7.4 nl/min. TF/P inulin did not change significantly, 1.25 ± 0.04 to 1.34 ± 0.05. When four hydropenic dogs were hemorrhaged 10–20% of blood volume, kidney GFR fell 39% and nephron GFR 34% (106 ± 7.5 to 70 ± 6.5). In four separate experiments in hydropenic dogs, GFR was reduced comparably by arterial clamping: kidney GFR fell 30% and nephron GFR 33% (72 ± 6 to 48 ± 4.8). Changes in proximal reabsorption were very similar, as indicated by a rise in TF/P inulin from 1.55 ± 0.1 to 1.80 ± 0.12 after hemorrhage and from 1.47 ± 0.07 to 1.78 ± 0.1 during clamping. We conclude: (1) Most of the increment in distal Na delivery from superficial nephrons after saline loading is due to increased filtration. (2) "Redistribution" of GFR toward superficial nephrons occurs during saline infusion and is reversed during subsequent clamping. No redistribution of GFR away from superficial nephrons occurred in hydropenic dogs during clamping or hemorrhage. (3) When renal hemodynamics was reduced similarly by clamping or hemorrhage, changes in proximal reabsorption were comparable. Thus, the effect of hemorrhage on proximal reabsorption can be explained without invoking volume-mediated extrarenal factors.

15. Inhibition of the Hypercalcemic Effects of Vitamin D with Imidazole. SUSAN AVERY* AND NORMAN H. BELL,* Indianapolis, Ind. (introduced by John F. Williams, Jr.).

Whereas it is well established that the mediator of biologic action of parathyroid hormone on bone and kidney is cyclic adenosine-3',5'-monophosphate (cAMP), there is little information concerning the mediation of vitamin D action. To investigate this, the effects on serum calcium (Ca) of imidazole, an activator of the enzyme phosphodiesterase which converts cAMP to adenosine monophosphate, were investigated in thyroparathyroidectomized (TPTX) rats. Animals were given a Ca-deficient diet and vitamin D_3 (80,000 U or 160,000 U) in doses sufficient to produce a normal or elevated serum Ca. Maximal decreases in serum Ca were observed 3 hr after imidazole. At doses of 30, 40, and 60 mg/100 g body wt, imidazole produced mean decreases in serum Ca of 2.0 mg/100 ml in vitamin D–treated TPTX rats. Comparable changes in serum Ca were produced in intact untreated rats. The maximal decreases in serum Ca were directly related to the serum Ca at the time of imidazole administration. The hypercalcemic action of vitamin D was potentiated and the hypocalcemic action of imidazole was diminished by theophylline (5 mg/100 g body wt), an inhibitor of phosphodiesterase, and by dibutyryl cAMP (10 mg/100 g body wt). Imidazole (40 mg/100 g body wt), administered 2 hr before sacrifice to weanling rats given vitamin D_3 (10,000 U), did not significantly alter ^{45}Ca transport in everted sacs of the proximal duodenum. The results suggest that cAMP is necessary for the hypercalcemic action of vitamin D.

16. Dialysance of Amino Acids and Related Compounds. ALEXANDER AVIRAM,* JOHN H. PETERS,* AND PAUL F. GULYASSY, San Francisco and Menlo Park, Calif.

Increasingly intensive hemodialysis in chronic uremia carries with it the risk of progressive depletion of solutes of major metabolic significance. To define the rate of loss and determinants of transfer of amino acids during dialysis, we measured dialysance of amino acids and several derivatives. Because of marked differences in relative contributions of membranes and of liquid films to permeability, we studied the standard Kiil and the Dow Hollow Fiber (HF) dialyzers. Direct dialysance (D_d), i.e. (dialysate flow rate × concentration in dialysate)/(concentration in incoming plasma), was used as the parameter which is correct *a priori*. Indirect dialysance (D_b or D_p), i.e. (blood or plasma flow rate × change in concentration of blood or plasma)/(concentration in incoming blood or plasma) was compared with D_d. 15 studies of 25 amino acids and derivatives showed that

these solutes were extracted solely from the plasma, since D_d/D_p was 1.01 ± 0.02 (SE) for the Kiil and 1.06 ± 0.04 for the HF dialyzer. Contrary to the widely held assumption, the same is true for urea and creatinine, for which D_d/D_p was not different from 1.0 for either dialyzer. D_d ranged from 76 (urea) to 28 (tryptophan) for the Kiil and from 112 to 33 for the HF at mean flows of 200 ml/min (blood) and 500 ml/min (dialysate). There was a linear relation to molecular weight (mol wt), as $D_d = 78 - 0.19$ mol wt for the Kiil ($r = 0.75$) and $D_d = 122 - 0.38$ mol wt for the HF ($r = 0.75$). An even stronger relation was found to diffusion coefficient (D_{25}), as $D_d = 13.1 + 46.5\ D_{25}$ for the Kiil ($r = 0.92$) and $D_d = 17.0 + 72.8\ D_{25}$ for the HF ($r = 0.90$). (Supported by USPHS contract PH-43-66-1132, USPHS HE-11350.)

17. Relation of Papillary Interstitial Cell Granules to Sodium and Urea in Renal Papilla during Water Diuresis. SYLVIA AZAR,* MASAO ISHII,* AND LOUIS TOBIAN,** Minneapolis, Minn.

Interstitial cells of renal papilla appear to be secretory cells with cytoplasmic lipid granules. The number of granules and the concentrations of sodium and urea are all three significantly reduced in experimental hypertension. To study this effect in a nonhypertensive setting, chronic water diuresis was produced in rats by giving all calories as dilute drinking fluid, thus causing in all rats a daily urine weight about three-quarters of body weight. In 14 control nondiuretic rats, the papillary granule count averaged 137 per 1000 tissue squares (0.7 mm^2); papillary sodium concentration averaged 147 mEq/kg wet wt; and papillary urea concentration averaged 171 mmoles/kg wet wt. In a group of 14 rats whose dietary solids contained 8% protein, 2 wk of massive diuresis produced an average granule count of 90; [Na], 93; [urea], 37. In a group of 14 rats whose dietary solids contained 25% protein, 2 wk of massive diuresis produced a granule count of 93; [Na], 94; [urea], 131. In a group of 16 rats whose dietary solids contained 17% protein, 11 wk of massive diuresis produced a granule count of 134; [Na], 141; [urea], 100. A 2 wk low-protein diuresis significantly lowered sodium, urea, and granules in the papilla. A 2 wk high-protein diuresis significantly lowered sodium and granules in the papilla but changed urea very little. An 11 wk average-protein diuresis significantly lowered urea in the papilla but did not change granules and sodium. Thus, the granules correlate well with sodium concentration in the papilla and clearly not with urea. When water diuresis lowered papillary sodium, it also lowered granules, regardless of urea changes. We speculate that interstitial cells may regulate their humoral secretion according to papillary sodium concentration.

18. In Vitro Lymphocyte Reactivity in the Presence of a Cell-Free Macrophage Supernatant. FRITZ H. BACH, BARBARA ALTER,* AND SUSAN SOLLIDAY,* Madison, Wis.

Previous experiments have suggested a role for macrophages in in vitro lymphocyte reactions, such as the Mixed Leukocyte Culture test. These experiments have, for the most part, utilized Rabinowitz purified lymphocytes. The purified lymphocyte preparations have shown varying degrees of reactivity, ranging from no activity to somewhat diminished activity. The fact that the purified preparations have in some cases responded leaves unclear the question whether added macrophages, which reconstitute the response, are essential to the reaction or simply facilitate it. We have purified lymphocytes in a two-step preparation procedure as described by Shortman. Such purified preparations show no response to purified allogeneic mitomycin C–treated stimulating cells. The addition of a mitomycin C–treated macrophage preparation, isogeneic or allogeneic, to the responding cells reconstitutes the response. To investigate the role of the macrophages further, we have tested a cell-free supernatant of macrophages for its ability to reconstitute the response of purified lymphocytes. In five experiments, the cell-free supernatant completely reconstituted the response; in two experiments, a partial reconstitution was achieved; and in two experiments, no reconstitution occurred. Each experiment involved a different preparation of macrophage supernatant. The activity of the supernatant decreases with dilution. The possible role of such a factor in disease states involving macrophage dysfunction may be of interest. (Supported by NIH grant GM-15422–03 and Office of Naval Research grant N00014-67-A-0128-003.)

19. The Effect of Hydrogen Peroxide Production in Polymorphonuclear Leukocytes on Reduced Glutathione Levels in Caucasian Glucose-6-Phosphate Dehydrogenase–Deficient Red Blood Cells. ROBERT L. BAEHNER,* DAVID G. NATHAN, AND WILLIAM B. CASTLE,** Boston, Mass.

Patients with G6PD-deficient red blood cells (G6PD-RBC) may develop hemolytic anemia during acute infections. One oxidant threat to G6PD-RBC induced by infection might be the generation of H_2O_2 by juxtaposed phagocytosing polymorphonuclear leukocytes (PMN). To test this hypothesis, G6PD-RBC and normal RBC were incubated in glucose-enriched buffer with resting and phagocytosing normal and chronic granulomatous disease (CGD) PMN and with or without KCN and latex spherules. RBC glutathione (GSH) was calculated from total GSH minus PMN GSH. Both PMN GSH and normal RBC GSH remained stable during a 2 hr incubation at 37°C. In contrast, GSH rapidly declined in G6PD-RBC when PMN were present, at rates directly proportional to the ratio of PMN to RBC in the mixture. This effect was enhanced by phagocytosis and KCN inhibition of catalase. In some G6PD-RBC, GSH was exhausted in 2 hr. CGD PMN do not produce H_2O_2 and they failed to diminish GSH in G6PD-RBC. The rate of GSH oxidation in catalase-inhibited G6PD-RBC by normal PMN permits calculation of H_2O_2 release from PMN during phagocytosis from: $H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O$. In two separate studies this value was 42.5 and 47.7 mμmoles H_2O_2 produced per 10^7 WBC per hr. This accounts for approximately 40% of H_2O_2 produced via the manometrically measured cyanide-insensitive "respiratory burst," a far greater amount than was previously suspected from the ^{14}C-formate method of Iyer and associates. These studies show that normal phagocytosing PMN release much more H_2O_2 than

was previously realized, enough to induce oxidant injury in adjacent G6PD-RBC. This process may contribute to hemolysis during infection in G6PD-deficient patients. The CGD cell does not produce H_2O_2 and hence presents no threat to G6PD-RBC. (Research supported by USPHS grant AI-08173.)

20. Anatomy of Red Cell Damage by *Plasmodium falciparum* in Man. STANLEY P. BALCERZAK,* JOHN D. ARNOLD,* and DANIEL C. MARTIN,* Columbus, Ohio, and Kansas City, Mo. (introduced by James V. Warren**).

Morphology of red cells from two patients infected with falciparum malaria was studied to gain a better understanding of mechanisms of parasite invasion and erythrocyte destruction. Surface structure of red cells was examined by scanning electron microscopy and correlated with findings from transmission electron microscopy of serial sections of similar specimens. Serial sections revealed that although the bulk of the parasite was covered by intact erythrocyte membrane, a portion of the parasite was usually in direct contact with plasma. The parasite frequently projected beyond the contour of the red cell at a point where the membrane appeared disrupted. Scanning electron microscopy revealed various-sized membrane defects (0.1–2.0 μ in diameter) which were often occupied by rounded objects with corrugated surfaces. The presence of these objects correlated well with the presence of parasites as demonstrated by examination of single cells by light and scanning electron microscopy. Other defects appeared empty, and examination of such cells by light and scanning electron microscopy revealed no parasites. These data suggest that the malarial parasite (1) disrupts red cell membrane possibly at the time of penetrating the cell, (2) in some fashion stabilizes red cell substance to prevent loss of red cell cytoplasm, and (3) is in direct contact with plasma although located mainly beneath intact red cell membrane. (Research supported in part by grants from the NIH and the United States Army.)

21. Essential Fatty Acids, Gallstones, and Phospholipid Metabolism. JOHN BALINT, DONALD BEELER,* DONALD TREBLE,* AND EMILIOS KYRIAKIDES,* Albany, N. Y.

Feeding a fat-free diet rich in glucose, or sucrose, results in the formation of gallstones in hamsters. In order to investigate changes in hepatic and biliary lecithin biosynthesis under these conditions, male hamsters were fed for 10 wk a fat-free diet, while control animals received a regular chow. At this time, groups of five control and five experimental animals were injected intraperitoneally with a mixture containing ^{3}H-choline-methyl, ^{14}C-methionine-methyl, and $^{32}PO_4$, and killed 1, 2, and 4 hr later. Bile was obtained by aspiration of the gallbladder. For each group at each time period, bile, plasma, and liver were pooled and lipids extracted. Hepatic phospholipids and their water-soluble precursors were purified by silicic acid and ion exchange chromatography, respectively. Further fractionation of phospholipids was achieved by argentation chromatography, and the fatty acids were quantified by gas chromatography. Cholesterol gallstones were found in 11 of 15 experimental animals. Biliary phospholipids and hepatic lecithins in these animals showed replacement of linoleic by oleic and of arachidonic by eicosatrienoic acid. Oleoyl and eicosatrienoyl lecithins in experimental animals took the place of linoleoyl and arachidonoyl lecithins, respectively, in the metabolic scheme. Pool size of water-soluble precursors and of hepatic phospholipids was unchanged. Specific activities of these compounds for all three isotopes in the experimental animals were higher than those of the corresponding substances in controls, suggesting more rapid turnover in the former. Biliary phospholipids attained higher specific activities for ^{32}P and ^{3}H than any hepatic lecithin in control animals, while the reverse was the case in experimentals, suggesting a possible excretory defect in the latter. (Supported by grants from the NIH.)

22. Renal Excretion of Bile Salts in Obstructive Jaundice. DHIRENDRA S. BANA,* LILLIAN HAGOPIAN,* AND LEAH M. LOWENSTEIN,* Boston, Mass. (introduced by Jacob Lemann, Jr.).

Bile salts can cause renal failure experimentally. Since bile salts, normally absent from the urine, may be present in the urine in jaundice, we investigated the role of the kidney in bile salt excretion during obstructive jaundice. Eight rats with bile duct ligation 12 hr previously (group BD) and six normal rats (group N) were anesthetized, then infused intravenously with tritiated inulin in saline at 46 μl/min. After three 20-min control clearance periods, ^{14}C–sodium cholate (0.122 nmole/min) was also infused. After 30 min equilibration, three 20 min clearance periods were collected. The mean control inulin clearance for group BD was 2.27 ± 0.23 (SE) ml/min, and for group N was 2.88 ± 0.38 ml/min. These clearances were not altered by the bile salt infusion. The mean renal clearance of radioactive bile salts not bound to serum protein was 478 ± 80 μl/min in group BD and 36 ± 14 μl/min in group N. The ratio of bile salt to inulin clearance was 0.215 ± 0.02 in group BD and 0.0135 ± 0.003 in group N, a 16-fold increase ($P < 0.001$). Thin-layer chromatography showed that in both groups of rats 50% of the serum cholate had been conjugated to taurocholate and glycocholate 20 min after the start of the bile salt infusion, increasing to 97% conjugated after 90 min. Over 90% of the bile salts in the urine of both groups were also conjugated. Thus, in obstructive jaundice renal excretion plays an important role in bile salt elimination. We have also found that bile salts accumulate in renal tissue in vitro. Since these compounds are nephrotoxic, they may contribute to the renal failure commonly observed in jaundice. (Work supported by USPHS grants AM-13058 and AM-11793.)

23. Precipitation by Anti-DNA Antibodies of Adeno-Associated Satellite Virions. EUGENE V. BARNETT, K. TORIKAI,* AND HEATHER D. MAYOR,* Los Angeles, Calif., and Houston, Texas.

The primary etiology of systemic lupus erythematosus (SLE), although mediated by immunologic mechanisms, may be persistent viral infection. To explore this possibility further, SLE sera, rabbit antisera, NZB mouse sera (all with antibodies to single-stranded [s-s] DNA), and control sera were tested by diffusion in agarose against a variety of cesium chloride–purified virions as well as DNA, RNA, and

other control antigens. Precipitins were noted between anti-s-s DNA sera and s-s calf thymus DNA (1.3 to 500 μg/ml), double-stranded (d-s) calf thymus DNA (100 to 500 μg/ml), adeno-associated Satellite virions (Satellite), buoyant density 1.43, containing 25 μg s-s DNA per ml, Satellite capsids, buoyant density 1.30, <0.2 μg DNA per ml, and Kilham rat virus, <0.2 μg s-s DNA per ml, but not with preparations of rhinovirus (s-s RNA), adenovirus (d-s DNA), reovirus (d-s RNA), or supernatants from uninfected cell cultures. Satellite capsids when diffused against anti-Satellite serum and antibody to s-s DNA gave a line of complete identity. Anti-Satellite serum formed no precipitins with DNA or RNA preparations but did form precipitins with Satellite capsids. These lines of precipitation did not cross those formed between anti-DNA sera and s-s calf thymus DNA, indicating partial identity. DNA extracted from Satellite virions in concentrations up to 10 μg/ml formed no precipitins with anti-s-s DNA, confirming physical data showing that DNA extracted from Satellite virions undergoes renaturation to the d-s state. The data presented here are consistent with the hypothesis that SLE antibodies detected by viral antigens in serologic tests may have been induced by autoimmunization. The hypothesis that persistent viral infection may result in antibodies cross-reacting with autoantigens is not excluded.

24. Histaminase Activity in Medullary Carcinoma of the Thyroid. STEPHEN BAYLIN,* MICHAEL BEAVEN,* KARL ENGELMAN,* AND ALBERT SJOERDSMA,** Bethesda, Md.

A highly sensitive and specific radioassay for histaminase based on release of ^3H_2O during deamination of β-^{3}H(side-chain label)-histamine has been applied to human sera and tissues. The well known progressive increase of histaminase in the sera of pregnant women was confirmed and detected as early as 9–23 days after conception. The mean serum value ± 2SD for 27 normal individuals was 1.4 ± 2.0 units (μμmoles histamine deaminated per g per hr). An extensive survey of serum activity in disease states uncovered a strikingly elevated value of 66 units in an 18-yr-old girl with metastatic (lung and bone) medullary carcinoma of the thyroid. This patient is a member of an unusual family in which many individuals are afflicted with this disorder. A cousin with widespread metastases had a serum level of 6.3 units, and three relatives with only localized tumors had values within the normal range, 0.9, 1.5, and 2.2 units. Tumor tissue was obtained from the original patient and two of the latter patients. Values in tumor ranged from 1385 to 1930 units as compared with 45–170 units in adjacent thyroid from the same individuals. Further studies in the girl revealed a severalfold increase in serum values after injection of heparin (100 mg i.v.), whereas an oral 10 mg dose of aminoguanidine, a specific inhibitor of histaminase, produced complete and long-lasting inhibition of serum activity. A possible role for the enzyme in tumor growth is being investigated by maintaining the patient on 30 mg aminoguanidine per day. To our knowledge, this study represents the first correlation of an increased activity of histaminase in tumor tissue and serum.

25. Defective Mineralization and Increased Resorption of Bone in Phosphorus-Deficient Rats. D. BAYLINK,* J. WERGEDAL,* M. STAUFFER,* AND C. RICH,** Seattle, Wash.

In order to evaluate the effects of phosphorus on the processes of bone formation, mineralization, and resorption, we used recently developed morphologic methods to measure these processes in the tibias of rats fed a low-phosphorus diet (0.2%). Because the test diet caused decreased food consumption, the control rats, which were given a 0.6% phosphorus diet, were pair-fed with the test animals. The results, given as the mean ± SD, for the control compared with the low-phosphorus group were: weight gain (g) 50.0 ± 5.1 vs. 38.9 ± 3.4, serum phosphorus (mg/100 ml) 10.3 ± 1.1 vs. 6.2 ± 0.7, serum calcium (mg/100 ml) 9.9 ± 0.3 vs. 11.1 ± 0.5, osteoblastic matrix formation (μ/day) 12.5 ± 1.3 vs. 11.1 ± 1.2, osteoid maturation rate (%/hr) 6.7 ± 0.9 vs. 4.6 ± 0.5, mineralization rate (%/hr) 2.27 ± 0.28 vs. 1.87 ± 0.20, and osteoclastic bone resorption rate (mm^3/day) 0.027 ± 0.009 vs. 0.060 ± 0.006. All the above differences were statistically significant, $P < 0.02$. Osteoid maturation, which must occur before mineralization can be initiated, and the subsequent rate of mineralization represent two stages of the process of mineralization, and both stages were inhibited by phosphorus deficiency. The changes in weight gain probably contributed to some of the observed changes in bone processes, because when the effect of body weight was removed by analysis of covariance, statistically significant changes were found in mineralization and resorption, but not in formation. Results similar to those shown above were obtained when this experiment was repeated in thyroparathyroidectomized rats. Therefore, the defective mineralization and the large increase in the bone resorption rate probably were the direct consequence of hypophosphatemia. (Research supported by USPHS grant AM-9096.)

26. Identification of an Epidemiologically Virulent Strain of Group C Meningococcus. HARRY BEATY* AND GEORGE COUNTS,* Seattle, Wash. (introduced by Robert G. Petersdorf).

In 1969 meningococcal infections appeared in epidemic form at Fort Lewis, Washington. Employing a combination of serologic identification, sulfadiazine sensitivity, and the new technique of meningocin typing, a single epidemic strain of group C meningococcus was identified as the agent responsible for colonization and production of disease. Three pieces of evidence favor this hypothesis: (1) Among 29 isolates from patients with meningococcal disease, all belonged to serologic group C and had identical meningocin types and sulfadiazine sensitivity. (2) In a carrier survey of 394 recruits followed throughout the 8 wk basic training period, carrier rates for meningococci were 13.6% initially and 37.9% at the completion of training. 23% of meningococci isolated initially and 40% of those recovered in the 8th week were group C. However, the carrier rate of the epidemic strain rose from 16% of group C isolates at the beginning of training to 80% at its completion. These data show that the epidemic strain had greater ability to colonize a susceptible population than other group C strains, and strains in other serologic groups. (3) Three cases of meningococcal disease

occurred in separate platoons that had carrier rates below 15% during the first 4 wk of training. These cases were caused by the epidemic strain and occurred only after that organism predominated among carriers in the respective platoons. This study provides the methods for characterizing meningococci precisely, and indicates that epidemics of meningococcal disease are not caused by heterogeneous strains within a serologic group, but are due to a single strain with the unique propensity for colonizing a susceptible population and causing disease. (Supported by grants from the USPHS.)

27. The Role of Cyclic Adenosine-3′,5′-Monophosphate in Lithium Inhibition of Thyroid Secretion. S. C. Berens,* J. A. Williams,* and J. Wolff, Bethesda, Md.

Goiter formation is a side effect encountered during the treatment of manic states with lithium salts. We have shown that acute treatment of rats with Li^+ depressed all parameters of iodine metabolism studied: the iodide-concentrating mechanism, ^{131}I uptake, and ^{131}I release. Since these are normally under the control of TSH, it seemed possible that Li^+ might inhibit adenyl cyclase. Stimulation of adenyl cyclase activity in purified beef thyroid membranes by 100 milliunits/ml of TSH was inhibited by LiCl: inhibition (50%) occurred at ~10 mM Li^+ and attained a maximum (80%) at ~25 mM. LiCl, in concentrations up to 50 mM, had no effect on the $Na^+ + K^+$ ATPase in these membranes. Li^+ also affects later steps in iodine mtabolism. Thyroids from lithium-fed mice showed decreased hormone secretion in vitro when incubated with either TSH or dibutyryl cyclic AMP. Inhibition was noncompetitive with respect to TSH or cyclic nucleotide and was enhanced by addition of 25 mM Li^+ to the medium. Inhibition was also produced by the injection of 8 meq/kg of Li^+ 3 hr before sacrifice without lithium in the diet. These findings suggest that Li^+ acts both at the adenyl cyclase step and at a later stage of thyroid activation, and that the predominant effect may be beyond the cyclase step.

28. Intestinal Ferritin Synthesis in the Rat. George M. Bernier,* Stanley G. Schade,* and Marcel E. Conrad, Washington, D. C.

Many investigators have postulated that ferritin plays an important role in the regulation of iron absorption, because there is more ferritin in the intestinal absorptive cells of iron-replete than of iron-deficient animals. It remains uncertain whether ferritin acts to facilitate absorption or to limit absorption and enhance excretion. Ferritin synthesis was measured using the incorporation of ^{14}-C-labeled amino acids into intestinal protein and species-specific antiferritin antiserum. Oral doses of iron produced more ferritin synthesis in the duodenum than in other parts of the gut. The quantity of ferritin synthesized was proportional to the log of the oral dose of iron (1–100 μmoles), suggesting a relation between the mucosal uptake of iron and ferritin synthesis. Conversely, parenteral injections of iron produced more synthesis of ferritin in the terminal ileum than in the proximal small intestine. Since little iron is absorbed in the ileum, this ferritin must be sequestered by intestinal cells for excretion. Cobalt inhibits iron absorption as effectively as equimolar quantities of carrier iron, and these metals seem to share a common intestinal absorptive pathway. Ferritin does not share this pathway because cobalt does not stimulate ferritin synthesis and is not incorporated into ferritin. The ability of ferritin to hold iron in intestinal cells and prevent the transfer of unneeded iron into the body serves as a mechanism for diminishing iron absorption which is unavailable to cobalt. These data support the hypothesis that ferritin in the intestinal cell functions in the storage and excretion of iron rather than as the primary regulator of iron absorption.

29. Role of Injury and Death of Cells in the Release of Fibrinolytic Activity and Inhibitors of Fibrinolysis. Maria B. Bernik* and Hau C. Kwaan, Chicago, Ill.

Studies in culture have shown that most tissues possess fibrinolytic activity which is due to activator(s) of plasminogen, and also contain inhibitor(s) of activator activity. Further studies have indicated that whereas production and release of activator(s) and inhibitor(s) appear to be primarily a function of live, metabolizing cells, release of these components may also occur through injury and death of cells. Studies in this area, however, have been hampered hitherto by lack of appropriate systems in which activator(s) and inhibitor(s) can be studied separately. Culture models from human tissues were developed to investigate such release and its physiological significance in tissue injury. Primary cultures of renal medulla provided cells rich in activator and virtually free of inhibitor(s), while serial propagation of lung and liver cells provided cultures rich in inhibitor and virtually free of activator activity. Cells adapted to serum-free medium and monitored for viability, mitotic activity, and activator and inhibitor content were subjected to trauma by mechanical scraping or spontaneous sloughing in nutrient-free medium. After trauma, cell-free supernatants of renal medulla yielded up to 4.0 CTA U/ml activator, while supernatants of lung and liver inhibited up to 3.0 CTA U/ml activator. Nonspecific proteolytic or antiproteolytic activity was elicited but rarely, in trace amounts. These findings indicate that in tissue injury there is release from cells of both activator(s) and inhibitor(s) of activation. Such combined release may be expected, if not necessary, to achieve a dual purpose: to provide activator for the local disposal of fibrin and yet maintain equilibrium within the fibrinolytic system. (Research supported by NIH grant AM-14018-01, and by a grant from the Otho S. A. Sprague Foundation.)

30. Subsidiary Influences on Deoxycorticosterone Production. E. G. Biglieri, J. R. Stockigt,* M. Schambelan,* T. Lee,* and W. F. Ganong,* San Francisco and Davis, Calif.

Adrenocorticotropin (ACTH) is the major determinant of deoxycorticosterone (DOC) secretion. DOC production was assessed by the exeretory rates of tetrahydrodeoxycorticosterone (THDOC) in normal man (24) and by adrenal venous levels in dogs (10) by the double-isotope dilution derivative technique. Intravenous infusion of ACTH (25 units over 8 hr/day) for short (8 hr) or long (up to 17 days) periods of time produced a sustained increase in

THDOC production, wnereas oral administration of dexamethasone (2 mg/day) for 2 days reduced THDOC production by 50%. The response to ACTH was greater during sodium restriction (20 mEq/day) and reduced after "escape" from 9α-fluorohydrocortisone as compared with values obtained on 120 mEq sodium intake per day. Sodium restriction alone for 5–7 days effected no increase in THDOC in normal subjects, but a 50% increase in an untreated patient with hypopituitarism. Infusion of ACTH for 3 days in this patient resulted in a subnormal and delayed increase in cortisol excretion, but the increase in THDOC excretion was normal and prompt. Furthermore, graded doses (0.16–1.6 μg/min) of angiotensin II amide administered intravenously to dogs (4) immediately after hypophysectomy and nephrectomy stimulated DOC production far above control levels after pretreatment with large doses of glucocorticoid hormones for 1 month. We suggest that the state of activity of the renin-angiotensin system (RAS) determines the maximum DOC response to ACTH. In the absence of ACTH the subsidiary regulatory influence of the RAS maintains the adrenal sensitivity (of DOC) to ACTH and enhances its response to angiotensin II. (Research supported by grants AM-06415 and AM-06704 from the NIH.)

31. Computer-Assisted Evaluation of Electrolyte and Acid-Base Disorders. HOWARD L. BLEICH,* Boston, Mass. (introduced by A. Stone Freedberg**).

A time-sharing computer program has been designed to assist the physician in understanding and managing electrolyte and acid-base disturbances. The program directs a dialogue during which the user supplies whatever appropriate laboratory and clinical information may be available. Explanatory comments are used as needed to insure that each entry is of proper form, within reasonable numeric limits, and consistent with all previous entries. As soon as the data are entered, the program generates an evaluation note that varies in length from four words to three pages, depending upon the complexity of the abnormalities presented and the completeness of the information available. When appropriate, the note includes an explanation of the pathophysiology, a list of diagnostic possibilities, general and specific therapeutic recommendations, precautionary measures required by the illness or by its treatment, suggestions for additional laboratory studies, and references to the medical literature. The program has been used in our hospital for approximately 6 months, and it is available for consultation or for education at virtually any hour of the day or night. The program was written for a PDP-9 computer that can service as many as 24 terminals simultaneously. It may be called from any general-purpose teletype-compatible terminal (including oscilloscopic displays) that can be connected to the telephone system. The time required to enter the data and to obtain the evaluation note is approximately 4 min, less if an oscilloscopic display is used. The cost is less than one dollar. (Supported by a grant from the John A. Hartford Foundation.)

32. Anti-inflammatory Effects of Estrogen. PHYLLIS BODEL,* G. MORRIS DILLARD,* SANDRA KAPLAN,* AND STEPHEN MALAWISTA,* New Haven, Conn. (introduced by Lawrence R. Freedman).

Remissions of certain inflammatory diseases such as rheumatoid arthritis frequently occur during pregnancy. Since estrogens alter a variety of cellular and metabolic functions in vivo, and since leukocytes are important mediators of inflammation, the effects of estrogens on leukocyte metabolism and function were investigated. In three separate studies, the presence of estrogen was associated with suppression of the usual cellular metabolic responses to phagocytosis. In the first set of experiments, leukocytes from normal males, obtained by dextran sedimentation, were incubated in a 12% serum-buffer medium with or without 200 μg of 17β-estradiol. After 1 hr, live or heat-killed staphylococci were added, and phagocytosis, leukocyte respiration, conversion of ^{1}C-glucose to CO_2, pyrogen production, and release of acid phosphatase from leukocyte granules were measured. Although in the presence of estradiol phagocytosis was not impaired, the normal increase in oxygen consumption and ^{1}C-glucose oxidation with phagocytosis was reduced. These effects were dose related, were augmented when progesterone was added, and were minimal when dehydroepiandrosterone, an androgen, was substituted. There was also significantly less pyrogen produced by estrogen-treated cells after phagocytosis, and granule lysis was diminished. Studies of respiration and CO_2 production after phagocytosis were also made on leukocytes from pregant subjects before and after delivery, from normal male and female volunteers, and from hospitalized patients. In all cases, activity of the hexose monophosphate shunt after phagocytosis was depressed in association with higher levels of estrogens. In two patients, one with periodic fever and another with the syndrome of familial Mediterranean fever, estrogen treatment was followed by dramatic suppression of febrile and inflammatory attacks. These data suggest that estrogens have naturally occurring as well as therapeutic anti-inflammatory activities. (Research supported by grants from the NIH.)

33. An Inactivator of Anaphylatoxin in Normal Human Serum. VIKTOR A. BOKISCH* AND HANS J. MÜLLER-EBERHARD, La Jolla, Calif.

Unlike a variety of animal sera, human serum has been known to lack the ability to generate anaphylatoxin activity. The action of anaphylatoxin results in increased capillary permeability, smooth muscle contraction, histamine release from mast cells, and chemotactic attraction of polymorphonuclear leukocytes. The activity has been shown to reside in two peptides of molecular weights 7000 and 10,000, which are derived from the complement proteins C3 and C5 in the course of an immunologic reaction. Both peptides, designated C3a and C5a, could be produced from their precursor proteins after isolation from human serum. The failure to generate the activities of C3a and C5a in whole human serum suggested the possibility of the presence in serum of an anaphylatoxin inhibitor or inactivator. Utilizing inactivation of C3a by serum fractions as an assay, a potent inactivator was detected and subsequently isolated and identified as an α-globulin with a molecular weight of approximately 300,000

and an approximate serum concentration of 50 μg/ml. The inactivator was shown to act enzymatically on C3a, C5a, and bradykinin, one molecule of enzyme inactivating 500 molecules of C3a in 2 min at 20°C. It was found to have carboxypeptidase B specificity and to hydrolyze hippuryl-L-arginine and hippuryl-L-lysine. Release of C-terminal arginine from C3a and bradykinin could be correlated with abolition of the anaphylactic and phlogogenic activities. The occurrence of the enzyme in human serum fully explains the failure to produce anaphylatoxin activity in whole human serum and suggests that this serum enzyme fulfills an important regulatory function in vivo. (Supported by NIH grant AI-07007, American Heart Association grant 68-666, and AEC contract AT(04-3)-730. Dr. Bokisch is a Helen Hay Whitney Fellow.)

34. Intravaginal and Intrarectal Screening of Antimitotic Drugs for Topical Effectiveness. R. H. BONDER* AND E. J. VAN SCOTT,** Philadelphia, Pa.

This study indicates that intravaginal (IVag) and intrarectal (IR) testing of drugs for antimitotic effects in mice can yield useful information on penetrative properties of drugs and may provide a practical screen for predicting topical actions on skin. Number of mitotic cells per cm mucosa of virginal young mice untreated and 6 hr after intraperitoneal (i.p.) podophyllin provided reference baseline data. Test drugs were instilled IR or IVag at time zero, repeated at 2 hr along with podophyllin i.p., and the animals were killed at 8 hr. When a high number of metaphase-arrested cells resulted, the test drug was readministered without i.p. podophyllin to ascertain whether the drug possessed primary metaphase-arresting action. The validity of the screen was established by testing drugs with known effect on the cell cycle, such as mechlorethamine, BCNU, podophyllin, Vinblastine, cytarabine, and methotrexate. Our previous work has shown that systemic administration of these drugs affects rectal and vaginal epithelium equally. Important qualitative differences are found when drugs are administered IVag or IR and may forecast the action of each drug on the epidermis when applied topically.

35. The Regulation of Adrenal Cholesterol in Man. A. J. BORKOWSKI,* C. DELCROIX,* AND S. LEVIN,* Brussels, Belgium (introduced by A. E. Renold**).

The kinetics of plasma and adrenal cholesterol equilibration was investigated by comparing their specific activities at various times after 4-^{14}C-cholesterol administration in patients undergoing adrenalectomy for mammary carcinoma. In the zona fasciculata the following daily fractional turnovers were obtained: those of free adrenal cholesterol respectively from free plasma cholesterol and from esterified adrenal cholesterol, $k_1 = 3.35$ and $k_2 = 6.88$; that of esterified adrenal cholesterol from free adrenal cholesterol, $k_3 = 0.23$, the local synthesis of free adrenal cholesterol and the entry of esterified plasma cholesterol into the pool of the adrenal esters being negligible. Similar parameters were found in the zona reticularis, with $k_1 = 2.38$, $k_2 = 2.90$, and $k_3 = 0.25$. The equilibration of plasma and adrenal cholesterol did not slow down under dexamethazone suppression, but was accelerated by ACTH stimulation. In the latter instance the specific activities of urinary cortisol followed closely those of free adrenal cholesterol. Under dexamethazone or ACTH the turnovers of the various adrenal esters were similar to one another as normally. Adrenal slices incubated for 3 hr in a Krebs-Ringer-bicarbonate medium with 2-^{14}C-acetate synthesized negligible amounts of cholesterol: means per mg of nitrogen from 8 studies, in the zona fasciculata 9 ng, in the zona reticularis 51 ng, the difference between the two zones being highly significant ($P < 0.001$). ACTH administered before the adrenalectomy increased this in vitro synthesis 35 times in the zona fasciculata and 9 times, i.e. significantly less ($P < 0.05$), in the zona reticularis, whereas dexamethazone suppressed it. The pattern of the in vitro esterification was comparable to that observed in vivo, but the tendency for the more unsaturated cholesteryl esters to have a higher specific activity was accentuated.

36. Effect of Cyclic Adenosine-3′,5′-Monophosphate on Bone Marrow Delta Aminolevulinic Acid Synthetase Activity. SYLVIA S. BOTTOMLEY* AND G. ANN SMITHEE,* Oklahoma City, Okla. (introduced by Stewart Wolf**).

It has been shown in this laboratory that erythropoietin (ESF) enhances δ-aminolevulinic acid synthetase (ALAS) activity in marrow cultures and that protein synthesis may be required for this effect. In this study the possible role of cyclic adenosine-3′,5′-monophosphate (cyclic AMP) in the control of ALAS was examined in rabbit bone marrow cultures. The marrow culture method and ALAS assay used were as described previously. All substances tested were added when the cultures were established, and ALAS activity was measured 24 hr later. Cyclic AMP (10^{-6} M) and dibutyryl cyclic AMP (DB-cyclic AMP) (10^{-7} M) increased ALAS activity 100% and 185%, respectively, above control cultures ($P < 0.01$). Lower or higher concentrations of each compound were progressively less effective. Adenosine-5′-phosphate (10^{-6} M) or adenosine-3′-phosphate (10^{-6} M) had no effect. Actinomycin D (0.4 μg/ml) or cycloheximide (5 μg/ml) completely inhibited the cyclic AMP effect on ALAS activity. Whereas DB-cyclic AMP (10^{-5} M) or ESF (0.3 units/ml) alone increased ALAS activity 129% and 167%, respectively, above controls, addition of both substances to marrow cultures increased enzyme activity 390% above controls. Theophylline (10^{-4} M) alone enhanced ALAS activity 37% above controls and potentiated the effect of ESF by 53%. These findings demonstrate that cyclic AMP increases ALAS activity in bone marrow in vitro and suggest that cyclic AMP enhances synthesis of the enzyme. ESF may be another hormone which exerts an effect on its target cell by influencing intracellular cyclic AMP levels. (Research supported by the Veterans Administration and NIH grant AM-13143.)

37. Cyclic Adenosine-3′,5′-Monophosphate and the Regulation of Human Granulocyte Function. HENRY R. BOURNE,* ROBERT I. LEHRER,* KENNETH L. MELMON, AND MARTIN J. CLINE, San Francisco, Calif.

In many tissues cyclic AMP controls important cell functions. We have identified adenyl cyclase and phosphodies-

terase, enzymes which respectively synthesize and degrade cyclic AMP, in the human granulocyte. The following evidence supports a role for cyclic AMP in controlling the complex anatomic and biochemical events associated with phagocytosis. (1) Granulocyte adenyl cyclase was stimulated by prostaglandin E_1 (PGE_1) and NaF. As in other tissues, the enzyme required magnesium and was inhibited by calcium, zinc, and alloxan. Granulocyte phosphodiesterase was inhibited by theophylline. (2) PGE_1, theophylline, and dibutyryl cyclic AMP (an analogue of cyclic AMP) inhibited the degranulation of human granulocytes that follows phagocytosis of bacteria and fungi in vitro. Degranulation was measured by the decrease of lysosomal enzyme activity (e.g., lysozyme and myeloperoxidase) within the cells and their granule fraction, and by movement of a portion of this activity into the extracellular fluid. (3) PGE_1, theophylline, and dibutyryl cyclic AMP significantly inhibited the burst of oxygen consumption that normally follows particle ingestion by granulocytes. (4) Theophylline and dibutyryl cyclic AMP consistently inhibited granulocyte candidacidal activity. Inhibition by PGE_1 was variable. None of these drugs inhibited phagocytosis per se. In other tissues PGE_1 and theophylline cause intracellular concentrations of cyclic AMP to increase, and dibutyryl cyclic AMP mimics the action of endogenous cyclic AMP. Our working hypothesis is that the same is true in granulocytes, and that intracellular cyclic AMP inhibits both the degranulation and the burst of oxygen consumption that follow phagocytosis. Inhibition of either or both of these events provides a way for endocrine and pharmacologic agents to inhibit the granulocyte's ability to kill a microorganism, *Candida albicans*. Such pharmacologic mechanisms also allow further investigation of the lysosomes of the granulocyte and their possible contribution to inflammation.

38. Factors Affecting the Uni- and Bidirectional Reaction of Mixed Leukocyte Cultures. BERTHA A. BOURONCLE,* LOUIS MALSPEIS,* AND JOAN F. ASCHENBRAND,* Columbus, Ohio (introduced by Charles A. Doan**).

The in vitro reaction of mixed leukocyte cultures provides a reliable test for assessing histocompatibility in humans. In research, it can provide insight into fundamental biological processes. As originally described, two populations of leukocytes are reacted in vitro and the blastic transformation of lymphocytes is measured by the incorporation of ^{3}H-thymidine into cells synthesizing DNA. The unidirectional reaction is obtained by inhibiting the response of one population of lymphocytes by mitomycin C. To improve the reliability of stimulation caused by histocompatibility, we investigated factors which affected the technique. We found that: pH maintenance of the cultures by exposure to a CO_2 humidified atmosphere is essential to obtaining reproducible results; incorporation of isotopically labeled thymidine in the blastoid cells steadily increases to a plateau after 18 hr of incubation, and progressively decreases after 24 hr; the maximum response of mixed leukocyte cultures occurs on the 6th, 7th, or 8th day; pure lymphocytes do not interact in mixed cultures, but the reaction is restored by the addition of macrophages, though the degree of response is not directly proportional to the percentage of macrophages in the cultures; maximum response is obtained when the cells are centrifuged at 130 *g*, and decreased at 220 *g*; the maximum response increases if the number of lymphocytes is increased in the cultures; the presence of antibiotics does not affect the response unless the individuals are allergic to penicillin. It is concluded that in order to apply the mixed leukocyte culture as a technique for quantitative evaluation of the lymphocyte reaction, attention should be given to the standardization of factors affecting maximum response. (Research supported by a grant from The Anderson Foundation.)

39. Potassium as a Mediator of the Adrenal Response to Sodium Depletion in the Rat. JOHN E. BOYD,* W. PEARSON PALMORE,* AND PATRICK J. MULROW, New Haven, Conn.

In the rat, sodium depletion results in an increased aldosterone secretion, an increased conversion of B [1] to aldosterone, and an increased zona glomerulosa width. The importance of angiotensin II in regulating aldosterone secretion in this species has been questioned. We have studied the importance of potassium, known to increase zona glomerulosa width, in mediating this response. Male Sprague-Dawley rats were used. Oral potassium loading (0.3 M KCl in 5% dextrose) increased aldosterone secretion from control levels of 6.0 ± 1.0 ng/min (mean $\pm$ SEM) to 15.6 ± 1.9 ng/min after 2 days, and 17.3 ng/min after 8 days of potassium loading. In the 2 day interval, the conversion of B to aldosterone, assayed in isolated adrenal mitochondria, increased twofold. Rats placed on a sodium-free diet for 7–9 days secreted aldosterone at 17.3 ± 2.2 ng/min, but when potassium was omitted from the diet before (2 wk) and during the period of sodium depletion, aldosterone secretion was only 0.6 ± 1.1 ng/min. In contrast, the secretion of B was similar in the two groups. Arterial plasma [K] was then measured during dietary sodium depletion. After 8 days, plasma [K] rose from 3.7 ± 0.3 mEq/liter to 4.5 ± 0.3 mEq/liter ($P < 0.001$). After only a 30–32 hr period on the sodium-free diet, [K] increased from 3.6 ± 0.1 to 4.1 mEq/liter ($P < 0.001$). Thus dietary potassium is essential for this response to sodium depletion, plasma [K] rises during sodium depletion, and potassium loading mimics sodium depletion. We therefore conclude that potassium is an important mediator of the adrenal response to sodium depletion in the rat. (Research supported by a grant from the NIH.)

40. Studies on the Physiological Role of Thyroxine-Binding Prealbumin. LEWIS E. BRAVERMAN, THEODORE AVRUSKIN,* MICHAEL J. CULLEN,* AND SIDNEY H. INGBAR, Boston, Mass.

Although in vitro evidence suggests that thyroxine(T_4)-binding prealbumin (TBPA) is less important than the thyroxine-binding globulin (TBG) in the binding of T_4, its role in T_4 transport in vivo is uncertain. Clinical states associated with alterations in TBPA binding are generally associated with changes in TBG and with other abnormalities that obscure the effects on T_4 metabolism of changes in TBPA, per se. In normals, norethandrolone increases TBPA markedly, but also decreases TBG, preventing an evaluation

[1] Corticosterone.

of the effects of increased TBPA on T_4 metabolism. To circumvent this difficulty, we have studied peripheral T_4 metabolism before and during norethandrolone administration in four patients with a congenital lack of T_4 binding by TBG. T_4-binding capacity of TBG was negligible prior to norethandrolone and was uninfluenced by its administration, whereas that of TBPA increased greatly (mean, 283 vs. 388 μg T_4 per 100 ml). Increased TBPA caused a small (mean, 1.8 vs. 2.4 μg per 100 ml) but highly significant increase in PBI, together with a decrease in T_4 clearance, so that total daily turnover of T_4 was unchanged. Decreased T_4 clearance was accounted for mainly by a change in T_4 distribution space (TDS; mean, 18.5 vs. 11.8 liters). Concomitantly, the percentage of free T_4 in serum decreased slightly but significantly (mean, 0.072 vs. 0.062). These changes indicate that TBPA plays a distinct but small role in T_4 metabolism in the absence of TBG and suggest, therefore, that its role is even less important when TBG is present.

41. Relation between Peritubular Capillary Hematocrit and Fluid Reabsorption by the Renal Proximal Tubule. B. M. BRENNER,* J. TROY,* I. UEKI,* AND J. WARD,* San Francisco, Calif. (introduced by T. B. Bradley, Jr.).

The oncotic gradient generated across the peritubular capillary wall as a consequence of glomerular ultrafiltration appears, in part, to determine net proximal reabsorption. Since peritubular capillary hematocrit should likewise exceed that in preglomerular blood, a potential for marked changes in peritubular capillary viscosity also exists. We therefore examined the effect of induced changes in peritubular capillary hematocrit on proximal reabsorption. Before micropuncture, each of seven hydropenic rats received 5 ml of rat plasma intravenously in an isovolemic exchange for whole blood. Fluid/plasma (F/P) inulin ratios from 20 late proximal tubules, and calculated absolute reabsorption (Cd) averaged 1.60 ± 0.06 (SE) and 16.8 ± 1.8 nl/min, respectively, differing significantly ($P < 0.005$) from values from 18 tubules from nine control rats that were not exchanged (2.73 ± 0.12 and 27.2 ± 2.5 nl/min). Mean differences in nephron and kidney GFR, arterial pressure, and arterial protein concentration between groups were not significant. Peritubular capillary hematocrit in controls averaged $61.1 \pm 0.5\%$ and always exceeded simultaneously measured arterial hematocrit ($50.1 \pm 0.8\%$). After exchange these measures averaged $45.6 \pm 1.4\%$ and $34.7 \pm 0.8\%$. A second isovolemic exchange then was performed in each experimental rat, using 5 ml of a 75–85% rat RBC suspension. Peritubular capillary ($57.4 \pm 1.1\%$) and arterial ($46.9 \pm 0.6\%$) hematocrits uniformly increased. Again, despite no mean differences in GFR, arterial pressure, and arterial protein concentration, changes in reabsorption paralleled changes in hematocrit. F/P inulin ratios increased in 18 of 20 tubules (mean re-collection/initial collection ratio $= 1.24 \pm 0.04$), and Cd increased in 15 of 20 (overall average $= +28\%$). Though these data provide no insight as to the mechanism whereby changes in hematocrit lead to corresponding changes in reabsorption, preliminary data indicate that, when measured, parallel changes in peritubular capillary protein concentration also occur. (Support: Veterans Administration; NIH grant AM-13888.)

42. Rapid Reversal of Smoking-Induced Arterial Hypoxemia at Altitude. GEORGE J. BREWER, JOHN W. EATON,* ROBERT F. GROVER,* AND JOHN V. WEIL,* Ann Arbor, Mich., and Denver, Colo.

"Normal" cigarette smokers in Leadville, Colorado (altitude 10,200 feet) have a significantly reduced mean arterial P_{O_2} (P_{O_2} 53.4 ± 5.8 [SD] mm Hg vs. 58.6 ± 4.2 in Leadville nonsmokers; $P < 0.05$; $n = 20$), and a significantly higher mean level of carboxyhemoglobin ($6.6\% \pm 2.7$) than smokers near sea level ($4.7\% \pm 2.2$; $P < 0.01$; $n = 62$). Smokers in Leadville, as compared with sea-level residents, have elevated levels of erythrocyte 2,3-diphosphoglycerate but do not have the decreased hemoglobin oxygen affinity usually present at this altitude, owing to carboxyhemoglobin, which increases hemoglobin oxygen affinity. Most individuals with excessive polycythemia in Leadville are cigarette smokers. The smoking-induced arterial hypoxemia is rapidly reversed upon cessation of smoking. Of ten "normal" volunteers who stopped smoking, eight showed a marked rise in arterial P_{O_2} (mean and SD of the eight $= 7.43 \pm 3.1$ mm), without significant change in mean arterial P_{CO_2}. Two volunteers showed no change in arterial P_{O_2}. We have previously shown a similarly rapid decrease in hemoglobin oxygen affinity after cessation of smoking, with a close temporal relation to decrease in carboxyhemoglobin levels. Two possible explanations for the arterial hypoxemia induced by smoking, and its rapid reversal upon cessation of smoking, are: (1) an airway effect which is rapidly reversible; (2) a carboxyhemoglobin effect (through increased hemoglobin oxygen affinity) in the presence of a small amount of arteriovenous shunting or ventilation-perfusion disturbances in smokers (as reported by Brody and Coburn). It is not known whether arterial hypoxemia is present in "normal" smokers at lower altitudes. However, hypoxemic effects of smoking are presumably important in patients with lung disease, whose arterial P_{O_2} are analogous to those of high-altitude residents.

43. Mechanisms of Neutropenia in Rheumatoid Arthritis. LEONARD H. BRUBAKER* AND WILLIAM S. IRVIN,* Columbia, Mo. (introduced by Charles E. Mengel).

Neutropenia (<1500 cells per mm^3) is a serious complication of rheumatoid arthritis (RA). After splenectomy an increase in the neutrophil count can occur in some patients, but relapses are frequent. In the present study autologous, ^{32}P-diisopropylfluorophosphate-labeled whole blood was reinfused into four patients with RA and neutropenia. Specific radioactivity (SR) was determined in white cells isolated from blood drawn 15 min, 1, 3, 5, 10, and 24 hr thereafter. Previous studies by others have shown that the SR determined at 15 min ranges in normals from 16 to 99% of that expected from simple dilution of labeled bag volume into blood volume. The missing cells are considered to be marginated, in equilibrium with circulating cells. SR later falls off exponentially with a normal $T_{\frac{1}{2}}$ of 4–10 hr. Patients 1, 2, and 3 had palpable splenomegaly and increased marrow neutrophil cellularity. Neutrophil kinetics showed increased margination (<10% circulating cells at 15 min) in patients 1 and 2, but the $T_{\frac{1}{2}}$ was indeterminate because of extremely low counts. Patient 3 was on massive steroids, but still had

a short $T_{\frac{1}{2}}$ of 3.8 hr. His percentage of margination was normal. The patients' mechanism of neutropenia, therefore, was increased peripheral utilization of cells. Splenectomy was performed in patients 2 and 3 because they had the lowest neutrophil counts (<600/mm³) and were febrile. Counts in both rose postoperatively (maximum neutrophils, 7200 in 2 and 16,000 in 3), but fell to neutropenic levels in 2 months and 2 wk, respectively. Postoperative neutrophil kinetics showed normal margination in both, and $T_{\frac{1}{2}}$ of 4.2 hr in 2 and 3.8 hr in 3 (steroids reduced). Patient 4 had decreased marrow granulocytes and no palpable splenomegaly. Neutrophil kinetics showed normal margination (90% circulating at 15 min) and survival ($T_{\frac{1}{2}} = 4.8$ hr). Her mechanism of neutropenia was decreased production of cells. These data suggest that at least two mechanisms of neutropenia exist in RA, (1) increased peripheral removal at sites other than the spleen alone, and (2) decreased production. (NIH grant FR-5387-07.)

44. Age-Dependent Immunoglobulin Alterations in Patients Susceptible to Chronic Respiratory Disease. C. E. BUCKLEY III,* F. C. DORSEY,* AND H. O. SIEKER, Durham, N. C.

Chronic respiratory disease (CRD) is often associated with chronic or recurrent infectious bronchitis. Very little is known of the changes in immunity which could account for this susceptibility to infection. Serum concentrations of IgG, IgA, and IgM were measured in duplicate by single radial diffusion in 592 patients with CRD and in 506 apparently healthy controls ranging in age from 14 to 92 yr. Computer programs for multivariate statistical analysis and categorizing and comparing frequency differences between patients and controls were used for analysis of these data. Serum IgG, IgA, and IgM were decreased in patients aged 25–54 yr. The significances of mean and age-covariate differences observed were: IgG, $P < 0.90\%$ and $P < 0.16\%$; IgA, $P < 3.43\%$ and $P < 2.70\%$; and IgM, $P < 0.45\%$ and $P < 0.16\%$. Patients generally had hyperglobulinemia in the 55–92 yr age range. However, only IgM was significantly increased ($P < 0.35\%$, $P < 1.20\%$). A χ_2 analysis of distributional differences revealed low IgG to be the most frequent alteration observed. These data provide direct evidence of age-related alterations in immunity in the natural history of CRD. The data suggest that decreased serum immunoglobulin concentrations are related to CRD early in adult life. Late in life, surviving patients exhibit varying degrees of hyperglobulinemia primarily involving IgM. Compromised immunity early in life and compensatory hyperglobulinemia late in life may both occur in patients with CRD. (Research supported by grant AI-07617 from the NIH.)

45. The Interaction of 2,3-Diphosphoglycerate with Certain Human Hemoglobins. H. FRANKLIN BUNN* AND ROBIN W. BRIEHL, Bronx, N. Y., and Boston, Mass.

2,3-Diphosphoglycerate (2,3-DPG), present in unusually high concentration in red cells, is a potent modifier of hemoglobin function, causing a "shift to the right" in the oxygen equilibrium curve and thus an increase in P_{50}, the partial pressure of oxygen required to half saturate hemoglobin. Benesch and Benesch have shown that 2,3-DPG binds in 1:1 molar ratio to deoxyhemoglobin, and have proposed that the strongly anionic 2,3-DPG interacts electrostatically with positively charged groups on the β-chains. A comparison of the interaction of 2,3-DPG with human hemoglobin variants and minor components of known structure might provide information regarding the binding site. Spectrophotometric oxygen equilibria were carried out on "stripped" hemoglobin (5×10^{-5} M tetramer) with and without 2,3-DPG in 0.1 M NaCl, 0.05 M *bis* Tris buffer, pH 7.20, 10°C. In the presence of 2×10^{-4} M 2,3-DPG, the P_{50} of hemoglobins A, A_2, S, and C increased about twofold, indicating a substantial and equal decrease in oxygen affinity. Furthermore, hemoglobins Chesapeake and $M_{Milwaukee-1}$, which have intrinsically high and low oxygen affinities respectively, also showed a twofold increase in P_{50} under the same conditions. In comparison with these, hemoglobins A_{Ic} and F_{II} were less reactive: P_{50} 2×10^{-4} M DPG/P_{50} stripped = 1.23 and 1.20, respectively. Hemoglobin F_I showed virtually no reactivity: P_{50} 2×10^{-4} M DPG/P_{50} stripped = 1.02. The N-terminal amino of each β-chain of hemoglobin A_{Ic} is linked to a hexose. In hemoglobin F_I the N-terminal amino of each γ-chain is acetylated. The low reactivity of these hemoglobins suggests that the N-termini of the non-α-chains are involved in the binding of 2,3-DPG to hemoglobin. Models derived from the high-resolution X-ray crystallographic data of Perutz and associates show that upon deoxygenation the cleft between the β-chains widens, making the entrance of 2,3-DPG into the central cavity sterically possible. Furthermore, the N-terminal amino groups of the β-chains move together by about 4 A, allowing binding to the phosphates of 2,3-DPG. (This work was supported in part by grants HE-07451 to R. W. Briehl and HE-39262 to H. F. Bunn from the National Heart Institute.)

46. Glucagon Stimulates Thyroid Function. GERALD BURKE,* Chicago, Ill. (introduced by Eric Reiss).

Recent investigations have broadened the spectrum of biochemical influence of glucagon in animals and man. We studied effects of glucagon on sheep thyroid adenyl cyclase activity, endocytosis, glucose oxidation, and phospholipogenesis in vitro, and on mouse thyroid radioiodine uptake and release in vivo. Glucagon, 10^{-6}–10^{-4} M, caused a dose-related 3- to 5-fold increase in thyroid adenyl cyclase activity. Combinations of glucagon and maximal doses of thyrotropin (TSH) reduced enzymic stimulation below that achieved with either peptide alone, whereas combinations of glucagon or TSH and maximal concentrations of sodium fluoride were additive on cyclase. The effects of TSH and glucagon, but not of NaF, on thyroid adenyl cyclase were abolished by adrenergic blockers. Glucagon, 10^{-6}–10^{-4} M, stimulated sheep thyroid slice glucose oxidation and phospholipogenesis in a dose-related manner. Combinations of submaximal doses of TSH and glucagon produced additive effects on thyroid slice metabolism, but maximally effective concentrations were not additive. Glucagon did not stimulate endocytosis and did not modify TSH effects thereon. Glucagon stimulated mouse thyroid radioiodine uptake and release in vivo. The magnitude and time course of ^{131}I release after low doses (100 μg)

were like those after 0.05 mU TSH, whereas high doses (500 μg) of glucagon had a long-acting effect. When added to TSH or long-acting thyroid stimulator (LATS), more potent stimulators of mouse thyroid ^{131}I uptake and release, glucagon significantly reduced the TSH or LATS effect thereon. The decrease in ^{131}I release was seen at 3 hr for TSH and at 3 and 24 hr for LATS. In summary, glucagon stimulates many phases of thyroid hormone secretion and appears to do so by interaction with receptor sites shared by TSH and/or LATS. (Research supported by NIH grant AM-11136.)

47. D-Glucose Binding by a Brush Border Fraction from Rabbit Renal Tubules. DIETRICH BUSSE,* LOUIS J. ELSAS,* AND LEON E. ROSENBERG, New Haven, Conn.

The kinetics of hexose transport in bacteria and of glucose reabsorption in the intact human kidney suggest that specific membrane proteins mediate sugar transport in these systems. Such proteins have, in fact, been isolated from bacterial cells, but their existence in renal tubular membranes remains speculative. Since the rate of intracellular catabolism of glucose prohibits investigation of its uptake in kidney slices or isolated tubules, we have examined the binding of ^{14}C-D-glucose to a brush border–rich subcellular membrane fraction isolated from rabbit renal tubules by the sequential use of collagenase, EDTA, and differential centrifugation. Glucose binding increased linearly for 5 min and was greater at 37° than at 4°C. Phlorizin (0.1 mM) and D-galactose (10 mM), known competitive inhibitors of renal glucose transport, inhibited binding of D-glucose (0.01 mM) by 43% and 65%, respectively, but the unnatural isomer, L-glucose (10 mM), did not affect binding of the D-isomer. Binding was virtually abolished by parachloromercuribenzoate, implying that membrane sulfhydryl groups were required. Deletion of sodium from the incubation medium impaired binding by 30%. This sodium dependence was not mediated by Na-K ATPase, since ouabain had no effect on glucose binding. The binding process was saturable. A kinetic analysis, performed over an 8000-fold range of substrate concentrations (0.01–80 mM), suggested the presence of at least two different binding sites with widely different affinities. These studies provide the first demonstration of specific binding of D-glucose to a cell-free fraction of mammalian kidney. This fraction should be useful in characterizing the postulated membrane proteins which catalyze renal glucose transport and which may be defective in such human diseases as renal glycosuria, glucose-galactose malabsorption, and the Fanconi syndrome.

48. Heterogeneity in Assembly of Human Macroglobulins. J. BUXBAUM,* S. ZOLLA,* M. D. SCHARFF, AND E. C. FRANKLIN, New York, N. Y.

Marrow aspirates or lymph node cells from nine patients with macroglobulinemia were incubated with ^{14}C-labeled amino acids in short-term tissue cultures. Secreted and cytoplasmic material from cells lysed with Nonidet P-40, and immunologic precipitates prepared from them with antisera to μ-chains or k-chains, were examined by electrophoresis on polyacrylamide gels in sodium dodecyl sulfate. In all specimens the major intracellular immunoglobulins identified in precipitates isolated with anti-μ sera were μL, $(\mu L)_2$, and $(\mu_2L_2)5$. Pulse chase studies revealed that the major intracellular protein after 1 hr of labeling was the 8S monomer, $(\mu L)_2$. Most of the 1 hr cytoplasms also contained small amounts of μL, which may be the major precursor of the 8S subunit. After 3 hr of labeling, two distinct patterns of assembly of 19S polymer $(\mu_2L_2)5$ were noted. In two of the cultures, less than 10% of intracellular immunoglobulins existed as the fully assembled 19S polymer, while in cells from five individuals the bulk of intracellular immunoglobulins was 19S polymer. In the two remaining samples only 8S monomer was seen after 1 hr. Intermediates between 8S monomer and fully assembled 19S polymer were sometimes seen. Regardless of the nature of the intracellular protein, the major constituent in secreted material was the 19S polymer with little or no 8S monomer. The ability to assemble intracellular 19S γM did not correlate with L or μ-chain type. It would then appear that there may be several pathways for assembly of the $(\mu_2L_2)5$ 19S polymer. In some cultures this may occur just before or at the time of secretion, whereas in others it may occur earlier. The mechanisms underlying these differences remain to be determined. (Supported by the NIH and the Health Research Council of the City of New York.)

49. Regulation of Antibody Formation by Serum Antibody. JEAN-CLAUDE BYSTRYN,* MARTIN W. GRAF,* AND JONATHAN W. UHR, New York, N. Y.

We have studied a possible "feedback" role of serum antibody by removing it specifically from immunized rabbits. This was accomplished by injecting rabbits intravenously with two immunologically unrelated bacteriophages in saline, and 3–6 wk later, when serum antibody levels were falling, by performing exchange transfusion with blood of rabbits immunized with only one of the bacteriophages. By this technique, serum levels of one antibody were reduced by 50–84% in eight rabbits studied, whereas the levels of the other (control) antibody were maintained. Subsequently, there was an increase, usually biphasic, in the levels of the depleted antibody. Peak titers were usually achieved 8 days after exchange and, unexpectedly, were 148–322% of their preexchange levels. In contrast, the levels of the control antibody continued to decrease slowly. The rebound was due primarily to increased levels of 7S Ig as determined by ultracentrifugation in a sucrose gradient and by susceptibility of antibody activity to mercaptoethanol. The rebound was not caused by redistribution of antibody from extravascular sites. Thus, antibody to a third bacteriophage which was passively administered several days before the exchange in four of these animals was redistributed within 36 hr and could account for 25% or less of the observed rebound. We therefore interpret the rebound as caused by an increased rate of specific antibody formation. These findings indicate that serum antibody plays a major role in regulating the rate of antibody formation to conventional antigens. (Supported by grants from the NIH and the NSF.)

50. Cholestyramine Enhances Digitalis Excretion and Protects against Lethal Intoxication. JAMES H. CALDWELL* AND NORTON J. GREENBERGER,* Columbus, Ohio (introduced by Arnold M. Weissler).

It has been established that digitalis glycosides undergo significant biliary excretion and intestinal reabsorption. We have confirmed this by demonstrating in rats that after the intraduodenal administration of ^{3}H-digoxin or ^{3}H-digitoxin, 45–50% of the labeled glycoside is excreted in the bile in 24 hr. Theoretically, interruption of the enterohepatic circulation should lead to decreased digitalis blood levels and a shortened physiological effect, and might also prevent mortality from lethal doses. To test these hypotheses, studies were carried out using cholestyramine, an anion exchange resin known also to bind neutral sterols. In vitro, at a glycoside concentration of 50 μg/ml, cholestyramine (40 mg/ml) bound 80% of the available ^{3}H-digitoxin and 50% of the ^{3}H-digoxin. Binding was independent of pH and temperature. Subcutaneous injection of digitoxin (10 mg/kg) caused death in 100% of the rats (20 of 20) and guinea pigs (10 of 10). By contrast, pretreatment with cholestyramine (80 mg p.o. before injection) resulted in 70% survival in rats (7 of 10) and 90% survival in guinea pigs (9 of 10); cholestyramine given after injection of digitoxin resulted in 22% survival in rats (4 of 18). In cholestyramine-treated rats, there was significantly increased fecal excretion of ^{3}H-digitoxin as compared with controls. In 10 normal human subjects, cholestyramine treatment resulted in a decreased metabolic half-life of orally administered ^{3}H-digitoxin (10 vs. 6½ days). These data indicate that cholestyramine can bind appreciable amounts of digitoxin in vivo and in vitro and protect against lethal doses of digitoxin in experimental animals. The effect is probably mediated by interruption of the enterohepatic circulation of cardiac glycosides and ultimately might prove to be of value in the treatment of digitalis-induced arrhythmias in man.

51. Regional Myocardial Perfusion in Man. PAUL J. CANNON,* RALPH B. DELL,* AND EDWARD M. DWYER, JR.,* New York, N. Y. (introduced by John H. Laragh).

We have devised a new method for quantitating the perfusion of various regions of the myocardium in man by measuring the clearance constants (k) of xenon-133 washout from multiple areas of myocardium with a multiple-crystal scintillation camera. In 29 patients, Xe-133 was injected into the right and/or left coronary artery and counts per second were recorded simultaneously from each of 294 scintillation crystals viewing the precordium through a multichannel collimator. The radii of view of each crystal at 3, 5, and 8 cm distances were 0.6, 0.8, and 1.3 cm. Count overlap from adjacent crystals was 3, 3, and 16% respectively. The slopes (k) and the SD of the initial monoexponential segment of the isotope washout curves were calculated by the method of least mean squares for each crystal on an IBM 360/91 computer. Myocardial blood flow was also calculated assuming a partition coefficient of 0.72. In each study the pattern of myocardial perfusion was superimposed over a tracing of the patient's coronary arteriogram; appropriate alignment was achieved by use of radioactive and radiopaque markers. In 14 patients with normal coronary arteries, k obtained from crystals overlying the left ventricle (LV) in any one subject varied by a mean of 15% (coefficient of variation) (range 12–18%). Mean LV flows averaged 68 ± 15 (SD) ml/100 g per min (range 51–92) and significantly exceeded those of right ventricles (49 ± 12 ml/100 g per min) and atrial regions (38 ± 11 ml/100 g per min). In patients with angiographically abnormal coronary arteries, k obtained from LV in each individual varied 23%, indicating nonuniformity of flow (range 14–39%). In eight subjects, k recorded from crystals overlying areas of myocardium distal to >75% arterial constrictions were significantly reduced, whereas in seven with <75% constrictions or abundant collaterals, regional perfusion was maintained. The data demonstrate heterogeneity of myocardial perfusion in coronary artery disease. This approach permits for the first time assessment of collateral flow and localization of areas of reduced capillary perfusion to discrete lesions of coronary vessels. (Research supported by grants from the NIH.)

52. Burkitt's Tumor: A Comparative Study of the Disease in the United States and Africa. PAUL P. CARBONE,* JOHN L. ZIEGLER,* CLARENCE H. BROWN,* RICHARD MORROW,* AND COSTAN BERARD,* Bethesda, Md., and Kampala, Uganda (introduced by C. Gordon Zubrod**).

Burkitt's tumor is a lymphoreticular neoplasm found commonly in central Africa and rarely in the United States. Using similar histopathologic criteria at the National Cancer Institute and Makerere University, 57 Ugandan children (UBT) and 16 American patients (ABT) were classified as Burkitt's tumor and treated with cyclophosphamide (CYT) 40 mg/kg every 3 wk for one to six doses. An additional six patients in Uganda (ULS) and four in the United States (ALS) were classified as lymphoblastic lymphosarcoma and treated similarly. Males predominated in all groups, and the median ages were: UBT, 7 yr; ABT, 10; ULS, 9; and ALS, 9. Facial and abdominal tumors were presenting signs in all groups, with mediastinal lymphadenopathy common to the ALS group primarily. Leukemic transformation occurred in 4 of 16 ABT and 4 of 4 ALS. Complete tumor regressions occurred in 74% UBT, 57% ABT, 33% ULS, and 25% ALS. The proportions of patients surviving 1 yr in remission with no maintenance therapy are 35% UBT, 44% ABT, 33% ULS, and 0 ALS. Burkitt's tumor can be diagnosed in the United States and Africa and differentiated from childhood lymphosarcoma. The responses to CYT in the United States and Africa are comparable. The proportion of long-term responders to CYT is equally impressive for UBT and ABT, making the diagnosis of Burkitt's tumor of therapeutic and prognostic value.

53. Different Activities of Anticellular Antibody. FRANCIS J. CAREY* AND DEAN L. MANN,* St. Louis, Mo., and Bethesda, Md. (introduced by Carl G. Harford**).

HeLa cells infected with parainfluenza virus type II lyse when incubated with antibodies against uninfected HeLa cells. Lysis of infected cells is not complement dependent and occurs with concentrations of antibody less than that re-

quired to lyse similar numbers of uninfected cells with complement. These observations suggest that anticellular antibody might contain antibodies with different activities. To investigate this possibility, antisera were prepared in rabbits against whole HeLa cells (WC-Ab) and an isolated antigenic component of HeLa cell membrane (I-Ab). This membrane component was solubilized with papain and separated by column chromatography. Both WC-Ab and I-Ab lysed uninfected HeLa cells with complement. However, only WC-Ab lysed parainfluenza-infected cells without complement. Both antisera cross-reacted with cultured human lymphoid cells. Absorption of the IgG fraction of WC-Ab with lymphoid cells completely removed the cytolytic antibody for lymphoid cells and decreased the complement-dependent cytolytic antibody for uninfected HeLa cells. The WC-Ab required to lyse approximately 10^6 HeLa cells increased from 0.2 mg to 0.6 mg. However, absorption with lymphoid cells did not reduce the cytolytic activity for infected cells. 0.15 mg of absorbed and unabsorbed IC-Ab lysed 10^6 infected cells. Thus WC-Ab contained at least two populations of antibodies. One lysed parainfluenza-infected HeLa cells and was not absorbed by lymphoid cells. The second was absorbed by lymphoid cells and might correspond to I-Ab. I-Ab did not lyse infected HeLa cells, but did lyse uninfected HeLa cells and cultured lymphoid cells with complement. These studies suggest that antibodies against different antigenic components of the cell membrane might alter structure or function differently, alterations resulting in lysis with complement in one instance and with virus infection in another. These findings also suggest another mechanism for "autoimmune disease," i.e., antibody-mediated injury of virus-infected cells. (Research supported by grants from the NIH.)

54. Connective Tissue "Activation" In Vitro. C. WILLIAM CASTOR, Ann Arbor, Mich.

A polypeptide fraction extracted from human leukocytes and fibroblasts induced metabolic hyperactivity in cultured human synovial cells resembling that in chronic rheumatoid synovitis. The altered metabolic activity included overproduction of hyaluronic acid and lactic acid, and increased glucose consumption. Connective tissue "activator" was demonstrated in extracts of human lymphocytes, polymorphonuclear leukocytes, platelets, spleen, liver, lymph node, skeletal muscle, and rheumatoid synovial membrane. A similar "activator" was extracted from cells grown in tissue culture, including normal and rheumatoid synovial cells, J-111 cells, Chang liver cells, and HEp-2 cells. Transformed human synovial cells, a hamster kidney strain, and the mouse L cell had no measurable activity. The "activator" material is destroyed by proteolytic digestion, and gel filtration indicates a molecular weight between 4000 and 10,000. CM-Sephadex C-25 retarded the "activator" on columns, suggesting that it is cationic at neutral pH. Iodoacetamide and *p*-chloromercuribenzoate destroy the biologic activity of the "activator," whereas dithiothreitol is without adverse effect, suggesting that sulfhydryl groups are vital to the biological function of this protein. Target fibroblasts respond to "activator" in a simple salt medium, suggesting that endogenous substrates are adequate to initiate the activation process. Addition of glucose, mannose, or glucosamine to the saline medium along with "activator" supports even greater metabolic stimulation. With galactose or fructose as substrate, "activator" causes a marked increase in hyaluronate formation but virtually no change in hexose uptake or lactate formation. These data indicate that the hyaluronate-stimulating effect of the "activator" substance is separable from and not solely dependent on stimulating energy metabolism in the target cell. The connective tissue "activator" substance may (1) have a central role in regulating the progression of the inflammatory reaction from the exudative to the reparative phase and (2) be important in perpetuating chronic inflammatory states.

55. Impaired Cellular Immunity in the Pathogenesis of Amyloidosis. EDGAR S. CATHCART,* MICHAEL F. MULLARKEY,* AND ALAN S. COHEN, Boston, Mass.

Though there is circumstantial evidence implicating immunologic dysfunction in amyloid disease, most data have precluded an etiologic role for circulating antibody and none are available concerning the role of delayed hypersensitivity. Therefore, to assess directly cellular immune function in the genesis of amyloidosis, the following experiments were performed. 12 Hartley guinea pigs were sensitized simultaneously to diptheria toxoid (Dpd) and casein in complete Freund's adjuvant by footpad injection, then tested by the in vitro macrophage inhibition test of David and associates. Macrophage inhibition of more than 60% to both antigens ($P < 0.01$) developed within 1 wk and persisted for 4 wk. A second series of animals were similarly sensitized to both antigens but also received 1 ml 10% casein subcutaneously, three times per week for periods ranging from 1 to 4 wk. These showed significant macrophage inhibition to Dpd by week 1 and to casein by week 2. However, by week 4 macrophage inhibition to both Dpd and casein was completely abolished. Finally, 24 guinea pigs were given multiple subcutaneous casein injections for periods varying between 8 and 52 wk before footpad immunization with Dpd and casein. Groups of four animals were sacrificed bimonthly for tissue examination by Congo red staining, and for evaluation of their response to Dpd and casein by macrophage inhibition. This last regimen produced increasing amounts of amyloid in the tissues at week 24 and thereafter. It was also found that unresponsiveness to casein persisted throughout the entire experimental period, whereas sensitivity to Dpd was not inhibited. These experiments provide the first clear evidence that impaired cellular immunity is present during the induction of amyloidosis and may contribute to its pathogenesis. (Research supported by grants AM-04599 and T1-AM-5285 from the National Institute of Arthritis and Metabolic Diseases.)

56. Therapeutic Effects of L-Carnitine in Experimental Intoxication with Diphtheria Toxin. DAVID R. CHALLONER,* WILLIAM C. ELLIOTT,* AND ISIDORE MANDELBAUM,* Indianapolis, Ind. (introduced by John B. Hickam**).

A significant factor in the mortality of infection with *Corynebacterium diphtheriae* is myocarditis produced by

diphtheria toxin (DT) and associated with fatty infiltration and carnitine (C) depletion. The present study was undertaken to determine whether exogenous carnitine might protect against the mortality of DT. Standard probit procedures for determination of LD_{50} were carried out by injecting 24 groups of six guinea pigs each (250–300 g) with DT and assessing mortality after 4 days, with and without 25 mg C, i.p. twice daily. Control LD_{50} was 172 mμl of DT (95% limits, 127–232) vs. 345 mμl (95% limits, 272–438) in the C-treated animals; potency ratio, DT/DT + C = 2.06 (95% limits, 1.4–2.9). Survival in guinea pigs was plotted after injection of 2 LD_{50} of DT ± C, 50 mg twice daily for 4 days. Of 114 DT animals, only 35% survived 4 days, 20% 8 days, and 20% 14 days. In 89 DT + C animals, 86.5% survived during the 4 days of treatment, 62.5% 8 days, and 47.5% 14 days. Pathology revealed myocardial necrosis and inflammation with some fatty infiltration. On the hypothesis that the survival effect of C was on the myocardium, dogs were injected with 30 LD_{50} DT i.v. and cardiovascular function was assessed 2–4 days later, before and after 1500 mg C i.v. The "sick" animals were hypotensive with low cardiac output. In three out of three closed-chest animals, arterial mean and pulse pressure, initially low, returned to normal levels within 30 min. In four open-chest dogs, C improved left ventricular function curves. In healthy dogs, in one dog with acute myocardial depression secondary to pentobarbital, and in one dog with chronic AV fistulae and failure, no effect of C was seen, suggesting that the carnitine effect may be specific. After tracheostomy, antibiotics, and antitoxin, the treatment of diptheria is only supportive. The present results in experimental animals provide evidence, for the first time to our knowledge, for an effect of C or any agent in decreasing the mortality of DT and in acutely improving the depressed cardiovascular function of diptheritic animals.

57. Acute Buffering of Carbon Dioxide by Tissues In Vivo. NEIL S. CHERNIACK,* NORMAN H. EDELMAN,* PETER G. TUTEUR,* AND THEODOR J. TRUEB,* Philadelphia, Pa. (introduced by Alfred P. Fishman**).

At equilibrium, each tissue has its own distinctive CO_2 buffering capacity. Attempts to use these steady-state buffering capacities to predict the rate of blood P_{CO_2} rise during acute exposure to CO_2 have been unsuccessful. The discrepancy might be explained by the failure of some or all of the tissues to exert their full buffering capacity during brief exposures to CO_2. This hypothesis was tested in 16 dogs and 6 goats by determining in vivo buffering capacity, (Δ tissue CO_2 concentration)/(Δ venous P_{CO_2}), in three different tissues—brain, spleen, and muscle—during transient states of CO_2 retention produced by apneic oxygenation or by ventilation with 3% CO_2. To determine CO_2 production, uptake, and buffering capacity, we analyzed arterial and venous blood from each organ for CO_2 content and tension and measured continuously arterial inflow (in the spleen, venous outflow as well) using electromagnetic flow probes. In goats, by ligating extracranial branches of the internal maxillary artery we could monitor cerebral blood flow with an electromagnetic flow probe. By 6 min of apneic oxygenation, the buffering capacities of the brain and spleen were 3.61 ± 0.67 and 4.22 ± 0.44 ml/kg per mm Hg P_{CO_2}, respectively, the same as their steady-state buffering capacity. The buffering capacity of muscle was much less (0.97 ± 0.19 ml/kg per mm Hg P_{CO_2}), but by 30 min of CO_2 exposure it had reached about the same values as were obtained for brain and spleen. Limited CO_2 diffusion or unequal distribution of perfusion within muscle is theoretically an unlikely explanation for the initially low CO_2 buffering capacity of muscle and its subsequent increase. Results are best explained if, unlike that in spleen or brain CO_2 buffering in muscle is rate limited by a chemical process (e.g., caused by carbonic anhydrase lack). (Supported by NIH grant 12962.)

58. Lipid Metabolism in the Human Fatty Streak Lesion. ARAM V. CHOBANIAN, FRANCISCO MANZUR,* AND ROBERT D. LILLE,* Boston, Mass.

The possible role of arterial metabolism in lipid accumulation in the early fatty streak lesion has been examined in arteries obtained from patients dying suddenly from accidental causes. Adjacent areas of normal intima and fatty streaks were incubated with 1-^{14}C-acetate and the incorporation of acetate to lipid was measured over a 3 hr period. Significantly greater incorporation of acetate to lipid was observed in fatty streaks than in adjacent normal intima when compared either on the basis of tissue weight (mean increase 123%) or on that of DNA content (mean increase 156%). All lipid classes and particularly the esterified fatty acids participated in the augmented lipid synthesis in the fatty streak. The major fatty acid radioactivity was recovered with palmitic acid and with an unidentified acid of chain length apparently greater than 20 carbons. The greatest relative increase in acetate incorporation occurred in the cholesterol ester fraction, where 2- to 10-fold greater incorporation rates were observed in the fatty streak than in normal artery. These radiochemical findings were similar to the results of chemical analyses, which demonstrated a 2- to 12-fold increase in cholesterol esters in the fatty streak. The relative concentration of cholesterol oleate was increased and that of cholesterol linoleate decreased in the fatty streak as compared with normal intima. These in vitro studies have demonstrated an apparent increase in lipid synthesis in the human fatty streak lesion. The findings suggest an important role of arterial metabolism in the unusual accumulation of cholesterol esters in the fatty streak. (Research supported by grants HE-12869-01 and HE-07299-09 from the NIH.)

59. The Thyroid Gland in Graves' Disease: Victim or Culprit? INDER J. CHOPRA* AND DAVID H. SOLOMON,** Torrance, Calif.

Hyperthyroidism in Graves' disease has been attributed to long-acting thyroid stimulator (LATS). Preliminary data showing dissociation of thyroid nonsuppressibility and serum LATS during antithyroid drug treatment have weakened this assumption. Accordingly, we examined two other corollaries of the hypothesis that LATS causes hyperthyroidism: (a) the degree of hyperthyroidism should correlate with serum LATS; (b) failure of correlation between suppressibility and serum LATS should be explicable by the presence or

absence of TSH. LATS assays were performed on 10-fold IgG concentrates of serum. (1) Thyroid function was assessed in 30 untreated hyperthyroid patients, divided into four groups based on serum LATS concentration (<0.13, 0.13–0.40, 0.41–0.80, >0.80 mU/ml). There was no significant relation between serum LATS and serum thyroxine, free thyroxine, T_3 resin uptake, 20 min or 24 hr ^{131}I uptake, or thyroid weight estimated from scintillography. (2) During antithyroid drug treatment, serum TSH became detectable on 28 occasions and remained undetectable on 45. When detectable, it invariably fell markedly after 7 days of T_3 administration. Suppressibility of 20 min thyroid ^{131}I uptake was, however, only slightly greater (statistically insignificantly) when TSH was initially detectable than when it was not. This was true whether or not LATS was detectable. On the basis of these and previous data, we conclude that the return of thyroid suppressibility during antithyroid drug treatment is determined neither by disappearance of LATS nor by reappearance of TSH in the serum. In addition, serum LATS in untreated hyperthyroidism correlates neither with thyroid size nor with thyroid function. These findings suggest that the primary fault in Graves' disease is an intrinsic thyroid abnormality which causes autonomous hyperactivity and, paradoxically, unresponsiveness to LATS, produced presumably as a result of the same intrinsic abnormality. (Research supported by grants from the NIH and the John A. Hartford Foundation.)

60. Necrotizing Angiitis in Young Adult Drug Addicts. B. P. Citron,* M. Halpern,* M. McCarron,* R. McCormick,* and B. J. Haverback, Los Angeles, Calif.

We have observed five drug addicts with necrotizing angiitis, a rare systemic disease of polymorphic clinical manifestations characterized histologically by inflammation and fibrinoid necrosis of small and medium-sized arteries. The two females and three males, ages 18–30, had employed the entire spectrum of stimulants, depressants, hallucinogens, and narcotics. All had injected metamphetamine and one had used it exclusively. The diagnosis of necrotizing angiitis in the fifth case, asymptomatic except for headache, was made during an evaluation of hypertension. Classical vascular changes consisting of small aneurysms and segment arteritis were noted in the kidneys in all patients by angiography or at pathologic examination. Similar lesions were observed in the arteries of the liver and pancreas in the two patients who had selective visceral angiography. Postmortem findings revealed generalized arterial changes of differing age including chronic and healed lesions in medium-sized and small arteries. It has been suggested that necrotizing angiitis represents a basic type of hypersensitivity reaction. It has occurred during treatment with sulfonamides, penicillin, iodine, thiourea, and diphenylhydantoin sodium. Metamphetamine appears to be the etiologic agent in our cases. However, a conclusion regarding the role of a particular drug in the pathogenesis of hippie necrotizing angiitis is premature owing to the multiplicity of injected substances and the high probability of contamination in "homemade" drugs. Additional study is required to clarify the specific role of metamphetamine in this disease.

61. Mutation Rate of Immunoglobulin-Producing Mouse Myeloma Cells In Vitro. Philip Coffino* and Matthew D. Scharff, New York, N. Y.

We have previously described a method for detecting, quantitating, and recovering rare variants of immunoglobulin-producing cells from continuous cultures of mouse myeloma cells. Cells are cloned in soft agar, and after colonies have developed, they are overlaid with specific antisera against either mouse light (L) or heavy (H) chains. The original culture contained primarily cells secreting both H and L chains (7S Ig2b). Variants which secrete only L chains can be visually identified, enumerated, and recovered from the agar to be grown up and then characterized biochemically. The rate of conversion from H + L chain production to L chain production only was determined to be 1.1×10^{-3}/cell per generation by fluctuation analysis (Luria and Delbruck). The "variance" calculated from an analysis of 40 colonies indicated that the mutation was spontaneous rather than induced by the assay. None of 23,000 L chain–producing clones showed reversion to H chain production, indicating a maximum reversion rate of 4×10^{-5}/cell per generation. Conversion of H + L chain producers to cells which produce neither H nor L chains producers to cells which produce neither H nor L chains (nonproducers) has not yet been detected. However, of 1000 L chain–producing clones, one lost its ability to produce both chains. Mutagenesis with nitrosoguanidine resulted in 6 out of 1000 L chain–producing colonies' becoming nonproducers. These findings suggest that there may be a stepwise loss of immunoglobulin chain production. However, the data do not indicate whether this is due to point mutation, chromosome rearrangement, cytoplasmic changes, or some other mechanism. (Research supported by grants from the NIH, the NSF, and the American Cancer Society.)

62. Arteriovenous Shunt and Capillary Blood Flow in the Finger. Jay D. Coffman, Boston, Mass.

Human cutaneous arteriovenous shunt (A-V) flow, which is important in body temperature regulation and possibly in certain diseases, and its relation to capillary flow have not been studied owing to a lack of appropriate methodology. In this study, the quantitation and control of A-V and capillary flow in the fingertip during reflex sympathetic nerve stimulation (total body cooling) or adrenergic nerve transmitter administration was investigated. Capillary flow was measured by the disappearance rate of a local depot of $Na^{131}I$, and total flow by venous occlusion plethysmography. Subtraction of capillary from total flow yielded A-V flow. In 10 subjects, total finger, A-V, and capillary flow in a 28.3°C room averaged 60, 49.8, and 10.2 ml/100 ml tissue per min respectively, and, after body cooling at 20°C, 33, 25.7, and 7.3 ml/100 ml. Only decreases in total and A-V flow were significant ($P < 0.01$). In five subjects, toe capillary flow averaged 10.5 ml/100 ml in the warm room and 4.0 ml/100 ml in the cool room ($P < 0.01$). In eight subjects, norepinephrine (0.125–0.25 μg/min) infused into the brachial artery decreased total, A-V, and capillary flow from 73, 65, and 8 ml/100 ml respectively to 35, 30, and 5 ml/100 ml. All three flows were significantly decreased ($P < 0.02$), although A-V flow decreased more than capillary flow. In

summary, A-V shunt flow averaged approximately 80% of total flow and decreased significantly in response to reflex sympathetic nerve stimulation and the sympathetic neurotransmitter, whereas capillary flow averaged about 20% of total flow and decreased significantly only with norephinephrine. Toe capillary flow was decreased significantly and consistently by reflex sympathetic stimulation. We conclude that sympathetic nervous system activity exerts its greatest effect on finger A-V shunt flow rather than on capillary flow, and therefore nutritional flow is not adversely affected. (Research supported by a grant from the American Heart Association.)

63. Sequenced External Counterpulsation in Cardiogenic Shock. LAWRENCE S. COHEN,* JERE H. MITCHELL, CHARLES B. MULLINS,* AND HAROLD D. ALSOBROOK,* Dallas, Texas.

Sequenced external counterpulsation to the extremities is a noninvasive ventricular assist technique for the therapy of cardiogenic shock. By means of this device external pressure is applied during ventricular diastole to cuffs located from distal to proximal sites on each extremity. This causes return of arterial blood to the central circulation, which augments central aortic pressure and possibly coronary flow. The extremity cuffs are rapidly deflated just before ventricular systole so that peripheral resistance is lessened, thereby decreasing left ventricular afterload. The cardiovascular effects of this method have been studied in anesthetized baboons with normal blood pressure and in cardiogenic shock, and in awake humans with normal blood pressure. During sequenced external counterpulsation in six baboons not in cardiogenic shock, cardiac output rose between 5 and 53% (average 25%). The ratio of diastolic to systolic area in the aortic pressure tracing changed from 0.47 at control to 0.97 during sequenced pulsation. Cardiogenic shock was induced by the injection of mercury (0.2–0.5 ml) selectively into the left coronary artery in 12 baboons. During sequenced pulsation cardiac output rose between 1 and 29% (average 17%). The ratio of diastolic to systolic area in the aortic pressure tracing changed from 0.57 at control to 1.54 during sequenced pulsation. In three normal volunteers cardiac output rose between 6 and 11% (average 8.3%). The ratio of diastolic to systolic area in the aortic pressure tracing changed from 0.46 at control to 0.79 during sequenced pulsation. External sequenced counterpulsation can augment cardiac output and aortic diastolic pressure and may prove useful in the therapy of cardiogenic shock. (Research supported by grants from the NIH and the Dallas Heart Association.)

64. Detection of Australia Antigen Au(1) and anti-Au(1) in Human Serum by Radioimmunoprecipitation with Iodide-125-Labeled Au(1). JOHN A. COLLER* AND IRVING MILLMAN,* Philadelphia, Pa. (introduced by Baruch S. Blumberg).

The association of Australia antigen (Au(1)) with viral hepatitis is well documented; it probably represents an antigenic determinant of the causative agent. This investigation was undertaken to develop a technique more sensitive than immunodiffusion (ID) or complement fixation (CF) for detecting Au(1) and anti-Au(1). Au(1) was purified by the method of Millman and associates from enzymatically treated plasma by column gel filtration and sucrose and cesium chloride density gradient ultracentrifugation. Iodide-125 was conjugated to purified Au(1). The assay of anti-Au(1) involves coprecipitation of soluble immune complexes of labeled Au(1) and human anti-Au(1) with rabbit anti-human IgC; the radioactivity in the precipitates is counted. Sera containing anti-Au(1) of CF titers $\leq 1{:}32$ were tested by radioimmunoprecipitation. Equivalence titers of 1:5000 and 1:20,000 were measured. Antibody was still detectable at 1:320,000. Less than 0.1 mμg of Au(1) was detectable by a coprecipitation-inhibition method. Nine patients with acute viral hepatitis, without anti-Au(1) or Au(1) as tested by ID or CF, were tested by radioimmunoprecipitation; three had Au(1) and four had anti-Au(1). Radioimmunoprecipitation permits (1) detection of anti-Au(1) antibody and Au(1) not detected by CF or ID; (2) detection of the simultaneous presence of Au(1) and anti-Au(1); and (3) the quantitative estimation of Au(1). To our knowledge, this is the first use of radioimmunoprecipitation in the clinical detection and quantitation of a virus-associated antigen. This concept and technique may have wide application in basic and clinical viral investigations beyond the scope of the current Australia antigen study. (This paper was supported in part by the NIH [GM-1540-04], by USPHS grants CA-06551, CA-08069, CA-06927, and by an appropriation from the Commonwealth of Pennsylvania.)

65. Autonomic Effects on Plasma Renin Activity in Hypertension. R. D. COLLINS,* M. H. WEINBERGER,* C. GONZALES,* G. NOKES,* AND J. A. LUETSCHER,** Stanford, Calif.

The intact autonomic nervous system is necessary for normal regulation of the plasma renin activity (PRA). The response of PRA to slow hemorrhage and sodium depletion is blunted in the denervated kidney. Patients with autonomic insufficiency may have impaired ability to respond to the upright posture with increased PRA. Norepinephrine causes release of renin from isolated juxtaglomerular cells. We have studied 34 hypertensive patients and five normotensive controls on a high-sodium diet, on a low-sodium diet, and with addition of chlorothiazide for further sodium depletion. PRA and norepinephrine excretion (NE) have been measured. In 23 hypertensive patients, PRA and NE increased normally during progressive sodium depletion. PRA and NE increased further when the patient stood. Those hypertensive patients whose PRA and NE were within the normal range had a significant positive correlation of PRA and NE at all levels of sodium intake and posture. In 11 patients PRA was subnormal at all levels of sodium intake and failed to increase normally with standing. In these 11 patients the increases in NE after sodium depletion and standing were smaller than those observed in the other hypertensive patients or the normotensive controls. The correlation between PRA and NE was insignificant on both high and low sodium intake and became positive only after sodium depletion. All groups of hypertensive patients lost compara-

ble amounts of sodium and had a similar fall in blood pressure. The failure of PRA to increase in the low-renin group appears to be related to lessened autonomic activity. There is no other evidence of an organic lesion of the autonomic nervous system. The findings could be related to reported expansion of extracellular volume in the low-renin hypertensives.

66. Whole-Mount Electron Microscope Study of Human Meiotic Chromosomes. DAVID E. COMINGS AND TADASHI A. OKADA,* Duarte, Calif.

A single-cell suspension of testicular cells was prepared by gentle dispersion of biopsy specimens from males without testicular disease. These cells were spread on a trough of distilled water, picked up on grids, stained with uranyl acetate, passed through ethanol washes to amyl acetate, dried in air or in the critical-point apparatus of Anderson, and examined by electron microscopy. Before fixation some cells were treated with DNase, RNase, or Pronase. The following observations were made: (1) The synaptonemal complex (SC) was clearly visualized and remained essentially unaltered after extensive DNase treatment had removed all the chromatin. Of especial importance, the central element, frequently considered to be the pairing surface of homologous DNA, was unaltered, indicating that it was not composed of DNA or chromatin. (2) The lateral elements of SC were composed of two protein filaments. (3) During leptonema, homologous axial lateral elements, although unpaired for most of their length, attached next to each other at the nuclear membrane. (4) Chromatin fibers attached in clusters to the lateral element, producing the chromomeres seen by light microscopy. (5) The chromatin fibers extended out from the SC as loops, producing a typical lampbrush-like configuration. Lampbrush chromosomes are thus not restricted to oogenesis, but occur during spermatogenesis as well. (6) Pairing is best visualized as a two-step process consisting first of chromosomal pairing, during which the proteinaceous SC pulls homologous chromosomes into approximate association with each other, and then of molecular pairing, which probably takes place in a random fashion by base pairing of short stretches of homologous single DNA strands, in the region around the SC (i.e., not at the central element) in a manner analogous to that observed in bacteriophage and bacteria. (Research supported by grant GM-15886 from the NIH.)

67. Effect of Pacing-Induced Tachycardia on Myocardial Force-Velocity Relations in Man. C. RICHARD CONTI,* JOHN D. GRABER,* WALTER D. GUNDEL,* AND RICHARD S. Ross, Baltimore, Md.

Ventricular function was evaluated in seven patients with severe coronary artery disease prior to selective coronary arteriography. Simultaneous high-fidelity left ventricular pressures (catheter tip manometer) and dp/dt were recorded at resting heart rates and during pacing-induced tachycardia (PIT). A bipolar electrode catheter positioned in the right atrium was used to increase heart rate by stages to 130 beats per minute or more. Force-velocity curves were constructed by plotting V_{CE}, defined as (dp/dt)/kp, against isovolumic pressures at control rates and at varying paced rates up to 160 beats per minute. From these curves, peak V_{CE} and extrapolated V_{max} were obtained. Peak V_{CE} and V_{max} initially rose with PIT in six of the seven patients studied. Two of these patients developed typical angina pectoris (Typ AP) during PIT. During this ischemic period there was a fall in peak V_{CE} and V_{max} despite continued pacing at a constant or increasing rate. In the five patients who did not develop Typ AP with PIT, peak V_{CE} and V_{max} continued to rise or remained constant with increasing heart rate. These observations indicate that peak V_{CE} and V_{max} normally increase with increasing heart rate but decrease when myocardial ischemia is produced. These changes may reflect changes in myocardial contractility, although other factors, such as changes in the series elastic properties of the myocardium, or changes in the symmetry of ventricular contraction, may be playing a role. There was good correlation between clinical assessment of the patients' exercise tolerance and results of force-velocity relations obtained during PIT. (Research supported by grant HE-05584 from the NIH.)

68. Complete Deficiency of Leukocyte Glucose-6-Phosphate Dehydrogenase with Defective Bactericidal Activity. MILES R. COOPER,* LAWRENCE R. DECHATELET,* MARIANO F. LAVIA,* CHARLES E. MCCALL, CHARLES L. SPURR,* AND ROBERT L. BAEHNER,* Winstom-Salem, N. C., and Boston, Mass. (introduced by Manson Meads**).

A 52-yr-old Caucasian female (F.E.) had hemolytic anemia, a leukemoid reaction, and fatal sepsis due to *Escherichia coli.* Leukocyte ingestion was normal. Granule hydrolase and peroxidase activities were normal. These enzymes shifted normally to the phagocytic vacuole, as observed by light and electron microscopy. Quantitation of degranulation was also normal. However, ingested *Staphylococcus aureus, E. coli,* and *Serratia marcescens* (peroxide-negative bacteria) were not killed, whereas an H_2O_2-producing bacterium, *Streptococcus faecalis,* was killed normally. Neither reduction of nitro blue tetrazolium nor activation of hexose monophosphate shunt (HMPS) activity (1-^{14}C-glucose to $^{14}CO_2$) occurred during phagocytosis, and H_2O_2 production (^{14}C-formate to $^{14}CO_2$) was very low. These observations suggested the diagnosis of chronic granulomatous disease (CGD). However, in contrast to control and CGD leukocytes, glucose-6-phosphate dehydrogenase (G6PD) activity was completely absent in F.E. leukocytes, whereas NADH oxidase and NADPH oxidase activities were both normal. A peroxide generation system restores HMPS activity to CGD cells, but as much as 2 mM methylene blue did not stimulate the HMPS in F.E. cells, nor was H_2O_2 production enhanced. Thus F.E. and CGD cells share a common bactericidal defect: they do not produce H_2O_2. The metabolic basis of their defects differs. CGD cells lack the oxidase which produces H_2O_2; F.E. cells have normal oxidase activity but fail to produce NADPH owing to complete G6PD deficiency. These data show that adequate NADPH production is essential for the accumulation of sufficient reduced pyridine nucleotide, the substrate for the H_2O_2-producing oxidase in leukocytes. (Supported by NIH grants RR-05404, 5-TO1-CA-05035-12, and AI-09169-01.)

69. The Pathogenesis of Burr Cells in Uremia. R. A. COOPER,* Boston, Mass. (introduced by J. H. Jandl).

Red cells with spicules occur prominently in abetalipoproteinemia ("acanthocytes"), severe hepatocellular disease ("spur" cells), and uremia ("burr" cells). In acanthocytes, membrane phospholipid composition is altered; however, normal red cells acquire neither the morphologic nor the lipid abnormality upon incubation in acanthocyte serum. In spur cells, membrane cholesterol is increased and normal red cells acquire both the morphologic and the lipid abnormalities in spur serum. The lipid and morphologic transformations of burr cells have not previously been examined. 10 consecutive patients in a renal transplant program were studied. The cholesterol and phospholipid content and the phospholipid composition of red cells were normal. Normal red cells became burred upon incubation in serum from each uremic patient, and burr cells became normal in normal serum. The degree of burr cell transformation in vitro was related directly to the ratio of uremic serum to normal cells (v/v). Morphologic changes in vitro were not associated with alterations in membrane lipids. The "burr cell factor" in uremic serum was heat labile at 60°C for 30 min. Burring was inhibited partially by procaine (4×10^{-3} M) and completely by chlorpromazine (1×10^{-5} M). The "burr cell factor" was not removed by dialysis against buffer or normal serum for 96 hr, and persisted in the plasma of patients after 6 hr of hemodialysis and during chronic hemodialysis for periods up to 3 months. It remained after bilateral nephrectomy, but disappeared from the serum of patients in whom renal function was restored by transplantation. These studies demonstrate for the first time that burr cells in uremia result from a nondialyzable factor in serum. This factor is inhibited by cationic amphiphilic substances (procaine and chlorpromazine) known to prevent spicule formation by anionic and nonionized amphiphils. In contrast to the serum factor which induces spur cells, the "burr cell factor" is heat labile. It is not elaborated by diseased kidneys, and persists until a transplanted kidney functions normally. (Research supported by grants HE-07652, AM-05391, FR-76, and 1-F3-AM-38,345 from the NIH.)

70. Inhibition of Cell Proliferation by a Factor Produced by Lymphocytes In Vitro. S. R. COOPERBAND,* J. A. GREEN,* AND A. M. BADGER,* Boston, Mass. (introduced by J. A. Mannick).

Recent studies have demonstrated that stimulated lymphocytes produce a variety of extracellular substances which influence the in vitro behavior of other cells. We have found that supernatants from cultures of PHA- or antigen-stimulated human blood lymphocytes inhibit the proliferation of other cells in tissue culture. This phenomenon occurred without cytotoxicity in the treated cells. Susceptible cells include human amnion, HeLa, HEp2, and mouse L cells. Inhibition of proliferation was determined by a reduction in (1) ^{3}HT incorporation into DNA, (2) the number of mitotic figures per culture, (3) the rate of new cell production (total cell counts), and (4) the replication rate of cells in individually observed microcolonies. Dilution of 1:20 of supernatants reduced DNA synthesis in target cells to 10–20% of that in control cultures. At dilutions up to 1:640, the proliferation-inhibiting factor (PIF) still inhibited DNA synthesis 50%. Addition of PIF to 100 seeded HeLa cells did not reduce the total number of colonies formed, but did reduce the number of cells per colony to 10–50% of that of the control. Similar inhibitory activity was present in supernatants from unstimulated lymphocyte cultures, but to a much lesser degree. The absence of cytotoxicity was established by the observations that treated cells were morphologically intact in situ, excluded trypan blue, and formed a normal number of microcolonies when cloned after treatment. Supernatants from cultures established with dead leukocytes and PHA, extracts of fresh leukocytes, and PHA alone failed to produce this effect. PIF was produced in proportion to the number of lymphocytes in culture, was heat stable at 85° C for 30 min, nondialyzable, nonsedimentable at 90,000 *g*, and destroyed by trypsin. (Supported by grants from the NIH and the Massachusetts Cancer Society.)

71. Selective Suppression by Ethanol of RNA Synthesis in Human Hematopoietic Cells In Vitro: Fact or Artifact? JOSÉ CORCINO,* SAMUEL WAXMAN,* ARNOLD RUBIN,* AND VICTOR HERBERT, New York, N. Y.

In studying in vitro effects of various drugs on bone marrow cell nucleic acid metabolism, we unexpectedly found that ethanol, used as a solvent for certain drugs, appeared to selectively reduce incorporation of ^{3}H-uridine (^{3}HU) into RNA. 25 experiments with various concentrations of ethanol were carried out. More than 10% suppression of ^{3}HU incorporation was observed in 17 out of 23 experiments (75%) using 23 normal human bone marrow aspirates, and in two experiments where folate-deficient marrows were studied. Low concentrations (0.8 mg/ml) usually produced 10–15% suppression; high concentrations (8 mg/ml) usually produced 15–25% suppression. In 12 marrows where ^{3}H-thymidine incorporation into DNA was simultaneously studied, suppression exceeding 10% was seen in only 3 (25%). Suppression of ^{3}H-leucine incorporation into protein was observed in 7 out of 19 experiments (37%). There was a 20% inhibition of ^{3}HU incorporation into RNA of lymphocytes, both in the resting state and after PHA stimulation. This inhibition did not seem to be restricted to any particular species of RNA, as demonstrated by sucrose gradient sedimentation analysis of newly synthesized RNA. Thus, in preliminary studies, ethanol, in concentrations within the range observed in the plasma of intoxicated humans, appeared to selectively suppress incorporation of ^{3}HU, as compared with incorporation of ^{3}H-thymidine and ^{3}H-leucine. These findings are consistent with the reports of cytoplasmic vacuoles in bone marrow cells of intoxicated alcoholic humans, and of dilated endoplasmic reticulum and focal cytoplasmic degradation in the ileal biopsy of a similar subject. It remains to be determined whether there is interference with *uptake* rather than, or in addition to, interference with *incorporation* of ^{3}HU into hematopoietic cells. (Supported in part by USPHS grants AM-13358 and AM-11048, and fellowship 1-F3-AM-39,795.)

72. Effects of Pregnancy and Placental Steroids on Plasma Insulin and Pancreatic Islet Insulin Secretion. N. V. COSTRINI* AND R. K. KALKHOFF,* Milwaukee, Wis. (introduced by W. W. Engstrom**).

Two studies were performed to assess the role of placental steroids in the development of diabetogenic stress and exaggerated plasma insulin responses to glucose during pregnancy. I. Three groups of 10 female rats received daily injections of steroid in oil for 3 wk: (1) estradiol benzoate, 2.5 microgram, (2) progesterone, 2.5 milligrams, (3) combination of (1) and (2). In these animals, compared with 10 control animals, each treatment induced 25–50% higher plasma insulin levels ($P < 0.05$) during 30 min intravenous glucose tolerance tests (GTT, 1 mg per gram body weight); this response resembled exaggerated GTT insulin responses of 10 rats in late gestation (3rd week). Unlike pregnant animals, steroid-treated animals had significantly lower glucose responses than control rats at 10 through 30 min. II. Animals were treated for 3 wk with varying dosages of estradiol (micrograms) and progesterone (milligrams). Pancreatic islets were isolated on day 21 by the collagenase method of Lacy. Results of 250 incubations of 10 islets in 300 mg/100 ml glucose buffer are expressed as microunits secreted above base line in 90 min. With dosages of 5.0, estradiol (816 ± 48) and progesterone (896 ± 39) increased islet secretion above control levels (572 ± 25); these results were comparable to those in late pregnancy (865 ± 51, $P < 0.001$). 1.25 and 2.5 dosages had no effect separately. Combined administration of 2.5 (679 ± 20) or 5.0 (1009 ± 40) enhanced secretion above control and exceeded either hormone's effect when injected alone ($P < 0.005$). We conclude that placental steroids augment plasma insulin responses and islet insulin secretion during pregnancy, and in combination have additive effects. Since both hormones depress GTT glucose responses while inducing hyperinsulinemia, their insulinogenic properties rather than contrainsulin effects are emphasized. (NIH grant AM-10305 supported this research.)

73. Metabolism of Cysteine and Methionine in β-Mercaptolactate Cysteine Disulfiduria. J. C. CRAWHALL,* K. BIR,* P. PURKISS,* AND J. B. STANBURY,** Montreal, Canada, and Boston, Mass.

Two dietary studies have been carried out on the previously described mentally retarded patient who excreted β-mercaptolactate cysteine disulfide. The patient was successively placed on the following regimes: a control diet of known composition, a diet low in cystine and methionine, the control diet with addition of pyridoxine (100 mg 5 i.d.), and the control diet with addition of neomycin (1 g b.i.d.). The second experimental period included addition of oral cysteine (L-cysteine hydrochloride 5 g daily) and addition of L-methionine (5 g daily). Each period lasted for 6 days, and 24 hourly collections of urine were analyzed for individual amino acids and for sulfate. It was found that the patient was excreting about 200 mg of the disulfide daily, and this was not affected by reduction of sulfur amino acids in the diet. The metabolic defect was not influenced by addition of pyridoxine as a possible cofactor and was not affected by sterilization of the bowel with neomycin. Oral cysteine caused an increase of mixed disulfide excretion to 400–500 mg, but no comparable increase occurred with oral methionine. Total urinary sulfate increased after both cysteine and methionine feeding from 600 to 1500 mg/day. L-Cysteine was fed to nine normal subjects in a comparable amount. In three of these subjects 30–70 mg of the mixed disulfide was excreted in 6 hr, and in the others only trace amounts appeared. The formation of the disulfide can thus be a normal response to oral cysteine. It was also increased in the patient after oral cysteine but not after oral methionine. This result may indicate an abnormality of methionine metabolism in the patient, or the mixed disulfide could arise from an intracellular pool of β-mercaptolactate, so that more mixed disulfide was excreted on cysteine feeding.

74. Direct Effect of Long-Chain Free Fatty Acids on Insulin Secretion. STEPHEN R. CRESPIN,* WILLIAM B. GREENOUGH III,* DAVID BOYNTON,* AND DANIEL STEINBERG,** Bethesda, Md., and La Jolla, Calif.

Using a continuous-flow centrifuge, long-chain free fatty acids (FFA) can be infused directly in vivo without adverse side effects. As previously reported, elevation of plasma FFA to 0.8–1.4 μEq/ml by infusion of sodium oleate into the systemic circulation in conscious dogs produced a 2- to 4-fold increase in plasma immunoreactive insulin (IRI) and a 20–30% fall in blood glucose. The present studies were undertaken to determine whether or not this represented a direct stimulation of the pancreas by FFA. Anesthetized dogs were connected by a carotid artery shunt to the centrifuge, which continuously separated the arterial blood into cells and plasma. Sodium salts of long-chain FFA were infused into the plasma line emerging from the centrifuge at rates of 1.0–2.1 μEq/kg per min. The FFA-enriched plasma was then continuously recombined with the emerging packed cells and infused into the dog's cannulated superior pancreaticoduodenal artery. Pancreatic arterial FFA levels rose to 1.1–2.1 μEq/ml. There was an immediate 3- to 10-fold increase in pancreatic venous IRI that reached peak levels within 10 min. The level then declined slightly, but remained elevated until the FFA infusion was discontinued (30 min), when both IRI and FFA fell quickly to control levels. Oleate, linoleate, laurate, and palmitate were approximately equally potent in stimulating IRI secretion. Subsequent intrapancreatic infusion of glucose produced a second large IRI response. During FFA infusion, both systemic FFA and pancreatic ketone levels were unchanged. Long-chain FFA can directly stimulate insulin release from dog pancreas. This effect may play a role as a feedback control on the rate of lipolysis in adipose tissue and also may help maintain basal insulin levels during starvation.

75. The Relation of Mitral Valve Closure to Diastolic Murmur Production. J. MICHAEL CRILEY* AND IRA M. FELDMAN,* Torrance, Calif. (introduced by Karlman Wasserman).

The crescendo presystolic murmur of mitral stenosis and the explosive mid-diastolic murmur heard in patients with dominant mitral regurgitation have traditionally been attributed to periods of maximum flow across the mitral orifice.

A combined hemodynamic, phonocardiographic, and cineangiographic study on 18 patients with rheumatic mitral valvular disease failed to confirm this hypothesis, and suggested an alternative mechanism. Three groups of patients with clear-cut diastolic murmurs were included in the study: group I, mitral stenosis with sinus rhythm (n = 10); group II, mitral stenosis with atrial fibrillation (n = 3); and group III, mitral regurgitation with mild or "relative" stenosis (n = 5). The period of crescendo murmur production did not coincide with the maximal left atrial–left ventricular gradient (and predicted maximal diastolic flow) in any case. In group I the murmur began more than 80 msec after the onset of atrial contraction, increased in intensity as the "a" wave declined and gradient diminished, and reached a maximum at the time of atrioventricular pressure crossover in early systole. In group II, a "presystolic" murmur was frequently recorded when the end-diastolic gradient was over 10 mm Hg, and the murmur began abruptly with the onset of ventricular contraction. In group III, the murmur began over 100 msec after mitral valve opening, and continued until diastolic pressure equilibration occurred in mid-late diastole. Cineangiograms revealed a consistent correlation between the onset of mitral valve closure and the abrupt onset of diastolic or "presystolic" murmurs. It is therefore proposed that these murmurs are not caused by periods of maximal volume flow, but instead by the onset of valve closure in the face of a pressure gradient. (Research supported by a grant from the American Heart Association.)

76. Hepatic and Erythropoietic Sites of Porphyrin Synthesis in Erythropoietic Protoporphyria. DEREK J. CRIPPS* AND WILLIAM N. MACEACHERN,* Madison, Wis. (introduced by Edgar S. Gordon**).

The site of porphyrin synthesis in erythropoietic protoporphyria (EPP) may be hepatic and erythropoietic. A brother (B) and sister (S) with EPP were studied. Quantitative erythrocyte and stool porphyrins were determined over 18 months, and on red cell membranes and hemoglobin supernatant after hemolysis and ultracentrifugation. ALA synthetase was determined on liver biopsies; and both liver and marrow were examined by fluorescence microscopy, by microfluorospectrophotometry, and histologically. Erythrocyte protoporphyrin (PP) accounted for less than 5% of the large quantity of PP in the stool. The calculated mean daily excretion of PP from erythrocytes was 144 μg from B (more photosensitive) and 95 μg from S; the mean daily total PP in the stool was 3050 μg for B and 12,650 μg for S. PP was recovered from the hemoglobin supernatant and not detected on the erythrocyte lipid membrane. ALA synthetase was increased, 121 and 159 mμmoles/g liver per hr as compared with two controls. The liver histology was normal in B, but in S, random focal porphyrin was deposited, identified as PP from its fluorescence peak (6338 A) with the microfluorospectrometer. Two populations of marrow cells were observed; the number of fluorescing erythrocytes in B was 15% (PP 1135 μg/100 ml) and in S 12% (PP 720 μg/100 ml), and the ratios of identified fluorescing to nonfluorescing normoblasts were in a similar proportion: 12:56 in B and 19:71 in S. Analysis of fluorescence which was in the cytoplasm indicated PP (6326 A). In conclusion, abnormal porphyrin synthesis was detected in liver and marrow in EPP. (Research supported by a grant from the NIH.)

77. Xeroderma Pigmentosum: Abnormal Monochromatic Action Spectrum and Radioautographic Studies. DEREK J. CRIPPS* AND COLIN RAMSAY,* Madison, Wis. (introduced by George C. Rowe**).

Xeroderma pigmentosum (XP) is characterized by sensitivity to sunlight and absent or reduced repair replication of DNA. This report indicates the most effective (MAX) wavelengths of radiation (λ) responsible for photosensitivity and the effect of λ on repair replication of DNA in XP and controls. Action spectra to determine the minimal erythema dose (MED) in millijoules (mj) was studied in 19 controls and one subject with XP, using a prism-grating monochromator with λ 250–410 nm (5–10 nm intervals, 4 nm band widths). For radioautograph studies, three controls and one XP were irradiated at 250 and 300 nm with 3 × MED. Skin biopsies of unirradiated sites and of sites 1½ and 24 hr after irradiation were compared after in vitro exposure to ^{3}H-thymidine (DNA precursor). Nuclei of basal and malpighian cells were counted as percentage of labeled to unlabeled nuclei (radioautograph index). In 19 controls the MAX λ was 250 nm (MED 3.9, SD ± 2.9 mj), peak intensity at 8 hr; no reaction occurred above 320 nm. The MAX λ above 275 nm was 293 nm (MED 5.6, SD ± 2.6 mj), peak at 24 hr. In XP, erythema was delayed; peak intensity for all λ occurred at 72 hr with a MAX λ at 293 nm (MED 1.1 mj), but at 24 hr the MAX λ was 296 nm (MED 1.5 mj). The MED for 250 nm was normal (2.3 mj). Erythema occurred for λ up to 340 nm. The radioautograph index in unirradiated sites in XP was 1.5% and in three controls 2.5–2.8%. At 24 hr after irradiation with 250 and 300 nm, no change was observed in XP (1.6%), in contrast to controls (3.2–5.9%). In XP, delayed UV erythema (almost normal MED) may be related to defective repair of DNA. (Research supported by a grant from the NIH.)

78. Biosynthesis of Thyroid Hormone. LESLIE J. DEGROOT AND AKIO NAGASAKA,* Chicago, Ill.

Iodide peroxidase–tyrosine iodinase derived from calf thyroid by enzymatic digestion and acetone extraction has a molecular weight of about 90,000. With dilution, low ionic strength buffer, and 0.01% Triton, enzyme dissociates into a quarter-size subunit of approximately 17,000 mol wt. Tetramer and monomer contain minimal heme protein and both are enzymatically active. In 0.005 M phosphate buffer at 4°C, enzyme is gradually inactivated; it is protected or restored to original activity by 0.1 M phosphate. Phosphate ion may prevent transition to a monomeric form which is labile. Fe^{++}, flavine, and heme neither protect nor restore activity, and other agents (EDTA, metals, reducing agents) offer no protection. During incubation of enzyme with H_2O_2 and iodide, triiodide is formed. On addition of tyrosine, MIT is formed nonenzymatically in amount equivalent to that of previously generated triiodide. Triiodide is an intermediate in the iodination of tyrosine in this in vitro incubation mixture. Ability of NADPH to stimulate iodination in thyroid

mitochondria and microsomes parallels NADPH–cytochrome *c* reductase activity of the preparations. Both activities decrease in parallel with heat or PCMB. NADPH–cytochrome *c* reductase was purified from thyroid and used to reconstitute an in vitro iodinating system, including soluble iodide peroxidase purified by chromatography, and NADPH, NADPH–cytochrome *c* reductase, and vitamin K_3, as an H_2O_2-generating system. Iodination requires all components. The system is catalase sensitive. We conclude that the thyroid peroxidase is a tetrameric structure; dissociation to monomer is associated with enzyme lability. The monomer and tetramer are active. Triiodide is formed by peroxidase and iodinates tyrosine. In vivo production of H_2O_2 required for the peroxidative reaction may involve NADPH–cytochrome *c* reductase. (Supported by USPHS grant AM-13,377.)

79. Disscciatien of In Vivo and In Vitro Responsiveness to Thyroid-Stimulating Hormone in Thyroid Adenomas. FREDERICK DE RUBERTIS,* KAMEJIRO YAMASHITA,* ANDREW DEKKER,* AND JAMES B. FIELD, Pittsburgh, Pa.

Responsiveness to thyroid-stimulating hormone (TSH) in vitro was studied in two human "cold" thyroid adenomas, which in vivo did not concentrate ^{131}I before or after TSH as determined by scan. These results were compared with those using adjacent normal tissue obtained at the time of surgery. Response to TSH in vitro was evaluated by measuring adenyl cyclase, cyclic adenosine-3′,5′-monophosphate (cAMP), ^{3}H-adenine incorporation into ^{3}H-cAMP, glucose oxidation, ^{32}P incorporation into phospholipids, and colloid droplet formation. Both "cold" follicular adenomas responded equally or more to TSH in vitro than normal thyroid. Base line adenyl cyclase and its response to TSH (10 mU) was greater in the adenomas than in the normal tissue. Although base line cAMP was similar, TSH (3 mU/ml) increased it 27-fold in adenomas but only 8-fold in normal thyroid. TSH augmented ^{3}H-adenine incorporation into ^{3}H-cAMP 15-fold in adenomas and 8-fold in normal. Basal glucose oxidation and ^{32}P incorporation into phospholipids was greater in adenomas, but was stimulated equally by TSH in both tissues. TSH in vitro stimulated colloid droplet formation similarly in adenoma and normal tissue. In contrast, tissue from two patients with medullary carcinoma, appearing as "cold" areas in vivo, did not respond to TSH in vitro, whereas their normal thyroid responded as expected. These studies demonstrate responsiveness of "cold" adenomas to TSH in vitro despite no in vivo TSH stimulation of ^{131}I uptake. This suggests that the defect in iodine metabolism of the follicular adenomas does not reflect lack of response of adenyl cyclase–cAMP to TSH. It is also possible that TSH stimulation of ^{131}I metabolism is independent of cAMP. (Research supported by grant AM-06865 from the NIH.)

80. Regional Differences in Sodium and Water Transport by the Human Large Intestine. G. J. DEVROEDE,* S. F. PHILLIPS,* C. F. CODE,** AND J. F. LIND,* Rochester, Minn.

Contributions to sodium and water transport by different regions of the large intestine are unknown. To quantify net sodium and water transport in the healthy rectum, 100 ml of isotonic electrolyte solution, containing PEG, was introduced caudad to a balloon occluding the rectosigmoid junction; samples were removed each 10 min. In five studies, PEG and sodium concentrations remained relatively constant for 60 min, indicating no absorption of sodium or water. Standard 3 lumen perfusion ($n=7$) of electrolytes and PEG 25 cm from the anal verge, with sampling 10 and 25 cm caudad, confirmed lack of rectal absorption of sodium and water. Unidirectional fluxes of ^{24}Na and deuterium oxide (D_2O) from different areas of the large intestine into blood (insorption) were then compared ($n=19$). Arterial blood was sampled serially after introduction into the bowel of 50 ml D_2O and 100 μc ^{24}Na (as 154 mM NaCl). Insorption was calculated by integrating arterial appearance of isotopes with their disappearance rates from blood after rapid injection by vein. Mean insorption rates (%/min) for ^{24}Na in cecum, transverse colon, left colon, and rectum were 2.69, 1.23, 0.81, and 0.23 ($P<0.01$), respectively; D_2O, 3.93, 3.61, 2.92, and 1.73 ($P<0.01$), respectively. Insorption rates from the entire large bowel ($n=3$), calculated from steady-state perfusions from cecum to rectum and corrected for mean transit time through the colon, were 1.45%/min for ^{24}Na and 3.53%/min for D_2O. In these studies, insorption and net transport of sodium and water were closely correlated ($r=0.89$, $r=0.77$). These results define greater insorption of ^{24}Na and D_2O in the proximal than in the distal colon and imply greater absorption of sodium and water there. The rectum does not absorb sodium and water from isotonic contents. (Research supported by grants from the NIH.)

81. Effect of Hypermagnesemia on Proximal Tubule Sodium Reabsorption in the Rat. GERALD F. DI BONA,* Iowa City, Iowa (introduced by George N. Bedell**).

The effect of hypermagnesemia on sodium and water reabsorption in the rat proximal tubule was measured using the re-collection micropuncture technique. Deoxycorticosterone was given to all rats. Control rats (C, $n=7$) received 120 mM NaCl at 0.05 ml/min during 60 min periods I and II. Experimental rats (E, $n=6$) had magnesium chloride added to the infusion during period II to a final magnesium concentration of 160 mEq/liter. All animals received equal fluid volumes. Arterial pressure and hematocrit were measured serially. In C, $(TF/P)_{In_{II}}/(TF/P)_{In_I}=0.99\pm0.01$ (mean ± SE, $n=28$). There was no change in nephron or kidney GFR, urinary sodium excretion, plasma magnesium concentration, or arterial pressure. Mean change in calculated plasma volume was $+3.7\pm0.9\%$. In E, $(TF/P)_{In_{II}}/(TF/P)_{In_I}=0.76\pm0.03$ ($n=23$). This represented a 25% fall in fractional and a 22% fall in absolute sodium reabsorption. There was no significant change in nephron or kidney GFR, filtered sodium load, or arterial pressure. Absolute sodium excretion increased in five of six E ($P<0.05$); fractional sodium excretion increased in all E ($P<0.05$). Two E were restudied after aortic constriction; proximal fractional sodium reabsorption fell 18% ($n=7$) and fractional sodium excretion increased despite reductions in GFR of 35 and 67%. Plasma magnesium concentration increased from 1.75 ± 0.07 to 5.27 ± 0.28 mEq/liter. Mean

change in calculated plasma volume was $+3.5 \pm 1.0\%$. These results indicate that hypermagnesemia decreases proximal fractional and absolute sodium reabsorption and increases fractional and absolute sodium excretion. These findings are not dependent on volume expansion or systemic hemodynamic changes. A direct renal tubular effect is postulated. (Research support: Iowa Heart Association; Veterans Administration Research and Education associateship.)

82. Metabolic Deficiency of Polymorphonuclear Leukocytes in Sickle-Cell Disease. NIKOLAY V. DIMITROV* AND FRIEDRICH DOUWES,* Philadelphia, Pa. (introduced by Frank H. Gardner**).

Bacterial infections have been observed to occur more frequently in patients with sickle-cell disease (SS) than in patients with normal hemoglobin. There has been no adequate explanation for the dysfunction of antibacterial factors including polymorphonuclear leukocytes (PMN). PMN from patients with SS were investigated in regard to their metabolic response and particle uptake during phagocytosis. The metabolic stimulation during phagocytosis was studied using 1-^{14}C-glucose incubations, O_2 consumption, and the nitro blue tetrazolium (NBT) test. The phagocytic index was determined using polystyrene latex particles. PMN from SS patients with repeated infections showed a decreased or absent stimulation of the hexose monophosphate (HMP) shunt and O_2 consumption. They failed to reduce NBT, although simultaneously prepared blood films revealed the presence of particles in PMN. Another group of SS patients demonstrated no metabolic stimulation, but showed an absence of particle uptake by PMN. Patients with SS and no history of repeated infections had normal stimulation of HMP shunt, O_2 consumption, and reduced NBT. PMN from patients with SC disease and histories of repeated infections had the same metabolic pattern as the patients with SS. PMN from patients with sickle-cell trait did not differ metabolically from the normal controls. To exclude the effect of the incubation medium, PMN from patients with SS were incubated simultaneously in autologous serum, normal serum, and Krebs-Ringer bicarbonate buffer. No difference was noted in the metabolic response. Incubation of PMN from normal individuals in SS serum and normal serum had a normal metabolic response and normal phagocytic index. These data suggest that patients with SS prone to infection may have a genetically determined defect of the PMN. This could be considered an additional factor contributing to the decreased defense mechanism of these patients.

83. The In Vivo Response of Pulmonary Tissue Water to Osmotic Transients. RICHARD M. EFFROS,* WILLIAM PERL,* JOAN ARBOIT,* AND FRANCIS P. CHINARD,** Newark, N. J.

The multiple-indicator dilution technique has been adapted to the measurement of water flow between pulmonary tissue and blood after intravenous injections of osmotically active agents. 2–4 ml of a solution containing a vascular indicator (T-1824 or ^{125}I-albumin), tritiated water (THO), and either 10–20 mmoles NaCl with ^{22}Na or 40 mmoles urea with ^{14}C-urea were rapidly injected into the jugular vein of a dog. Carotid artery blood was pumped into sample tubes on a moving collection rack. Osmotic and indicator concentrations in each tube were measured and divided by the quantities of each injected to give "fractional" concentrations. The extravascular water volume (ΔV_{THO}) was determined from the vascular and THO curves. If no solvent drag occurs, the flow of water (Q) induced by a rise in vascular osmolality may be calculated from the equation: $Q = (C_a + C_i - C_v)fF/(C_v - C_i)$, where C_a and C_v refer to fractional arterial and venous osmotic concentrations, C_i designates fractional concentration of ^{22}Na or ^{14}C-urea, f is the water content of blood, and F is blood flow calculated from the vascular indicator curve. That ^{22}Na and ^{14}C-urea remained largely intravascular during a single circulation suggests minimal solvent drag and reflection coefficients (σ) of nearly 1.0. In all 12 studies, the initial movement of water from tissue to blood was followed by a more gradual restoration of water toward equilibrium levels. Using an upslope technique to analyze the early response of tissue water to the osmotic bolus, and for $\sigma = 1$, the osmotic permeability was $(5.36 \pm 1.56) \times 10^{-5}$ cm^3 sec^{-1} (cm H_2O)$^{-1}$ per ml of ΔV_{THO}, and with a pulmonary capillary surface area of 300 cm^2 per ml of ΔV_{THO}, 0.261 ± 0.076 cm/sec.

84. The Gastrointestinal Tract as an Organ of Hormone Clearance: Corticosteroids, Insulin, and Growth Hormone. RICHARD H. EGDAHL, ARNOLD H. HERMAN,* PETER H. SONKSEN,* J. STUART SOELDNER,* AND JOHN R. MCCORMICK,* Boston, Mass.

The liver has been assumed to be the major organ responsible for splanchnic clearance of hormones, although the gut has occasionally been implicated. This study was designed to assess quantitatively the importance of the upper and lower gut in hormone clearance. Clearance was studied in 13 dogs by constant infusion of the hormones under steady-state conditions and over a wide range of physiologic systemic hormone concentrations. Portal vein plasma flow was measured with an electromagnetic flowmeter. Corticosteroids (CS) were measured by the competitive protein-binding radioassay method, and insulin and growth hormone by double-antibody immunoassays. The metabolic clearance rate (MCR) was calculated for each hormone at each systemic concentration level. The gut fractional clearance (hormone concentration: aorta–portal vein/aorta) of CS was 0.431, the clearance was 181 ml/min, and the MCR was 512 ml/min. The gut fractional clearance of insulin was 0.271, the clearance was 111 ml/min, and the MCR was 379 ml/min. The growth hormone fractional clearance was 0.105, the clearance was 26.1 ml/min, and the MCR was 76.0 ml/min. The gut thus accounts for 35% of total CS clearance, 29% of insulin, and 34% of growth hormone total clearance. Both gut hormone clearance and MCR were independent of the systemic hormone concentration. The mechanisms involved in hormone clearance operate linearly within the wide range of concentrations covered in our studies. These studies demonstrate that the gut has a role quantitatively comparable to that of the liver in splanchnic clearance of CS, insulin, and growth hormone, and that the upper gastrointestinal tract is more important than the lower in hormone clearance. (Supported in part by NIH grants

AM-08726 and AM-09748, United States Army contract DADA C-7149, and NIH training grant GM-01498.)

85. Effect of a Morphine Analogue on Phagocytosis and Some of Its Accompanying Metabolic Activities. PETER ELSBACH, NANCY WURSTER,* SHARON LEBOW,* PENELOPE PETTIS,* AND ERIC J. SIMON,* New York, N. Y.

Studies on bacteria have suggested that morphine-like drugs have direct effects on the cell membrane. To determine the effect of this class of drugs on a mammalian cell, we selected the rabbit peritoneal exudate granulocyte, which undergoes striking membrane changes during phagocytosis. We examined in vitro the effect of the morphine analogue levorphanol (LEV) on phagocytosis and on metabolism by granulocytes incubated with and without polystyrene particles or live *Escherichia coli*. LEV (2×10^{-3} M) decreased (1) acylation of medium lysolecithin (which is stimulated about 2-fold during phagocytosis) both at rest (40%) and during phagocytosis (60%); (2) killing of live *E. coli* (10-fold); (3) $^{14}CO_2$ production from 1-^{14}C-glucose both at rest (50%) and during phagocytosis (80%); (4) K^+ content of granulocytes (35%); (5) oxidation of 1-^{14}C-linoleate by 50%, and its incorporation into triglyceride by more than 80%. However, LEV stimulated incorporation of 1-^{14}C-linoleate or palmitate into phosphatidylethanolamine 2- to 3-fold and into phosphatidylcholine by about 50%. Further, glucose uptake, lactate production, and ATP content are not affected by the drug. Thus LEV does not appear to exert its effect through generalized metabolic suppression. Antagonists of morphine do not counteract LEV, but have similar inhibitory effects. Removal of LEV by 2× resuspending of granulocytes completely reverses all inhibition, and in several experiments produced greater stimulation by polystyrene of acylation of lysolecithin than was seen with granulocytes not exposed to LEV. In line with observations on bacteria, it appears that the complex effects of LEV on granulocytes may be due at least in part to an effect on the cell membrane rather than on over-all energy production. (Supported by NIH grants AM-05972 and MH-10227.)

86. Macrophage Augmentation of Interferon Production in Cultures of Human Lymphocytes. L. B. EPSTEIN,* M. J. CLINE, AND T. C. MERIGAN, San Francisco and Palo Alto, Calif.

In studies to determine the blood cell types responsible for interferon production induced by PHA, the following observations were made. (1) In cultures containing 96–100% pure macrophages derived from blood monocytes, no interferon was detected in either the presence or the absence of PHA for up to 92 hr. (2) In cultures of 99.5–100% pure lymphocytes, low levels of interferon were detected in the presence but not in the absence of PHA. (3) 2- to 25-fold increases in interferon titers occurred when pure lymphocytes were combined with the macrophages in culture with PHA. The peak response of interferon occurred at 68 hr after the initiation of the combined cultures. For maximum response, PHA was required for the duration of the culture, and both cell types in association were necessary. Media from PHA-stimulated macrophages or lymphocytes could not substitute for the corresponding intact cell. However, frozen thawed macrophages in combination with lymphocytes and PHA produced an intermediate interferon response. An increase in either cell type produced an increased response in the range studied: lymphocytes, $0.45–1.8 \times 10^6$/ml; and macrophages, $0.5–2.1 \times 10^5$/ml. (4) Although all cultures producing interferon showed some degree of transformation (^{3}H-thymidine incorporation into DNA), no direct correlation between the degree of PHA-induced lymphocyte transformation and the interferon titers was observed. These studies indicate for the first time that two cell types may interrelate in the production of interferon, and suggest a parallel between the antibody and interferon systems in that under certain conditions the same two cell types—the macrophage and the lymphocyte—interact in the presence of a foreign material to produce a humoral substance active in host defense.

87. Decreased Synthesis of an Alpha Chain Hemoglobin Mutation (Alpha^I). G. J. FOLAYAN ESAN,* JOYCE V. O'DONNELL,* AND ARTHUR BANK,* New York, N. Y. (introduced by Paul A. Marks**).

The mechanisms responsible for the diminished amounts of abnormal hemoglobin (Hb) in heterozygotes for α-globin chain mutations have been investigated in a 23-yr-old Negro female and her newborn son with Hb I ($\alpha^{16\ Ly\rightarrow Glu}$). Cellulose acetate electrophoresis revealed 23.8% α^I-containing Hb in the mother's blood, as compared with 33.3% α^I-containing Hb in the newborn. The relatively higher percentage of α^I-containing Hb ($\alpha_2^I\gamma_2$) in the newborn than of α^I-containing Hb ($\alpha_2^I\beta_2$) in the mother suggests that there may be a greater affinity of α^I-chains for γ-than for β-chains. Hb synthesis in peripheral blood (PB) and bone marrow (BM) were measured in vitro by incubation with radioactive valine and determination of counts incorporated into purified globin chains. In the mother, α^A/α^I specific activity (sp. act.) in cpm at OD 280 was 0.96 in PB, and 0.94 in BM; the ratio in the newborn's PB was 1.03. With 3 times as much α^A as α^I in PB, the α^A/α^I sp. act. should approach 0.33 if the two chains are synthesized at similar rates. The α^A/α^I sp. act. of close to 1.0 suggests that α^I is produced in significantly lower amounts than α^A. The constant α^A/α^I sp. act. in BM and PB indicates that during erythroid cell maturation changes in the capacity for hemoglobin synthesis do not preferentially affect either α^I or α^A production. These data also make it appear unlikely that selective destruction of α^I-containing cells leads to diminished amounts of α^I in PB. The results can be explained by either the presence of more mRNA for α^A than for α^I secondary to an increase in the number or activity of α^A-genes relative to α^I-genes, or decreased synthesis time for α^I-chains. (Supported by grants from the NIH, the NSF, the American Cancer Society, and the Rockefeller Foundation.)

88. Diphenylhydantoin and Potassium Transport in Isolated Nerve Terminals. A. V. ESCUETA* AND S. H. APPEL, Durham, N. C.

In experiments designed to explain the anticonvulsant action of diphenylhydantoin sodium (DPH) stimulation of

synapse membrane, Na-K ATPase activity was observed with high sodium-potassium ratios. At more physiological ratios DPH produced little or no effect, and at low sodium-to-potassium ratios DPH was inhibitory. The present studies were undertaken to determine whether DPH alters potassium transport in synaptosomes in vitro in a manner predicted by the ATPase experiments. A rapid filtration technique was used to assess potassium transport in isolated synapses. With 50 mM sodium and 10 mM potassium, DPH (10^{-4} M) in vitro had no effect on total potassium accumulation although it completely reversed the ouabain inhibition of potassium transport. Varying stimulation of potassium uptake was observed with optimal or high sodium and low potassium concentrations. At 50 mM sodium, DPH stimulated potassium uptake 72% at 0.2 mM potassium, 32% at 0.75 mM potassium, and 25.8% at 1 mM potassium; moderate inhibition (42%) occurred at 2 mM potassium. With 0.2 mM potassium, DPH had no effect at zero sodium, but it enhanced potassium uptake 10% with 10 mM sodium, 72% with 50 mM sodium, and 44% with 100 mM sodium. At 50 mM sodium and 0.2 mM potassium, DPH enhanced total potassium accumulation to the same extent to which it stimulated its ouabain-sensitive compartment. DPH stimulatory effects appeared over a range of concentrations from 10^{-4} M to 10^{-10} M. After two daily intraperitoneal injections of DPH, potassium transport in synaptosomes isolated from rat cortex was similar to that observed with DPH added in vitro. The present data provide direct evidence for stimulation or inhibition of potassium transport in a pattern which could be explained by stimulation or inhibition of sodium-potassium ATPase. However, these results do not specify the mechanism involved, since DPH could interact with the membrane in a manner which directly or indirectly altered function of the sodium-potassium pump.

89. Inhibition of the Neuromuscular Junction by Porphobilinogen and Porphobilin As Compared with Uroporphyrins I and III. DANIEL S. FELDMAN,* RICHARD D. LEVERE,* JAMES S. LIEBERMAN,* RUTH A. CARDINAL,* AND C. J. WATSON,** Brooklyn, N. Y., and Minneapolis, Minn.

The chemical transmitter acetylcholine (ACh) is released spontaneously in packets by the motor nerve terminal. These miniature end-plate potentials (m.e.p.p.) related to ACh-induced depolarization may be recorded at the neuromuscular junction (NMJ) by microelectrodes inserted in the muscle fiber. Frequency of m.e.p.p. is a measure of presynaptic motor nerve release of ACh. Increasing K^+ in the bathing solution augments m.e.p.p. frequency. Porphobilinogen (PBG) and porphobilin (PB) had no effect on the resting m.e.p.p. frequency but significantly reduced the rate with high K^+ concentration. With PBG and PB linear relationship was noted. PB was most effective, $ED_{50} = 0.008$ μg/ml; PBG was intermediate in activity, $ED_{50} = 0.60$ μg/ml; uroporphyrin I did not show a linear response, concentrations from 0.05 to 1.0 μg/ml giving about 25% decrease in frequency. The basis of this response is not clear. Uroporphyrin III at similar concentrations was without effect. Rat diaphragm muscle twitches elicited in vitro on phrenic nerve stimulation were reversibly inhibited by PBG and PB at concentrations comparable to those causing reduced m.e.p.p. frequency. PBG produced effects at concentrations comparable to those which might reasonably be expected in the sera of patients with clinical and chemical exacerbations of acute intermittent porphyria. The effect of these compounds upon excitability in this model system exceeds that found with other amines and amino acids and suggests that they may have a role in the development of the symptom complex of acute intermittent porphyria. (Supported in part by NIH grants AM-09838, AM-10539, and NB-01004, and Health Research Council of the City of New York grant I-502.)

90. Evidence for a Glucose-Alanine Cycle: Amino Acid Metabolism during Muscular Exercise. PHILIP FELIG* AND JOHN WAHREN,* New Haven, Conn., and Stockholm, Sweden (introduced by P. K. Bondy**).

Alanine is released by resting forearm muscle to a greater extent than all other amino acids. Since alanine comprises only 5–10% of the amino acid residues in muscle protein, the basis for the primacy of alanine output is unclear. To characterize the pattern further and identify the factors regulating peripheral amino acid release, amino acid exchange across leg tissue was studied in seven healthy subjects before and after 10 and 40 min of exercise (bicycle ergometer, 400 kg/min). Simultaneous arterial, femoral venous, and hepatic venous blood samples were obtained. At rest, there was significant net release from leg tissue of 14 out of 19 plasma amino acids. By far the largest A-V difference across the leg was that for alanine (-71 ± 13 μmoles/liter), accounting for 40% of total amino acid output. Arterial concentration of alanine, but not of other amino acids, correlated directly with arterial pyruvate. During exercise, net release was demonstrable only for alanine, and was accompanied by a rise in arterial alanine concentration which correlated directly with simultaneously rising arterial pyruvate and lactate. Arterial levels of all other amino acids were unchanged. Splanchnic uptake of alanine exceeded that of all other amino acids, and rose by 60–70% with exercise. The results indicate that peripheral formation and release of alanine are not solely dependent on protein dissolution, but are related to utilization of glucose by muscle. Accordingly, it appears that alanine is synthesized by transamination of glucose-derived pyruvate and may thus constitute an important and previously unrecognized end product of glycolysis. The primacy of alanine in splanchnic amino acid exchange suggests further the existence of a cycle whereby glucose-derived alanine is taken up by the liver for reconversion to glucose.

91. A Soluble Cyclic Adenosine-3′,5′-Monophosphate–Responsive Protein Kinase from the Adrenal Cortex. JAMES J. FERGUSON, JR., Philadelphia, Pa.

The molecular mechanism by which cyclic adenosine-3′,5′-monophosphate (AMP) causes adrenal corticosteroidogenesis is currently unknown, but it is thought to involve an enhancement of the rate of conversion of cholesterol to pregnenolone. We have identified in extracts from beef and rat adrenal cortex a soluble protein which catalyzes the transfer of the gamma phosphate from adenosine triphosphate to added pro-

tein acceptors. This activity resides primarily in the cytoplasmic fraction, and requires added divalent cation for activity. Histone and protamine are phosphorylated much more rapidly than endogenous adrenal protein or other proteins tested. The rates of phosphorylation of histone and protamine are approximately doubled in the presence of 10 micromolar cyclic AMP. The natural phosphate acceptor for this enzyme, as well as its physiological functions in the adrenal cortex, are as yet unknown. It is superficially similar to the cyclic AMP–responsive protein kinases described in muscle, liver, brain, adipose tissue, and *Escherichnia coli* by other investigators. The sensitivity of this kinase to cyclic AMP stimulation suggests for it a significant functional role in mediating the hormonal effects of the cyclic nucleoside phosphate. (Supported by grant AM-07207 from the NIH.)

92. Bacteremia in the Pathogenesis of Ascending Pyelonephritis in the Rat. JOSHUA FIERER* AND ABRAHAM I. BRAUDE,** San Diego, Calif.

Most acute pyelonephritis in humans is caused by *Escherichia coli.* It is assumed that infection usually ascends to the kidney from infected bladder urine, but only one method has consistently created such an infection in the rat. We have reexamined this model to define the role of bacteremia in this infection. Female rats, dehydrated overnight, were given 1.0 ml of a log phase broth suspension of 10^7–10^9 *E. coli* 0111:B4:NM into the bladder via the urethra, which was occluded for 5 min. Reflux to the renal pelvis occurred in nearly all rats. Blood cultures of 27 out of 33 rats were positive 3 min after the infusion, with more than 10^4 bacteria per ml in 22. No animals with negative blood cultures developed pyelonephritis. Gross pyelonephritis developed in 42 of 44 kidneys with bacteremia of 10^4/ml or more, and in 7 of 10 with 10^2–10^4 bacteria per ml. Microscopic and gross lesions were those usually attributed to ascending infection. There were hemorrhages in the fornix, wedge-shaped abscesses from fornix to cortex, pyelitis, and occasionally papillitis. Bacteria appeared in renal vein blood during the urethral infusion only if the ureter was patent. They must, therefore, reflux to the renal pelvis, leave via damaged pelvic veins, and recirculate to the kidney. The "ascending" lesion was produced in 14 of 16 kidneys with 1.0 ml of sterile broth via the urethra followed by 10^7 or more *E. coli* i.v. With 10^6 bacteria, 10 of 14 kidneys were affected, and with 10^4, 8 of 22. The invariable association of bacteremia with pyelonephritis in this model, and creation of the lesion by intravenous bacteria, suggest that bacteremia causes the pyelonephritis. These rats may be particularly susceptible to hematogenous infection because of damage to fornix and internal hydronephrosis. These observations reopen the issue of the role of bacteremia in the pathogenesis of pyelonephritis. (NIH grant 7-RO1-HE-12967-01.)

93. Metabolic Effects of Proinsulin and Single-Component Insulin in the Human Forearm. S. EDWIN FINEBERG* AND THOMAS J. MERIMEE,* Boston, Mass. (introduced by Robert W. Wilkins**).

With the forearm as an experimental model, the effects of proinsulin and insulin were studied in man. The forearm was perfused for 30 min with either insulin or proinsulin, and simultaneous blood samples were collected from the brachial artery (A), a deep vein (DV), and a superficial vein (SV). These samples were analyzed for glucose, free fatty acid (FFA), potassium (K^+), and total immunoreactive insulin-proinsulin. Net alterations from the basal state were used as an index of the hormone's biologic action, with changes over the deep venous bed (A-DV) reflecting primarily muscle, and those over the superficial bed (A-SV) reflecting primarily adipose tissue. The final plasma concentration achieved was 1.76×10^{-9} mmoles/ml for proinsulin and 1.96×10^{-9} mmoles/ml for insulin. On an equimolar basis, insulin was more potent in the same individuals than proinsulin. However, the relative potency upon adipose tissue and muscle differed. From 0 to 60 min the mean arteriovenous concentration difference with proinsulin for FFA was 1.92 μM-min across adipose tissue and 0.80 μM-min across muscle, and for K^+, 0.69 μM-min across fat versus 1.32 μM-min across muscle. Glucose uptake in adipose tissue was more sensitive to proinsulin than that in muscle (A-SV 0.73 μM-min versus A-DV 0.38 μM-min). These findings contrasted with those from studies utilizing insulin. Insulin had a much greater effect on muscle than on adipose tissue, particularly for glucose. For glucose, uptake by adipose tissue equaled 2.19 μM-min versus uptake by muscle of 6.13 μM-min. We conclude that under physiologic conditions, insulin is more potent than proinsulin, but whereas the major effect of insulin is upon muscle, the major effect of proinsulin is upon adipose tissue.

94. A Lipoprotein Structural Abnormality in Type IV Hyperlipoproteinemia. WALDO R. FISHER,* Gainesville, Fla. (introduced by Leighton C. Cluff**).

In 14 of 15 subjects with hyper-pre-β-(type IV)lipoproteinemia the isolated serum lipoproteins contained two or more lipoproteins belonging to the low-density lipoprotein (LDL) class (density 1.006–1.063 g/ml). By contrast, additional LDL components were rarely found in the lipoproteins isolated from normals or subjects with hyper-β-(type II)lipoproteinemia. Five separate LDL components have been isolated by preparative ultracentrifugation from four of the type IV subjects. These lipoproteins have been characterized by their sedimentation coefficient, buoyant density, and electrophoretic behavior, and immunologically are β-lipoproteins. They have been isolated repeatedly from these subjects, and their physical properties remain relatively constant despite large fluctuations in the patients' lipemia. A maximum of four LDL have been isolated from the serum of individual subjects on specific occasions. Electron micrographs of these isolated lipoproteins demonstrate spherical macromolecules. Hydrodynamic measurements of the LDL indicate five subclasses with average molecular weights of 4.2, 3.7, 2.7, 2.1, and 1.1×10^6 and corresponding Sf values of 20, 17, 10, 4, and 0. These lipoproteins increase in buoyant density with increasing molecular size, and their measured lipid composition is consistent with their differing densities. A structurally abnormal LDL subunit is postulated in familial hyper-pre-β-lipoproteinemia which undergoes a typical self-association yielding multiple discrete species. This postulation is strengthened by studies on isolated LDL from seven subjects with

hyper-β-lipoproteinemia where only single species of LDL have been found. (Research supported by the NIH and the American Heart Association.)

95. Isolation of a Tumor Factor Responsible for Neovascularization. JUDAH FOLKMAN,* EZIO MERLER,* CHARLES AEBERNATHY,* AND GRETCHEN WILLIAMS,* Boston, Mass. (introduced by Fred S. Rosen).

The growth of solid tumors cannot be sustained without neovascularization of the tumor when it attains a diameter of 3 mm. A diffusible factor which produces rapid neovascularization has been isolated from tumor cells. Tumor cells in suspension (4×10^9) were lysed by nitrogen cavitation and the resulting mixture was centrifuged at 165,000 *g* for 1 hr. The supernatant fluid was fractionated on a column of Sephadex G-200 and the excluded volume dialyzed against Ringer's solution and concentrated (fraction I). Fraction I was assayed by continuous injection through a tubular Millipore filter fixed on the broad expanse of a dorsal subcutaneous air sac in rats. After 48 hr a dense growth of new capillaries and engorged feeder vessels encircled the millitube. Tritiated thymidine injected into the air sac together with fraction I was concentrated only in capillary endothelial cells. New vessels were formed without significant stromatization or cellular infiltration. A similar response was obtained when fraction I was injected into rabbit ear chambers. Other subcellular components were inactive except for nuclei, which showed weak activity. Other biological fluids used as controls were negative. The active fraction was separable from Walker 256 sarcoma, mouse melanoma, and human neuroblastoma, but not from HeLa cells, or from normal tissues such as kidney. Fraction I from thymus lymphocytes displayed weak activity. This factor is nondialyzable. It is stable at room temperature, but inactivated at 56°C. It is inactivated by ribonuclease and by subtilisin but not by trypsin. Our work supports the concept that tumor vessels are elicited by a factor common to many solid tumors and suggests that this factor is an RNA-lipoprotein complex which stimulates mitogenic activity in endothelial cells. (Supported by grants from the NIH [CA-08185 and AI-05877] and from the American Cancer Society [Massachusetts Division] and the Merck Company.)

96. Intracellular Phosphoribosyl Pyrophosphate Depletion by Allopurinol in Man. IRVING H. FOX,* WILLIAM N. KELLEY,* AND JAMES B. WYNGAARDEN, Durham, N. C.

In addition to its inhibitory effect on xanthine oxidase, allopurinol inhibits the *de novo* synthesis of purines. Although the mechanism for this latter effect is unclear, it is independent of the effect on xanthine oxidase. Phosphoribosyl pyrophosphate (PRPP), an essential substrate for the initial and rate-limiting step of purine biosynthesis, has been assayed in human erythrocytes by a method involving the conversion of labeled adenine to its nucleotide in the presence of a partially purified adenine phosphoribosyl transferase enzyme. We have found that (1) the normal concentration of PRPP in erythrocytes ranged from 1 to 4×10^{-6} M, which is substantially less than the known K_m values for this substrate. (2) Allopurinol at a concentration as low as 2×10^{-5} M depleted intracellular PRPP in vitro. (3) The administration of allopurinol in a single dose ranging from 2.1 to 4.0 mg/kg to six patients with normal hypoxanthine-guanine phosphoribosyl transferase activity produced a significant decrease in erythrocyte PRPP content. The maximum decrease occurred 3–5 hr after administration of the drug; and the values observed ranged from 42 to 76% of control values, with a mean of 55%. This effect preceded any significant change in the serum urate concentration. (4) Oxipurinol, the major product of allopurinol metabolism, had no effect on erythrocyte PRPP content after its in vivo administration. The intracellular depletion of PRPP observed in vitro and in vivo provides a likely mechanism for the inhibitory effect of allopurinol on purine biosynthesis. More importantly, since PRPP is an essential substrate for at least six other enzymes involved in nucleotide synthesis, the depletion of this substrate by allopurinol may produce similar alterations in a number of different biochemical pathways.

97. Inhibition of Hematopoietic Stem Cell Proliferation by Cellular Interaction. WALTER FRIED,* WILLIAM H. KNOSPE,* AND FRANK E. TROBAUGH, JR.,* Chicago, Ill. (introduced by John S. Graettinger**).

300 R X-irradiation with a leg shielded (300RLS) results in an increased ability of surviving hematopoietic stem cells (HSC) to endogenously repopulate the same mouse. The number of transplantable HSC in the unirradiated marrow, however, remains unaltered. We report our investigations of this phenomenon. Response to Vinblastine (VLB), which damages cells during mitosis, and to hydroxyurea (HU), which interferes with DNA synthesis, was determined to assess the percentage of HSC in cycle. HSC were assayed by the spleen colony method. Sensitivity of HSC in irradiated and in nonirradiated femoral marrow to VLB and to HU was determined 4 days after 300RLS. Response of HSC in nonirradiated mice was also determined. About 50% of the HSC in nonirradiated marrows remained 14 hr after injection of VLB to both unirradiated mice and mice exposed to 300RLS. Less than 25% of the HSC in irradiated marrows survived. HSC in marrows of unirradiated mice and in unirradiated marrows of mice exposed to 300RLS were unchanged 2 hr after injection of HU, whereas only 50% of those in the irradiated marrows survived. This suggests that HSC in cell-depleted sites only are stimulated to enter cell cycle after the HSC population is decreased by X-irradiation. Perhaps depletion of the HSC compartment releases a stimulus to remaining HSC, whose response is modified by interaction with other cells in the environment. Accordingly, 300RLS does not change the number or cycle time of HSC in nonirradiated limbs. After migration to cell-depleted sites, however, they proliferate rapidly. (This work was supported by the Leukemia Research Foundation and by NIH grants C-RO1-CA-04144-12 and AM-12936-01.)

98. Daily and Alternate-Day Methylprednisolone Treatment of Rabbit Skin Allograft Recipients. ELI A. FRIEDMAN,* Brooklyn, N. Y. (introduced by David M. Kydd**).

Daily treatment with 1 mg/kg methylprednisolone sodium succinate (MP) delayed ear skin allograft rejection in 17

rabbits to a mean of 23.4 days (range 11–42 days), a significant prolongation, $P < 0.01$, as compared with 28 control allografts rejected in 8.9 days (range 6–17 days). In contrast, 10 rabbits given MP, 2 mg/kg, on alternate days evinced no prolongation, rejecting allografts in a mean of 12.3 days (range 6–22 days). Combining daily 1 mg/kg for 5 days and alternate-day MP 2 mg/kg treatment from the 6th day until graft rejection prolonged graft viability about 5 days to a mean of 13.6 days (range 11–18 days) in 9 rabbits, $P < 0.05$. Control spleens in 10 rabbits weighed 0.78 ± 0.13 mg/g body weight. A reduction of about 50% of splenic mass to 0.40 ± 0.03 mg/g occurred 24 hr after one dose of MP, 2 mg/kg ($P < 0.01$), in 10 animals. No further decrease was noted at 48 hr. MP, 1 mg/kg for 19 days, decreased spleen weight in 6 rabbits to 0.42 ± 0.05 mg/g, but 6 animals receiving MP, 2 mg/kg, on alternate days had significantly higher spleen weights of 0.53 ± 0.03 mg/g ($P < 0.01$). Adrenal weight in 11 animals 24 hr after one dose of MP, 2 mg/kg, increased to 0.13 ± 0.016 mg/g ($P < 0.01$), as compared with adrenal weight in 10 control animals of 0.089 ± 0.005 mg/g. Adrenal weights in daily or alternate-day MP-treated rabbits did not differ from controls. We conclude that alternate-day treatment with MP, 2 mg/kg, does not prolong skin allograft survival and has a lesser lympholytic effect than daily treatment with 1 mg/kg. (Research supported by a grant from the Kidney Foundation of New York.)

99. Conformational Differences among Immunoglobulins from Myeloma, Hypogammaglobulinemia, and Normals. D. FROMMEL,* G. W. LITMAN,* W. D. TERRY, A. ROSENBERG,* AND R. A. GOOD,** Minneapolis, Minn.

Circular dichroism (CD) has been applied to conformational analysis of various proteins. We studied human immunoglobulins (IgG) and myeloma proteins of four subclasses in order to test differences in CD spectra between homogeneous and heterogeneous populations, to establish whether myeloma proteins could be used as reference standards for analyzing heterogeneous populations of molecules, and to analyze the influence of interchain disulfide linkages upon the secondary structure of a 4-polypeptide chain molecule. CD analyses revealed pronounced increase of negative optical activity for monoclonal IgG and for IgG of naturally or artificially restricted heterogeneity (IgG from hypogammaglobulinemic patients, normal IgG fractions from preparative zone electrophoresis) as compared with normal IgG. Increase in optical activity reflects predominance of peptide bonds in quasi-analogous states in homogeneous populations as opposed to variable distribution in normal heterogeneous IgG population. Among subclasses, only IgG3 shows a maximal optical activity at 212.5 mμ, in contrast to all other immunoglobulins studied, which have a maximum at 217 mμ. This phenomenon probably originates in peculiar organization of interchain linkages known to exist in IgG3 and reflects influence of disulfide bonds upon spatial intramolecular interaction and optical transition attributable to aromatic residues. Analyses of subunits of IgG led us to postulate that the structural profile of an intact IgG molecule is predominantly derived from inter- and intrachain disulfide distribution together with the contributions from various regions. Modern biophysical approaches, such as spectropolarimetry and spectroscopy, provide tools for establishing new insights into relations between myeloma, purified antibody, immunoglobulin from immune-deficient patients, and normal IgG. (Aided by the NSF, The National Foundation, the NIH, and the American Cancer Society.)

100. Increased Ureteral Back Pressure Enhances Tubular Sodium Reabsorption. MILFORD FULOP* AND PAUL BRAZEAU,* Bronx, N. Y. (introduced by Irmin Sternlieb**).

Although increased ureteral back pressure (UBP), such as may occur in pregnancy or ascites, usually causes larger decreases of sodium excretion than of GFR, the increased UBP has not generally been considered to enhance tubular sodium reabsorption directly. The effect of unilateral increases of UBP of 10–23 cm water on tubular sodium reabsorption was studied in anesthetized dogs during isotonic saline infusion (0.5 ml/kg per min). Urine was collected from the ureters at high flow rates, and C_{In}, the estimate of GFR, and C_{Na} were measured for each kidney. The C_{In} of the kidney with elevated UBP decreased less than 12% in 21 experiments, did not change in 8 experiments, and increased by 3–8% in 7 experiments. Urinary flow and C_{Na} of the kidney with elevated UBP decreased in all experiments, including 10 in which supramaximal amounts of pitressin and/or DOC were administered to the dogs. Results were independent of plasma inulin values because measurements were made simultaneously in the control kidney and in the kidney with elevated UBP. From these results we conclude that the decrease of C_{Na} occurring with increased UBP in the face of unchanged or increased C_{In} must be attributed to enhanced tubular reabsorption of sodium.

101. Immunochemical Localization of Aldose Reductase. KENNETH H. GABBAY* AND EDGAR S. CATHCART,* Boston, Mass. (introduced by Isadore N. Rosenberg**).

We previously showed that aldose reductase (AR), the enzyme responsible for conversion of glucose to sorbitol, is localized to specific tissue sites, i.e. Schwann cell and kidney papilla. Thus, compartmentalized sorbitol accumulations correlate with the sites of pathology in diabetic nerve and kidney. Furthermore, our previous report that 3,3-tetramethyleneglutaric acid (TMG), an inhibitor of AR, blocks sorbitol formation in lens and prevents cataract formation lends credibility to the concept that the sorbitol pathway is important in the formation of other diabetic complications. In the present study, two aldose reductases from beef kidney papilla were isolated by $(NH_4)_2SO_4$ precipitation and DEAE-cellulose and hydroxyapatite chromatography. The latter column separated AR into two forms, AR-A and AR-B, with AR-B predominating. AR-A had low activity with glucuronate as substrate. Sephadex G-100 chromatography gave 200-fold purification and a specific activity of 34 moles NADPH per kg protein per hr with glyceraldehyde as substrate. Rabbits were immunized with AR-B, and after adsorption by nonspecific tissue antigens, a monospecific antiserum to AR-B was demonstrated by Ouchterlony and immunoelectrophoretic techniques. The antiserum totally

inhibited AR activity. Rates of inhibition were similar when different substrates were used, i.e. glyceraldehyde, glucuronate, glucuronolactone, or xylose. The antiserum cross-reacted and showed immunologic identity with AR-A, as well as purified AR and crude enzyme extracts from brain, retina, lens, liver, and nerve. Indirect fluorescence microscopy using this antiserum and sheep anti-rabbit globulin was used to localize AR in kidney papilla. Thus, the ability to recognize the site of aldose reductase by immunological techniques provides a new and sensitive tool for further study of the accumulation of sorbitol in other diabetic tissues.

102. The Blank Problem in Competitive Protein-Binding Assays. V. K. GAMJAM,* M. MARVIN,* AND B. E. P. MURPHY, Montreal, Canada.

In the routine assays carried out for thyroxine, total corticoids, progestins, and androgens by competitive protein binding (CPB), only low blank values due to the solvents were encountered. Usually these were negligible if solvents were freshly redistilled. When chromatography was employed to achieve separation of individual steroids, the resulting blank values were very high, especially with thin-layer (TLC) as compared with paper chromatography (PC). A critical study was undertaken to locate those steps which could possibly contribute to the final blank value, starting from initial extraction to final assay. The effects of the following on b'ank values were examined: (i) solvents singly, in combination, and with silica gel; (ii) heating the silica gel at 550°C for 1 hr in a muffle furnace; (iii) washing the silica gel with nanograde solvents; (iv) employing nanograde solvents all through extraction to elution in place of redistilled solvents; (v) drying extracts and eluates, and running the chromatogram in an atmosphere of N_2 instead of air; (vi) prerunning of the thin-layer plates in a solvent system before final chromatography; and (vii) time of running the chromatogram. The blank values could be considerably reduced when the silica gel was prewashed, heated to 550°C before plates were made, and used promptly after activation of the plates following an initial chromatography. However, they were still too high and erratic to be satisfactory. Where PC was employed, very low blank values were found for progestins and corticoids (which bind to transcortin), but those for androgens (which bind to the sex steroid-binding globulin) were still too high. Preliminary studies using column chromatography (Sephadex LH-20) reduced the blank values to those of the solvents alone. As compared with PC and TLC, this method was simpler and less time-consuming to carry out. Sephadex chromatography thus provides a valuable adjunct to CPB assays. (Supported by the Medical Research Council of Canada.)

103. Evidence for a Primary Disturbance of Membrane Transport in Bartter's Syndrome and Liddle's Syndrome. J. GARDNER,* A. LAPEY,* A. SIMOPOULOS,* AND E. BRAVO,* Bethesda, Md. (introduced by R. S. Gordon, Jr.).

In Bartter's syndrome (idiopathic hyperaldosteronism, hypokalemia, increased renin and angiotensin) and in Liddle's syndrome (idiopathic hypoaldosteronism, hypokalemia, decreased renin and angiotensin), the primary defects are unknown. Primary abnormalities of renal sodium transport have been postulated, but not demonstrated, for both syndromes. If these syndromes reflect a generalized abnormality of sodium transport, other tissues should be involved. Therefore, we measured sodium content and the major components of sodium outflux in erythrocytes from normal subjects and from patients with Bartter's syndrome, Liddle's syndrome, primary hyperaldosteronism (hypokalemia, decreased renin and angiotensin), or secondary hyperaldosteronism (hypokalemia, increased renin and angiotensin). We also measured the in vitro effect of D-aldosterone (2×10^{-6} M, 6 hr) on sodium outflux in human erythrocytes and, for comparison with cells capable of protein synthesis, in avian erythrocytes and rabbit reticulocytes. Erythrocyte sodium content was increased in Bartter's (15.6 ± 1.9 mmoles/liter) and low in Liddle's (7.9 ± 0.5) syndrome relative to controls (9.6 ± 1.5). Total fractional sodium outflux was decreased in Bartter's (0.31 ± 0.02/hr) and increased in Liddle's (0.60 ± 0.02) relative to controls (0.44 ± 0.07). In Bartter's, ouabain-sensitive fractional outflux was decreased. In Liddle's, ouabain-insensitive fractional outflux was increased. Patients with other aldosterone abnormalities had normal sodium content and fractional outflux. These data indicate that the abnormal sodium transport measured in Bartter's or Liddle's syndrome was not attributable to hypokalemia or circulating aldosterone, renin, or angiotensin. Furthermore, there was no detectable effect of aldosterone on human or avian erythrocytes or rabbit reticulocytes. Thus, the decreased ouabain-sensitive fractional sodium outflux in Bartter's syndrome and the increased ouabain-insensitive fractional outflux in Liddle's syndrome may reflect primary generalized (genetic?) disturbances of membrane transport.

104. Use of the Megathrombocyte as an Index of Thrombopoiesis. SUDERSHAN K. GARG,* EDWARD L. AMOROSI,* AND SIMON KARPATKIN, New York, N. Y.

Previous work in this laboratory prompted the use of the large platelet (megathrombocyte) on EDTA peripheral smear (diameter $> 2.5\ \mu$) or in platelet-rich plasma (volume $> 13\ \mu^3$ by Coulter Counter) as an index of megakaryocyte turnover. The number of megathrombocytes was correlated with the number of megakaryocytes in respective bone marrow smears (per low-power "spicule" field or per 1000 nucleated cells). An excellent correlation was obtained in the 133 patients studied (except for megaloblastic anemia), $r = +0.6$, $P < 0.001$. An increase in the percentage of megathrombocytes was found in all patients with thrombocytopenia (except aplastic anemia), including ITP (22), drug-induced thrombocytopenia (4), hypersplenism (10), and megaloblastic anemia (13) of 3.6-, 3.6-, 2.7-, and 3.6-fold, respectively. This was associated with an increase in megakaryocyte number of 4.6-, 4.5-, 2.6-fold, and 0, respectively. An increase in percentage of megathrombocytes was also found in Fe deficiency anemia (18 of 24) and SLE (8 of 14); platelet counts were normal in (21 of 24) and (8 of 14), respectively. This increase was associated with an increase in megakaryocytes of 2.1- and 2.6-fold, respectively.

A compensated thrombocytolytic state was postulated in several patients with chronic ITP in remission (5), SLE (4), easy bruising (2), and vasculitis (1) because of normal platelet counts with increased megathrombocytes and megakaryocytes. This postulate was documented in 6 of 6 of these patients by in vivo $DF^{32}P$ platelet survival studies. These revealed an exponential decline in radioactivity ($T_{\frac{1}{2}}$ 32–66 hr) as compared with 10 normal controls with a linear decline in radioactivity ($T_{\frac{1}{2}}$ 115 hr). 10 patients recovering from thrombocytopenia were followed daily with platelet counts and determinations of percentage of megathrombocytes. A reciprocal relation was consistently found between the percentage of megathrombocytes and the platelet count. Platelets from two normal volunteers were cohort-labeled with ^{75}Se-methionine and separated into large and small platelet populations on 4 days. The data obtained were consistent with the megathrombocyte's being the young platelet. These data reveal the clinical usefulness of the "young" megathrombocyte as an index of thrombopoiesis. (Research supported by the New York Heart Association.)

105. Inhibition by Diphenylhydantoin (Dilantin) of Folic Acid Absorption in Man. C. D. Gerson,* G. W. Hepner,* N. Brown,* N. Cohen,* V. Herbert, and H. D. Janowitz,** New York, N. Y.

Controversy exists concerning the mechanism of diphenylhydantoin (DPH)-induced folate deficiency in man. Recent evidence that DPH does not inhibit folate conjugase activity has cast doubt on the concept that DPH prevents polyglutamate breakdown prior to absorption. We have studied the effect of DPH on intestinal absorption of pteroylglutamic acid by perfusing tritiated folate (^{3}HPGA) in a triple-lumen perfusion system. The control solution contained ^{3}HPGA 25 ng/ml in isosmotic glucose and saline. The test solution also contained DPH at 20 μg/ml or 100 μg/ml. DPH at the 20 μg/ml concentration was studied in four subjects with control followed by test solution. ^{3}HPGA absorption was 35.9% in the controls, and DPH significantly reduced this to 19.2%. Five subjects were studied in reverse order, at the same DPH concentration, DPH solution preceding control solution. ^{3}HPGA absorption was 17.9% with DPH and 28.0% in the control period. Though three of the five subjects appeared to have a sustained effect of DPH during control perfusion, only one failed to show increased absorption during the control run. ^{3}HPGA absorption in these nine subjects was significantly decreased from 31.4% in controls to 18.4% in DPH perfusions. In four subjects DPH 100 μg/ml had an inhibitory effect on ^{3}HPGA absorption similar to that seen at 20 μg/ml. One subject was studied with ^{3}HPGA 100 ng/ml, and inhibition by DPH 20 μg/ml remained impressive. There was no significant effect of DPH on net transport of sodium, chloride glucose, or water. These findings suggest that inhibition of pteroylglutamic acid absorption from the intestine may be a critical step in the pathogenesis of DPH-induced folate deficiency. (Supported by the Foundation for Ileitis and Colitis and the Nuffield Foundation.)

106. Coagulation-Associated Consumption of Complement: A Case with Dissociation between Serum and Plasma Complement Activities. Henry Gewurz,* Joseph Baron,* Oscar D. Ratnoff,** and Stanley Yachnin, Minneapolis, Minn., Chicago, Ill., and Cleveland, Ohio (introduced by Paul Quie).

Despite numerous investigations for factors and/or pathways common to the complement and coagulation systems, interrelations between these systems remain unclear. Recently we observed a 62-yr-old man with mild thrombocytopenia and eosinophilia whose freshly collected blood showed normal complement activity; prothrombin, partial thromboplastin, and thrombin times were normal. However, when his blood was incubated at room temperature, complement depletion as well as coagulation rapidly occurred, even when the formed elements had been removed. Complement depletion was totally inhibited by anticoagulants such as EDTA and heparin and partially inhibited by citrate. When fresh citrated plasma was recalcified, >90% decomplementation occurred in < 15 min. Cl was reduced by 90% and hemolytic C4 and C2 became virtually undetectable, but classical C3 (C3–C9) activity and C4 protein concentration remained unaffected. Complement consumption was not inhibited by EACA, hirudin, or soybean trypsin inhibitor, pointing against plasmin or thrombin, both of which induce similar complement depletion profiles, as the anticomplementary agent. However, small amounts of heparin had potent inhibitory activity. To our knowledge, normal plasma levels with markedly decreased serum complement levels have not previously been observed. The condition described herein is readily separable from hereditary angioedema (HAE), in which the early-acting complement components also are selectively consumed, since the C1-esterase inhibitor concentrations were normal by protein, antienzyme, and anti-C1 assays, and blood from HAE patients drawn into EDTA showed exaggerated rather than reduced depletion of complement. Hence, this patient seems to display a new abnormality of the complement system which evolves and/or is greatly magnified in association with coagulation. The underlying basis for this abnormality, and its relations to the clinical symptoms and to factors activated during hemostasis, are not yet clear.

107. A Computer Teaching Program in Hemostasis. Barbara Gilchrest* and Daniel Deykin, Boston, Mass.

This report describes the operation of a computer-aided instruction program in hemostasis. The program (CLOT) is on-line with an IBM 360/65 computer. It is written in CAILAN, a text-oriented language. CLOT is informal and conversational. It presents a patient with a bleeding problem, guides the student in eliciting a detailed history, and stresses the approach to the patient. CLOT then discusses with the student the interpretation of the standard coagulation assays and the differential diagnosis of selected bleeding disorders. The program uses natural language communication rather than multiple-choice questions, and the student phrases his answers from a large vocabulary list. The program pace is determined by the student, who may call for reference material (interpretation of clotting tests) from the

program. CLOT analyzes student answers and discusses individually both appropriate and inappropriate responses. The learning experience is active, and no two sessions are identical, although CLOT exposes each student to the same subject matter. CLOT monitors student performance, and the student may request an evaluation from the program. CLOT is an experiment in the teaching of hematology. It has been incorporated on a trial basis into the Harvard Medical School course in pathophysiology as a supplement to formal lectures on hemostasis. Initial student response to on-line program evaluation questions has been highly favorable. (Supported by NIH grant HE-11414.)

108. A Cyclic Adenosine-3′,5′-Monophosphate–Dependent Protein Kinase from the Adrenal Cortex: Comparison with a Cyclic Adenosine-3′,5′-Monophosphate–Binding Protein. GORDON N. GILL* AND LEONARD D. GARREN, La Jolla, Calif.

Current evidence indicates that cyclic adenosine-3′,5′-monophosphate (cAMP) is the intracellular mediator of the action of ACTH on the adrenal cortex. We have recently identified an adrenal cortical protein which tightly and specifically binds cAMP. In several mammalian tissues cAMP-activated protein kinases have been described, and these enzymes have been proposed as the site of action of cyclic AMP. In the present studies the possible association of a cAMP-dependent protein kinase with the cAMP-binding protein was investigated in the adrenal cortex. A cAMP-activated protein kinase was demonstrated in adrenal cortical tissue, and the enzyme was purified 50-fold. The adrenal cAMP-activated enzyme catalyzes the phosphorylation (from ATP) of histone, protamine, phosphorylase, kinase, and casein. Enzyme activity revealed a half maximal stimulation (K_m) at a cAMP concentration of 1.4×10^{-8} M. Only nucleotides with the 3′,5′ cyclic ring intact are able to substitute for cAMP in activating the protein kinase; the efficiency of substitution appears related to the structural similarity of the entire molecule to cAMP, with 3′,5′-IMP most efficient. Binding and protein kinase activities were compared throughout the purification procedures. The most highly purified preparation of binding protein (200-fold purified) sediments at 4S on sucrose gradients; the most highly purified protein kinase sediments at 7S and contains a parallel peak of 7S binding protein. Mixing experiments suggest that the 4S binding protein preparation can suppress the protein kinase reaction and cAMP completely relieves this suppression. These studies indicate that cAMP functions in part in the adrenal through activation of a protein kinase; in addition, the studies suggest that cAMP-binding protein may function as a regulator of the protein kinase.

109. Depression of Proximal Tubular Sodium Reabsorption by Beta Adrenergic Stimulation. J. R. GILL, JR.,* AND A. G. T. CASPER,* Bethesda, Md. (introduced by J. H. Baxter**).

There are probably beta adrenergic receptors in the kidney, but their effect on renal function is uncertain. Phenoxybenzamine 0.09 μg/kg per min was infused into the left renal artery of hypophysectomized, cortisone-treated dogs, and urine was collected from each kidney by ureteral catheters. Water diuresis was produced by infusion of 1 liter of 2.5% dextrose rapidly, then at 8 ml/min. After three control clearance periods at stable urine flow, isoproterenol (I) 0.018 μg/kg per min was infused into the left renal artery to stimulate beta receptors, until there was no further increase in urine flow, four to six periods; three or four periods were obtained after I was stopped. Mean C_{In} was 18 ± 1 (SE) ml/min (right) and 17 ± 1.3 ml/min (left) during control, and remained essentially unchanged with I (16 ± 1 ml/min, right; 17 ± 1.3 ml/min, left) and during postcontrol (14 ± 2 ml/min, right; 16 ± 1.5 ml/min, left). Mean C_{H_2O}/100 ml GFR was 3.9 ± 0.9 ml/min (right) and 5.5 ± 1.2 ml/min (left) during control; with I, the right was 4.0 ± 0.6 ml/min but the left increased to 8.1 ± 0.7 ml/min, $P < 0.02$; during postcontrol the right was 3.9 ± 1.1 ml/min but the left decreased to 6.0 ± 1 ml/min. The ratios of left to right for C_{H_2O} were 1.47 (control), 2.27 (I), and 1.54 (postcontrol). Mean V/100 ml GFR was 6.8 ± 1.2 ml/min (right) and 8.7 ± 1.7 ml/min (left) during control; during I, the right was 7.4 ± 0.7 ml/min but the left increased to 12.5 ml/min, $P < 0.02$; during postcontrol, the right was 7.8 ± 1.1 but the left decreased to 10.7 ± 1.0 ml/min. The ratios of left to right for V were 1.28 (control), 1.69 (I), and 1.37 (postcontrol). Mean C_{PAH}, filtration fraction, noncortical plasma flow, and $U_{Na}V$ in the left kidney were essentially unchanged during I, and mean arterial pressure was stable. The results suggest that renal beta adrenergic stimulation decreases proximal but not distal tubular reabsorption of sodium. This can be explained by vasodilatation in a segment of the renal vasculature such as to increase the transmission of arterial pressure to the peritubular capillary.

110. A New Biochemical Lesion in Acute Intermittent Porpyhria: Deficiency of Steroid Δ^4-5α-Reductase Activity. P. N. GILLETTE,* H. L. BRADLOW,* T. F. GALLAGHER,* AND A. KAPPAS, New York, N. Y.

Steroid metabolites of the 5β-H type are potent inducers of δ-aminolevulinate synthetase (ALAS), the rate-limiting enzyme in porphyrin-heme formation. This enzyme activity is excessive in the livers of patients with the genetic disease acute intermittent porphyria (AIP). We have studied the metabolism of two natural steroid hormones in AIP patients to determine whether there were alterations of hormone biotransformation in these subjects which were capable of generating disproportionate amounts of 5β-H steroid metabolites Testosterone is, in normals, metabolized to 5β-H (etiocholanolone) and 5α-H (androsterone) derivatives in approximately equimolar ratio. In six AIP patients in clinical remission, however, ^{14}C-testosterone metabolism was diverted substantially away from the 5α-H and toward the 5β-H pathway in each, with 5β-H/5α-H ratios of isolated metabolites ranging from 1.7/1 to 4.3/1. These abnormal 5β-H/5α-H ratios were restricted to the AIP subjects; four patients with acquired porphyria produced, by comparison, normal or substantially increased proportions of the 5α-H metabolite. These findings indicated a defect in steroid Δ^4-reductive transformation in AIP; to examine this defect further, the metabolism of ^{14}C-11-OH-androstenedione was

studied. This steroid is, because of its structure, metabolized almost exclusively in normals to a 5α-H derivative. In the AIP patients, however, metabolism of this compound via the 5α-H pathway was greatly diminished (less than one-third normal), indicating a gross deficiency of steroid Δ^4-5α-reductase activity in this genetic disease. This enzyme deficiency, which resembles that observed in certain endocrinopathies, diverts the metabolism of structurally appropriate endogenous hormones in AIP toward the preferential formation of metabolites known to have potent ALAS-inducing properties. Such metabolites may, in these genetically susceptible individuals, contribute to the excessive hepatic ALAS activity which characterizes their disease.

111. Polyarteritis and the Australia Antigen: A New Association. DAVID J. GOCKE,* KONRAD HSU,* COUNCILMAN MORGAN,** STEFANO BOMBARDIERI,* MICHAEL LOCKSHIN,* AND CHARLES L. CHRISTIAN, New York, N. Y.

Three patients have been observed with polyarteritis associated with Australia antigen. Their illnesses were characterized by fever, hepatic and renal damage, peripheral neuropathies, and hypertension. A diagnosis of polyarteritis was based in each on typical vascular lesions in muscle and liver. Australia antigen was detected in the serum of all three. The best-studied case occurred in a 48-yr-old female who received a transfusion of blood containing Australia antigen during a routine gynecologic operation. 3 wk later she returned because of fever (104°F), and Australia antigen had appeared in her serum. Over the next 2 months the fever persisted, progressive hepatic enzyme abnormalities developed, and ulnar and peroneal neuropathies appeared. Liver biopsy revealed active hepatitis and inflammatory changes in arterioles characteristic of polyarteritis. Muscle biopsy confirmed the diagnosis of polyarteritis. At 3 months an acute renal crisis occurred, with hypertension, pulmonary edema, hematuria, and azotemia. Steroid therapy resulted in defervescence of fever and improvement in hepatic function. However, hypertension, renal and hepatic abnormalities, and Australia antigen are still present 6 months after the transfusion. The titer of Australia antigen rose when the patient was relatively well and declined during periods of exacerbation. Serum complement levels were depressed only during the renal crisis. The patient's serum was examined for immune complexes by zonal centrifugation (35–50% sucrose, 157,000 *g* for 15 hr). Pellet fractions were Australia antigen negative by immunodiffusion. However, electron microscope examination of negatively stained suspensions of the pellets revealed particles characteristic of those described in association with Australia antigen. Immunofluorescence studies of muscle carried out with fluorescein-labeled anti-Australia antigen, anti-IgM, and anti-β_{1c} antisera revealed specific fluorescent deposits in the walls of small blood vessels. Control specimens were negative. These observations suggest that the diffuse vasculitis seen in these patients was caused by an immunologic mechanism involving deposition of complexes of Australia antigen, immunoglobulin, and complement in small blood vessels. Current evidence indicates that Australia antigen is intimately associated with a virus which is one of the etiologic agents of hepatitis. Thus, this may be the first recognition of a systemic vascular disease in humans mediated by an immunologic reaction to a viral agent.

112. Absorption of Synthetic "Cold" and Tritium-Labeled Pteroylheptaglutamic Acid. HERMAN A. GODWIN* AND IRWIN H. ROSENBERG,* Boston, Mass. (introduced by William B. Castle**).

Studies of conjugated (dietary) folate have been impeded by lack of well defined compounds for human and in vitro investigation. We have synthesized (in collaboration with J. Meienhofer, Children's Cancer Research Foundation, Boston, Mass.) pteroylglutamylgammahexaglutamic acid (Pt-Glu_7) and pteroyl-3′,5′-^{3}H-glutamylgammahexaglutamic acid (^{3}H-Pt-Glu_7), specific activity 175 μc/μmole. Purity was demonstrated by paper chromatography, electrophoresis, ultraviolet spectrum, and amino acid analysis. Predicted yields of *Streptococcus faecalis* and *Lactobacillus casei* activity followed deconjugation with chick pancreas extract. Free folate activity before deconjugation was less than 0.3% of the total. ^{3}H-Pt-Glu_7 was over 99% radiochemically pure. Absorption studies in seven normal subjects using 550–825 μg Pt-Glu_7 (equivalent to 200–300 μg pteroylmonoglutamic acid) orally with blood samples obtained during fasting and hourly for 4 hr after administration resulted in an average peak serum increment of 11.1 mμg/ml by *L. casei* assay. In four of these seven, serum was also assayed with *Str. faecalis;* similar peak increments were found, suggesting conversion during absorption to nonmethylated pteroylmonoglutamic acid. Identification of ^{3}H-pteroylmonoglutamic acid by electrophoretic mobility and microbiological assay as a product of in vitro incubation of ^{3}H-Pt-Glu_7 with homogenate of human intestinal biopsy adds evidence to this interpretation. Three normal adults were given an AEC-approved dose of ^{3}H-Pt-Glu_7 (30 μc, 0.5 μmole). 6-hourly blood samples, daily urine collections, and 72 hr stool samples were obtained. After 4 hr a 15 mg "flushing" dose of unlabeled folic acid was given parenterally. Urinary excretions in 48 hr for the three subjects were 43%, 54%, and 46% of the administered dose. Addition of stool radioactivity accounted for over 90% recovery of the original dose. These studies with ^{3}H-Pt-Glu_7 form the basis for development of a safe, quantitative test of conjugated folate absorption and metabolism. (Research supported by grants AM-00795, AM-05391, AM-05413, HE/AM-07652, AM-09115, and FR-0076 from the NIH.)

113. Etiologic Role of Dialysis in Stimulating Hyperparathyroidism during Chronic Hemodialysis. R. S. GOLDSMITH,* A. FOURNIER,* W. J. JOHNSON,* AND C. D. ARNAUD,* Rochester, Minn. (introduced by R. G. Sprague**).

We have systematically examined the role of PTH in the development of bone disease in 28 patients with renal insufficiency being treated by chronic hemodialysis for 6 months or longer. Initial observations clearly demonstrated that dialysis against a calcium concentration of less than 5.7 mg/100 ml was very likely a significant etiologic factor in the bone disease, since only one patient dialyzed against a

higher calcium concentration developed radiographic bone disease. Predialysis serum immunoreactive PTH (SIPTH) (1) was greatly elevated in all patients, but was significantly higher ($P < 0.05$) in patients with bone disease than in those without, (2) was negatively correlated with dialysate concentration of calcium (DCa) ($P < 0.05$), and (3) was positively correlated with serum phosphate concentrations (Sp) ($P < 0.05$). To assess directly the influence of DCa and Sp on SIPTH in individual patients, a 2×2 factorial study was conducted in which DCa and Sp were varied independently, each of the four regimens lasting 2–3 wk. SIPTH was found to be lowest with the high DCa–low Sp regimen and highest with the low DCa–high Sp regimen, confirming the initial observations. SIPTH decreased only slightly during 4 hr calcium infusions and somewhat more during dialysis against a DCa of 6.5 mg/100 ml. We conclude that (1) PTH plays a major etiologic role in renal osteodystrophy, and (2) PTH may be decreased substantially by using a high DCa and reducing Sp. (Supported by grants from the NIH.)

114. Survival of Cultured Fibroblasts from Xeroderma Pigmentosum and Normals after Ultraviolet Irradiation. SAMUEL GOLDSTEIN,* Hamilton, Ontario, Canada (introduced by John W. Littlefield).

Human diploid fibroblasts cultured in vitro provide an excellent model for the study of aging. Using colony formation from individually plated cells as an index, we have reported that survival of cells decreases with repeated passage (i.e. aging in vitro). Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder in which the skin shows extreme sensitivity to UV and sunlight, followed eventually by severe atrophy and an increased rate of carcinogenesis. Recently it was shown that skin fibroblasts cultured from patients with XP have a defect in an early stage of repair of DNA damage consequent to UV irradiation. In exploring possible molecular mechanisms in aging, we have studied survival after UV irradiation of early-passage skin fibroblasts from normal individuals and from subjects with XP. Survival of XP cells was reduced below 1% of nonirradiated controls after a low dose of UV (20 ergs/mm^2). At the same dose, survival of normal cells was enhanced by 25–50% as compared with nonirradiated controls. Repeat studies have localized this effect to 8–20 ergs/mm^2. At 60 ergs/mm^2, survival was decreased to 15–20% of controls, approaching 1% beyond 200 ergs/mm^2. These data show that deficient DNA repair in XP is expressed phenotypically as decreased survival of cells after low doses of UV. The biochemical basis for enhanced survival at the same doses in normal cells is unknown; however, it may be explained by the converse, increased DNA repair. Furthermore, deficient DNA repair may be a factor, not only in the decreased survival of cultured fibroblasts aging in vitro, but also in the cell loss and increased carcinogenesis observed in the skin and internal organs of individuals with XP, and some normal persons during the aging process. (Supported by a grant from the Medical Research Council of Canada during the tenure of a MRC scholarship.)

115. Intestinal Microflora in Laennec's Cirrhosis. S. L. GORBACH,* D. LAL,* AND R. LEVITAN, Chicago, Ill.

Antibiotics are widely used in treating hepatic coma, presumably to supppress urea-splitting bacteria in the gastrointestinal tract. However, no data are available on the types or distribution of such bacteria in the intestine of patients with Laennec's cirrhosis. To study this question, small bowel contents obtained by peroral intubation and stools from 14 chronic alcoholics with biopsy-proved cirrhosis were quantitatively cultured in aerobic and anaerobic conditions. All patients had signs of hepatic decompensation but none were in coma. Large numbers of urea-splitting bacteria were found in 12 of 14 patients, *Klebsiella* in 11 and *Proteus* in one. Seven of these patients had colonization of the upper small bowel by urea-splitting *Klebsiella:* the most proximal site was the stomach (3), followed by the duodenum (1) and jejunum (3). Four of these seven patients also had obligate anaerobes (*Bacteroides*) in the upper jejunum. The mean concentration of *Klebsiella* in the upper jejunum was $10^{4.2}$/ml. pH of gastric juice (basal) was 5.0 or higher in the three patients with coliforms in the stomach. Four other patients had *Klebsiella* confined to the ileum ($10^{5.9}$/ml) and stool ($10^{7.5}$/ml). One patient had *Proteus* ($10^{7.0}$/ml) in the stool. All strains of aerobic urea splitters were inhibited by easily achievable concentrations of neomycin (<25 μg/ml). *Klebsiella* and *Proteus* were either absent or less than 10^3/ml in the small bowel and stool in a control group of normal subjects. The prevalence of neomycin-sensitive urea splitters in the GI tract of cirrhotics may provide the explanation for the efficacy of this antibiotic in the management of hepatic coma. (Research supported by grants from the NIH, the National Aeronautics and Space Administration, and Veterans Administration Research Funds.)

116. Tolerance to Hypnotic Action of L-Methylphenobarbital Induced by Its Hypnotically Inactive Stereoisomer. E. GORDIS,* New York, N. Y. (introduced by Vincent P. Dole**).

Most pharmacological effects of asymmetric addicting drugs are highly stereospecific. Recently, several asymmetric barbiturates have been resolved, and the stereospecificities of their hypnotic action demonstrated. Methylphenobarbital, NF, has been used in racemic form for many years as an anticonvulsant. Its optical antipodes have been separated, and only the levorotatory form was found hypnotic. Barbiturate tolerance develops after repeated use in two ways. The nervous system becomes less sensitive to the drug, and drug degradation becomes faster as hepatic drug-metabolizing enzymes are induced. To study the stereospecificity of methylphenobarbital tolerance, we prepared the antipodes by Knabe's method of fractional crystallization of their methylquinine salts. White male rats in three groups received four daily injections of L-methylphenobarbital, D-methylphenobarbital, or saline. Only rats which received L-methylphenobarbital slept, and during the 4 days their mean sleeping times decreased from more than 4 hr to less than 10 min. On the 5th day all rats received L-methylphenobarbital. Those that had been pretreated with saline slept 2½ hr, whereas the rats which during the previous 4 days had received either isomer slept 11 min. Tolerance to the hypnotic

effect of the active isomer was produced equally efficiently by both isomers. Also tested were the abilities of both isomers to induce another hepatic enzyme activity, aminolevulinic acid synthetase. Both isomers induced this enzyme in chick embryo liver and rat liver almost as well as allylisopropylacetamide, a potent inducer. In summary, tolerance to the hypnotic action of L-methylphenobarbital has been produced by pretreatment with the hypnotically inactive D-methylphenobarbital. (Research supported by New York State Narcotics Addiction Control Commission grant C-25498.)

117. Extravascular Distribution of Diffusible Substances in Well Perfused Organs. CARL A. GORESKY, WILFRED H. ZIEGLER,* AND GLEN G. BACH,* Montreal, Canada.

Well perfused visceral organs are unique in having a high perfusion rate and capillary density, and an extravascular space of dimensions such that "diffusible substances" entering from the blood will at once be uniformly distributed in the lateral direction. We have attempted to gain insight into this physiological situation by obtaining an analytical solution to flow and diffusion in a model of a capillary situated in such an organ, when the extravascular passage of material is limited by the permeability of the barrier. The outflow concentration response to a single injection of diffusible substance is found to consist of two parts: an exponentially damped spike emerging at the transit time of a vascular reference substance (material which has not left the capillary), and a tail function, spread out over later time (material which has entered the extravascular space and is consequently delayed at the outflow). With increase in permeability, the proportion of the diffusible material under the tail function increases, and finally, when the capillary wall presents no barrier, this material emerges as a wave delayed relatively to a vascular substance (the flow-limited case which we have previously described). Consideration of the distributions of capillary and large-vessel transit times of vascular indicators enables this modeling to be extended to the interpretation of experimental studies in whole organs. The two possible extremes have been explored in detail: that in which the capillary transit times are uniform, and that in which the large-vessel transit times are identical. Present experimental observations appear to exclude the former possibility and imply that in most of the well perfused visceral organs, neither the capillary lengths nor the large-vessel transit times are uniform.

118. Studies on the Inherited Protein Defect in Abetalipoproteinemia: The Occurrence of Very Low Density Lipoproteins. A. M. GOTTO,* R. I. LEVY,* K. JOHN,* AND D. S. FREDRICKSON, Bethesda, Md. (introduced by Theodore Cooper).

Abetalipoproteinemia (ABL) is an inherited disorder in which low density lipoproteins (βLP), very low density lipoproteins (VLDL), and chylomicrons are absent from plasma, and normal transport of triglyceride from intestine and liver to the circulation does not occur. No βLP or normal protein moiety (apoβLP) can be detected immunochemically. It has recently been found that less than 50% of the protein moiety of VLDL is composed of three proteins (D_1, D_2, D_4) which also occur in small quantities in the high density lipoproteins (HDL). HDL was isolated from three patients with ABL, delipidated, and fractionated by gel filtration and chromatography on DEAE-cellulose. Bands having the migration of D_1, D_2, and D_4 were present on polyacrylamide gel electrophoresis in the fractionated and unfractionated protein. The occurrence of D_4 was demonstrated by the isolation of a highly purified protein which formed precipitin lines identical with those of normal D_4 and which contained N-terminal serine. The protein had the same amino acid composition as D_4, including the absence of cystine and the presence of only traces of isoleucine. Fractions corresponding to D_1 and D_2 were obtained in a partially purified state. The first formed precipitin lines identical with those of normal D_1. Thus, of the major protein constituents of VLDL—D_1, D_2, D_4, and apoβLP—D_1, D_4, and probably D_2 circulate in ABL, while apoβLP appears to be selectively absent. It seems likely, therefore, that the specific underlying protein defect in ABL is restricted to apoβLP and that in the absence of this protein the transport of triglyceride and formation of VLDL particles cannot occur.

119. Mechanical Properties of Esophageal Wall. RAJ K. GOYAL,* PIERO BIANCANI,* ARIS PHILLIPS,* AND HOWARD M. SPIRO, New Haven, Conn.

Experiments were performed on over 200 rats to define the mechanical characteristics of the esophageal wall and its components in order to provide an understanding of the esophageal response to intraluminal stresses. The mean $\pm$ SD breaking pressure was 1190 ± 78 g/cm^2 at a rate of pressure change (RPC) of 200 g/cm^2 per sec, and it was significantly lowered to 864 ± 137 g/cm^2 at RPC of 5 g/cm^2 per sec. The breaking pressure of the mucosa alone was 58% of that of the whole esophagus. The mean outer diameter of the esophagus was 1.61 ± 0.12 mm, the mucosal folds unfolded at 1.94 ± 0.11 mm, and the mucosa contributed negligibly until the esophageal diameter became 2.75 mm and held a pressure of 50 g/cm^2. Despite quantitative differences, the pressure-diameter curves for the middle part of the entire esophagus and of the mucosa were qualitatively similar, showing a nonlinear relation, the compliance decreasing with increasing loads. The diameter at a given pressure was dependent on load, rate, time, and history. The loading and the unloading curves followed different paths, showing hysteresis. The area of the hysteresis loop decreased with repeated cycles and was most marked for the "active" muscle component. The mucosa and the "passive" muscle component showed small change with repeated cycles. The results show that the "active" muscle component determines the esophageal diameter at lower loads and contributes little to the strength at higher loads and at breaking pressure. The major contribution to the strength at breaking pressure is provided by the mucosa and to a lesser extent by the passive muscle component.

120. In Vivo Studies on the Role of the Liver in Endotoxin Fever and Tolerance. SHELDON E. GREISMAN AND CELESTE L. WOODWARD,* Baltimore, Md.

The granulocyte has long been considered the major source of endogenous pyrogen responsible for endotoxin fever. Re-

cently, endogenous pyrogen elaboration was reported from isolated rabbit hepatic Kupffer cells incubated in vitro with endotoxin. A major role for the liver in endotoxin fever was thereupon postulated. The present studies test this concept in vivo. Laparotomies were performed on anesthetized rabbits, employing sterile and pyrogen-free precautions. A polyethylene catheter was threaded into a small mesenteric vein, secured, and brought out through the abdominal wall. 4 wk later, animals were acclimatized for pyrogen studies. Terminally, trypan blue marker was injected to confirm proper catheter placement. *Escherichia coli* endotoxin injection via the rabbit ear vein evoked classical biphasic febrile responses; tolerance induced by daily ear vein injections entailed diminution or elimination of the second fever peak but characteristic maintenance of the initial peak. In contrast to the ear vein route, when the endotoxin was perfused directly through livers of such endotoxin-tolerant rabbits, the initial fever spike was consistently reduced or eliminated; in nontolerant rabbits, the same phenomenon was also occasionally observed. The initial fever spike appears, therefore, to be mediated by extrahepatic sources, with tolerance induction evoking enhanced hepatic uptake of directly perfused endotoxin, thus precluding its reaching these sources. Of greater importance, in both normal and tolerant animals, the second fever spike was not impaired, and indeed at times was enhanced, by direct liver perfusion; the second fever spike after endotoxin administration thus appears primarily hepatic in origin. It is concluded that classic pyrogenic tolerance, induced by single daily systemic intravenous injections of endotoxin, is based not upon diminished responsiveness of extrahepatic mechanisms of fever, but rather upon refractoriness of the hepatic endogenous pyrogen-generating mechanisms. (Sponsored by NIH grants AI-07052 and 2-K3-HE-15,237.)

121. Effects of Aurintricarboxylic Acid on the Initiation of Hemoglobin Synthesis. ARTHUR P. GROLLMAN AND MOU-TUAN HUANG,* New York, N. Y.

The mechanism by which new peptide chains are initiated in animal cells is still obscure. Experiments were designed to test the hypothesis that initiation of globin chains during hemoglobin biosynthesis requires release of mRNA from the ribosome. Aurintricarboxylic acid (ATA), a dye which inhibits the attachment of mRNA to *Escherichia coli* ribosomes, was used to study the initiation of globin synthesis in lysates and fractionated cell-free extracts prepared from rabbit reticulocytes. ATA binds to reticulocyte ribosomes, preventing the attachment of polyuridylic acid and the subsequent synthesis of polyphenylalanine. The dye also inhibits hemoglobin synthesis in crude lysates by 50% at concentrations of 10^{-5}–10^{-6} M and induces the systematic breakdown of polyribosomes to single ribosomes. ATA does not affect binding of aminoacyl tRNA to ribosomes or the enzymatic activities of peptidyl transferase or translocase. Peptide formed in the presence of ATA cannot be distinguished from normal globin chains. We conclude that ATA specifically inhibits initiation of globin synthesis in rabbit reticulocytes by preventing the attachment of hemoglobin mRNA to ribosomes. The results suggest that a ribosome cycle occurs during hemoglobin synthesis with release of mRNA after completion of each peptide chain.

122. Direction of Portal Venous Flow in Cirrhosis. R. GROSZMANN,* B. KOTELANSKI,* I. M. KHATRI,* AND J. N. COHN, Washington, D. C.

The direction of blood flow in the portal venous system depends on the relation between intrahepatic (IHR) and extrahepatic portal-systemic (PSR) vascular resistances. PSR is the resultant of parallel resistances in the splenic collateral (SC) and mesenteric collateral (MC) beds. As portal pressure rises in the cirrhotic patient, development of collateral channels leads to a progressive fall in PSR. If PSR becomes lower than IHR, forward flow in the portal vein ceases. Flow was assessed in patients with alcoholic cirrhosis by injecting a bolus of ^{131}I-labeled serum albumin into the percutaneously catheterized splenic (S) and superior mesenteric (SM) arteries while hepatic venous (HV) blood was withdrawn through a polyethylene coil in a well scintillation counter. The following patterns were demonstrated in different subjects: (1) No appearance of isotope in HV after either SM or S injection (PSR < IHR) (total portal-systemic shunting); (2) appearance after SM but not S injection (SC < IHR < MC); (3) appearance after S but not SM injection (MC < IHR < SC). Anatomical patency of the portal system was proved in patients exhibiting each pattern. In seven patients with total shunting (pattern 1), albumin injected into the SM artery appeared in the left renal vein, indicating retrograde flow in the portal vein due to opening of a spontaneous low-resistance splenorenal shunt. We conclude that the parallel resistances in the portal system vary independently in cirrhosis and lead to striking alterations in flow direction in the portal vein. (Supported in part by a grant from the NIH.)

123. Studies on the Mechanism of Genetic Control of the Immune Response. F. CARL GRUMET,* BRUCE W. CHESEBRO,* GRAHAM F. MITCHELL,* AND HUGH O. MCDEVITT, Stanford, Calif.

The ability of inbred mice to respond to branched, multichain, synthetic polypeptide antigens (such as [T,G]-A--L) is under the direct control of a gene or genes closely linked to the H-2 locus in the ninth mouse linkage group. We have attempted to determine the mechanism of gene action by comparing the numbers of antibody-forming cells, and the avidity of the antibody produced, in responder, nonresponder, and (responder × nonresponder) F_1 mice. Antibody-producing cells were enumerated by a modification of the Jerne plaque-forming assay, and the avidity of serum antibody was measured by determining the antigen-binding capacity of a series of serum dilutions when incubated with a series of antigen dilutions by the method of Celada. Responder (CSW) mice formed large number of plaque-forming cells in both the "primary" and "secondary" phases of response. Anti-(T,G)-A--L antibody from these mice was of high antigen-binding activity and high avidity. In contrast, nonresponder mice produced far fewer plaque-forming cells and their antibody was of low titer and low avidity. (Responder × nonresponder) F_1 mice were similar to responder mice. When nonresponder mice were immunized with (T,G)-A--L complexed with methylated bovine serum albumin, the nonresponder mice assumed the characteristics of responder animals and produced high-titer, high-avidity antibodies.

These results suggest that nonresponder mice are capable of synthesizing antibody of the same specificity as responder mice, but lack the ability to recognize some of the antigenic determinants on (T,G)-A--L. When the antigen is complexed with a recognizable carrier, such as methylated bovine serum albumin, the recognition defect in the nonresponder is circumvented. This implies that genetic control of antigen recognition operates independently of any possible genetic control of antibody specificity. (Research supported by grants from the NIH and the Arthritis Foundation.)

124. Functional Asymmetry of Sugar Transport Carriers in Human Erythrocytes and in *Escherichia coli*. MATTEW J. GUY* AND DAVID SCHACHTER, New York, N. Y.

Facilitated diffusion of sugars by mobile carriers involves reversible formation of sugar-carrier complexes at both the outer and inner cell membrane faces. Given rate constants of formation and dissociation, respectively, of k_1 and k_{-1} at the outer face and k_{-3} and k_3 at the inner face, for an equilibrating transport the dissociation constants k_{-1}/k_1 and k_3/k_{-3} are equal. However, it is not necessary to assume rate constant symmetry with $k_{-1} = k_3$. Indeed, the development and application of a method for evaluating k_{-1}/k_3 demonstrates that $k_{-1} \neq k_3$ for mechanisms in human erythrocytes and *Escherichia coli*. If J^m_i and J^m_o are maximal rates of initial influx and efflux, respectively, then with no sugar on the opposite side of the membrane one can show that if $J^m_i = J^m_o$, $k_{-1} = k_3$; if $J^m_i/J^m_o > 1$, $k_{-1}/k_3 < J^m_o/J^m_i$; if $J^m_i/J^m_o < 1$, $k_{-1}/k_3 > J^m_o/J^m_i$. ^{14}C-glucose flux across human erythrocytes showed $J^m_i/J^m_o = 0.15$ (three observations, range 0.14–0.16) and thus $k_{-1}/k_3 > 6.7$. Correspondingly, ^{14}C-thiomethylgalactoside flux across *E. coli* treated with metabolic inhibitors showed $J^m_i/J^m_o = 0.21$ (four observations, range 0.13–0.39) and thus $k_{-1}/k_3 > 4.8$. With equilibrium concentrations of sugar on the opposite side of the membrane, J^m_i/J^m_o should equal 1, and a mean value of 0.83 (range 0.71–0.92, three observations) was observed with human erythrocytes. Evaluating k_{-1}/k_3 may help classify facilitated diffusion mechanisms and permit exploration of the molecular basis of the asymmetry. (Supported by NIH grants AM-01483 and AM-04407.)

125. Phenobarbital-Induced Acceleration of Vitamin D Metabolism. THEODORE J. HAHN,* JOHN G. HADDAD,* STANLEY J. BIRGE,* AND LOUIS V. AVIOLI, St. Louis, Mo.

It has been observed that administration of compounds which stimulate microsomal metabolism of drugs also stimulates the microsomal hydroxylation of steroid hormones. Our studies of the metabolism of ^{3}H-vitamin D_3 in human subjects treated with phenobarbital showed a marked increase in the rate of conversion of ^{3}H-vitamin D_3 to more polar metabolites. Sprague-Dawley rats were injected intraperitoneally with phenobarbital 50 mg/kg b.i.d. for 4 days. Microsome fractions prepared from pooled livers of control and phenobarbital-treated animals were incubated aerobically with ^{3}H-vitamin D (20,000 dpm/unit) in pH 7.4 phosphate buffer in the presence of TPN, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and $MgCl_2$. Pretreatment with phenobarbital markedly accelerated the in vitro microsomal conversion of ^{3}H-vitamin D to more polar metabolites. Parallel incubations employing hexobarbital as the substrate showed similar increases in hexobarbital oxidation by microsomes from the phenobarbital-treated animals. These data demonstrate that phenobarbital administration produces accelerated conversion of vitamin D_3 to polar metabolites in vivo and increased microsomal conversion of vitamin D_3 in vitro, suggesting a similarity between hepatic mechanisms for metabolism of vitamin D and certain of the steroid hormones. (These studies were supported in part by NIH grant AM-11674.)

126. The Conversion of Cholesterol to 3α,7 α-Dihydroxy-5β-cholestan-26-oic Acid and Chenodeoxycholic Acid in Man. RUSSELL F. HANSON* AND JAMES B. CAREY, JR., Minneapolis, Minn.

The two primary bile acids in man (cholic and chenodeoxycholic acid) are normally produced in approximately equal amounts. In liver injury associated with certain liver diseases, the synthesis of chenodeoxycholic acid exceeds that of cholic acid. Chenodeoxycholic acid is also the immediate precursor of the secondary bile acid, lithocholic acid, which is a potent hepatotoxin in experimental animals. To study the synthetic pathway of chenodeoxycholic acid in man, we administered C-26-^{14}C-cholesterol to a bile fistula patient and isolated C-26-^{14}C-3α,7α-dihydroxy-5β-cholestan-26-oic acid from the bile after hydrolysis and Celite column chromatography. Identity was established by infrared and mass spectroscopy. We then prepared this compound by electrolytic coupling of chenodeoxycholic acid and D,L-methyl succinic acid, after which it was labeled with tritium by the Wilzbach method. After purification, 104,500 dpm in 4.0 mg of the compound were injected intravenously into a patient with a T-tube bile fistula. In the first 8 hr of the experiment 70% of the injected radioactivity was excreted in the bile. The bile acids were isolated after hydrolysis and column chromatography. Chenodeoxycholic acid was identified by thin-layer chromatography, m.p., infrared and mass spectroscopy. After isolation, chenodeoxycholic acid was crystallized to a constant specific activity which did not decrease after addition of a known quantity of unlabeled chenodeoxycholic acid. The chenodeoxycholic acid fraction accounted for 45% of the radioactivity injected. Approximately an equal amount of radioactivity in the bile was present as unchanged 3α,7α-dihydroxy-5β-cholestan-26-oic acid. Presumably the inactive isomer that was injected was excreted unchanged. These experiments demonstrate (1) that chenodeoxycholic acid is the major metabolic product of 3α,7α-dihydroxy-5β-cholestan-26-oic acid in man, and (2) that this 27 carbon bile acid is an intermediate in the conversion of cholesterol to chenodeoxycholic acid in man. (Research supported by grants from the NIH.)

127. Studies of Platelet and Fibrinogen Consumption. LAURENCE A. HARKER,* Seattle, Wash. (introduced by Clement A. Finch**).

Platelet and fibrinogen kinetics have been concurrently measured in 15 normal subjects and 72 selected patients with "consumptive coagulopathy." Platelet destruction was mea-

sured in terms of platelet survival time (^{51}Cr in vitro labeling) and platelet turnover (platelet count divided by platelet survival). Similarly, fibrinogen destruction was assessed as the average time in circulation (the $t_{\frac{1}{2}}$ disappearance time of the ^{125}I-fibrinogen divided by the natural logarithm of 2) and fibrinogen turnover (fibrinogen concentration divided by mean survival time of fibrinogen). Isolated platelet destruction occurred in 10 patients with immune thrombocytopenic purpura (survival 0.02–0.83 days; turnover 3–8 × normal); in 16 patients with artificial heart valves (survival 2.7–7.0 days, turnover 2–3 × normal); and in 4 patients with plastic AV shunts (survival 3.5–5.5 days, turnover 2–3 × normal). Fibrinogen kinetics remained normal in these patients. Conversely, destruction was limited to fibrinogen in 4 patients given urokinase (survival 0.3–0.5 days, turnover 15–20 × normal). In the remaining patients, destructive of platelets and of fibrinogen were combined. Equal rates of platelet and fibrinogen consumption were found in 6 patients with bacteremia (3–8 × normal turnover), and in 12 patients with metastatic malignancy such as carcinoma of the prostate (2–6 × normal turnover). In contrast, in the 14 patients with vascular injury (including hemolytic-uremic syndrome, TTP, and drug arteritis), platelet consumption greatly exceeded fibrinogen destruction (3–6 × normal compared with 1–2 × normal). Such platelet vascular consumption uniformly decreased with steroid therapy but was little changed by coumarins or heparin. These results indicate that platelets and fibrinogen are independently consumed and the relative involvement of each reflects the underlying mechanism. Comparable consumption of fibrinogen and platelets results from the activation of coagulation (intravascular coagulation), whereas predominant platelet consumption indicates vascular damage. (Research supported by grants 5-RO1-HE-11775, 5-RO1-HE-06242 from the NIH.)

128. Human Plasma Alpha$_2$ Macroglobulin: An Inhibitor of Plasma Kallikrein. PETER C. HARPEL,* New York, N. Y. (introduced by Ralph Nachman).

The mechanisms by which the human plasma kallikrein enzyme system is regulated remain uncertain. $C\bar{1}$ inactivator inhibits kallikrein and $C\bar{1}$ in purified systems. Activation of $C\bar{1}$ has been documented in the plasma of patients with hereditary angioneurotic edema (HANE) who lack $C\bar{1}$ inactivator, but activation of kallikrein has not. These findings may be explained by the results of the present study, which have demonstrated that HANE plasma inhibits plasma kallikrein, and suggest that the inhibitor responsible is the α_2-macroglobulin (α_2-M). Kaolin-activated kallikrein TAMe esterase activity in the plasma of four patients with HANE fell with time to 70% of peak activity and became progressively more resistant to inhibition by soybean trypsin inhibitor (SBTI). Acid treatment of HANE plasma destroyed the factor responsible for these effects. Purified α_2-M inhibited the esterase, rat uterine-contracting, and vascular permeability-inducing activities of purified plasma kallikrein. Chromatographic experiments indicated that this inhibition was accompanied by the formation of a SBTI and $C\bar{1}$ inactivator-resistant, high molecular weight complex, analogous to that formed between α_2-M and trypsin. Purified α_2-M, obtained from kaolin-activated plasma, contained 240 times the TAMe esterase activity of the α_2-M isolated from plasma containing Trasylol and SBTI, inhibitors of kallikrein and of its activation. The ability of the α_2-M, a known inhibitor of thrombin and plasmin, to inhibit plasma kallikrein provides a new example of an interrelation between the coagulation, fibrinolysis, and kallikrein pathways. This inhibitor may play an important role in modulating the relative participation of these complex enzyme systems in human pathophysiologic states. (Supported by NIH grant NB-03346-09.)

129. Regulation of Plasma Bicarbonate Concentration during Acid Ingestion; Characterization of the "Chronic Mineral Acid Response Curve." JOHN T. HARRINGTON,* RUI C. DE SOUSA,* EDMOND S. RICANATI,* JOEL W. SHELKROT,* AND WILLIAM B. SCHWARTZ,** Boston, Mass.

This study was designed to determine what factors control the degree of acidosis which develops during chronic ingestion of a standard acid load and to quantify the relation between the level of acid intake and the steady-state plasma [HCO_3]. In the initial studies, 7 meq/kg per day of H^+ was fed to dogs for at least 2 wk to evaluate the influence on acid-base equilibrium of both the accompanying anion and the dietary intake of NaCl. The data demonstrate that if the diet contains no NaCl, the character of the anion is the critical determinant of the plasma [HCO_3], average [HCO_3] showing no reduction in response to HNO_3, a reduction of 3.7 meq/liter with H_2SO_4, and a reduction of 8.6 meq/liter with HCl (differences between the groups significant at the 0.01 level). When H_2SO_4 and HCl were given in association with a normal NaCl intake, the differences attributable to the character of the anion were abolished. It is concluded that an interplay of hydrogen ion load, anion characteristics, and NaCl intake determines the degree of acidosis which develops with the chronic ingestion of mineral acids. Having defined these relations, we then undertook to characterize the physiologic "chronic mineral acid response curve," using HCl (3.5, 5.25, and 7.0 meq/kg per day) and an intake of 3.0 meq/kg per day of NaCl. The data demonstrate that the correlation between acid load and plasma [HCO_3] is best defined by a linear regression about the origin described by the equation $Y = 0.90\ X$. The 95% confidence interval of the slope fell in the strikingly narrow range of −1.00 to −0.80. These data provide a new framework for a study of the mechanisms which regulate the adaptive response of the organism to mineral acid loads. Analysis of the slope of the chronic response curve also provides a possible explanation for the difficulty in detecting the signals responsible for the renal response to normal variations in dietary acid intake.

130. Heterogeneity of Very Low Density Lipoproteins in Man: Evidence for a Functional Role of a Beta Migrating Fraction in Triglyceride Transport and Its Relation to Broad-Beta Disease (Type III Hyperlipoproteinemia). WILLIAM R. HAZZARD,* DANIEL PORTE, JR., AND EDWIN L. BIERMAN, Seattle, Wash.

Plasma very low density lipoproteins (VLDL) (Sf 20–400) have been considered as a class rich in triglyceride

(TG), with uniform α_2 (pre-β) electrophoretic mobility. Thus the finding of cholesterol-rich, β-migrating VLDL in broad-β disease has led to the suggestion that a unique, abnormal lipoprotein may underlie this disorder. To test this hypothesis, VLDL subfractions (Sf 100–400, 60–100, 30–60, and 20–30) were isolated by density gradient ultracentrifugation from subjects with endogenous (type IV) lipemia (n = 5) or broad-β disease (n = 3) and analyzed after starch block electrophoresis. In both disorders a continuum of decreasing mobility and increasing cholesterol (C) content (with reciprocally reduced TG) was observed as a function of declining Sf rate: at extremes of this spectrum, Sf 100–400 lipoproteins migrated rapidly (0.7 relative to albumin; α_2) and were TG rich (TG/C = 6), whereas Sf 20–30 lipoproteins were slow (0.4; β) and C rich (TG/C = 1). β-VLDL was qualitatively similar but comprised a smaller proportion of total VLDL in endogenous lipemia (2–16%) than in broad-β disease (18–59%). In subjects from both groups, β-VLDL concentration increased 20–50% after heparin, while α_2-VLDL declined. Oral fat (2 g/kg) also increased levels of β-VLDL (100–200%) at 12 hr, after an early rise in α_2-VLDL, evidence for a contribution of exogenous TG to both α_2- and β-VLDL. High-carbohydrate diets which elevated endogenous TG levels also produced a rise in β- as well as α_2-VLDL. Thus β-VLDL appears to be a normal intermediate in VLDL catabolism, with a role in both endogenous and exogenous TG transport in man. Acceleration of α_2-VLDL turnover increases β-VLDL concentration. Accumulation of this subfraction in broad-β disease suggests impairment in VLDL degradation at approximately Sf 20–30. (Supported by Veterans Administration and NIH research grants.)

131. Effect of Normal Pregnancy on Calcium Kinetics. ROBERT P. HEANEY AND THOMAS G. SKILLMAN,* Omaha, Nebr., and Columbus, Ohio.

During the final trimester of pregnancy the human fetus acquires about 25 g of calcium from its mother. While this demand significantly stresses maternal calcium homeostasis, the mechanisms of maternal response have not previously been assessed. In this study, stable ^{48}Ca was given intravenously to measure internal components of calcium metabolism, and standard metabolic ward procedures were used to measure external balance. ^{48}Ca from serum, urine, and stool was analyzed by activation to ^{49}Ca in a nuclear reactor. 38 studies were done: 24 in normal pregnant women between 20 and 40 wk gestation; four in postpartum women; nine in control nonpregnant women; and one during estrogen-progesterone "pseudopregnancy." There was significant retention of Ca, P, and N throughout the last 20 wk of gestation. Both miscible pool and intestinal calcium secretion rose progressively during gestation, but reached values significantly higher than control only during the last 5 wk. Both absolute calcium absorption and fractional absorption efficiency were twice control values at 20 wk and remained high throughout pregnancy. Increased bone calcium accretion was noted by the 20th week and remained at twice control values during the entire 3rd trimester. Bone resorption was initially lowered, but then rose in parallel with accretion. Fetal contribution to pool size and accretion cannot be separated from maternal by these techniques. Nevertheless, both the change in maternal intestinal calcium handling and the increase in accretion antedated the onset of fetal calcification, suggesting maternal mechanisms which anticipated fetal need. None of these changes was induced by estrogen-progesterone "pseudopregnancy." This suggests that other hormones (e.g. placental lactogen) account for calcium metabolic changes of pregnancy.

132. Fasting Intestinal Contents: Volume, Flow Rate, and Velocity. RICHARD J. HEBERT* AND KONRAD H. SOERGEL, Milwaukee, Wis.

Excessive intestinal fluid production is implicated in several diarrheal diseases, but physiologic fasting intestinal contents (FIC) (succus entericus) flow rates are unknown. Similarly, concepts of intestinal electrolyte absorption need to incorporate site and rate of FIC production. Fasting adults were intubated with a multilumen tube. Isotonic PEG 4000 in D-mannitol was infused (1.1 ml/min) at the ligament of Treitz (LT); 15 min samples were collected for 4 hr from tips located 70 cm (J) and 140 cm (I) distally. After PSP bolus injections at LT, 5 min samples were obtained from J and I. In 14 studies, FIC flow rate at J was 2.52 ± 0.26 (SEM) ml/min; at I, 1.41 ± 0.36 ml/min. This aboral flow gradient accounts for the apparent jejunoileal difference in water absorption from saline. Dye dilution curves indicated bolus flow through a single compartment. The flow velocity was 1.77 ± 0.11 cm/min at J; 1.63 ± 0.16 cm/min at I. The total FIC volume (mean transit time × flow rate) from ligament of Treitz to ileocecal valve was approximately 240 ml, assuming linear decrease in flow rate along 210 cm of intestine. In a patient with Zollinger-Ellison syndrome and diarrhea, FIC flow rate was 14.5 and 11.4 ml/min at J and I, respectively; FIC volume, 1130 ml. During atropinization flow rate decreased 47% at J with no change at I; flow velocity decreased by 71% at J and 65% at I. FIC accumulated in the intestine; the volume increased by 122%. Effective balloon occlusion of the distal duodenum caused a decrease in flow rate of 33% and in velocity of 22% at J and I; volume declined by 21%. FIC represents mainly small intestine rather than gastroduodenal secretions; its formation is unaffected by atropine. The mechanism responsible for the aboral decline in FIC flow rate remains to be identified. (Support: NIH research and postdoctoral fellowship grants.)

133. Cineangiocardiographic Prediction of the Unsuccessful Mitral Commissurotomy. STANLEY J. HELLER* AND RICHARD A. CARLETON, Chicago, Ill.

Mitral commissurotomy is the preferred surgical treatment of mitral stenosis (MS). Commissurotomy may fail because of severe distortion of the mitral valve (MV) complex, including the leaflets, chordae tendineae, and papillary muscles. Such patients are recognized preoperatively only when significant calcification is present. Attempting to recognize the noncalcified cases preoperatively, we studied 35 consecutive patients with pure MS who had left ventricular (LV) cineangiocardiograms. These patients have impaired LV function, evidenced on cineangiocardiograms by increased end-systolic volumes and decreased ejection fractions, with

distortion, rigidity, and decreased mobility of the posterobasal area of the LV adjacent to the MV. Study of surgical and autopsy specimens has permitted recognition of the cineangiocardiographic appearance of specific posterobasal abnormalities: fibrosis and endocardial thickening of the LV wall, thickening and fusion of the chordae tendineae, and shortening of the LV inflow tract by a contracted mitral complex. Cineangiocardiographically normal LV were seen in only five patients; none required surgery. Mild to moderate LV cineangiocardiographic abnormalities, principally posterobasal wall changes, were seen in 24 patients. Four did not require surgery, seven had prostheses implanted because of valvular calcification, nine had commissurotomies considered satisfactory at surgery, and in four dense scarring either prevented adequate commissurotomy or led to significant mitral insufficiency. Severe LV cineangiocardiographic abnormalities, principally marked chordal fusion and inflow tract shortening, were seen in six patients. Two had prostheses implanted because of calcification. Four had unsatisfactory commissurotomies. In two of these the severely scarred MV and chordae could not be opened at all; both patients died postoperatively. Severe derangement of the subvalvular components of the mitral complex precludes successful commissurotomy and is identifiable preoperatively by selective LV cineangiocardiography. (Supported by USPHS training grant HE-05714-04 from the National Heart Institute.)

134. Colony Inhibition of Fibroblasts from Chimeric Dogs Mediated by the Dogs' Own Lymphocytes and Specifically Abrogated by Their Serum. I. HELLSTRÖM,* K. E. HELLSTRÖM,* R. STORB,* AND E. D. THOMAS,** Seattle, Wash.

Previous studies using the colony inhibition test have shown that antigenic tumors may grow progressively in vivo because humoral serum factors (enhancing antibodies?) are present which protect the neoplastic cells from attack by immune lymphocytes. A similar type of mechanism appears to provide part of the explanation why antigenically foreign mouse embryos are not rejected by their mothers' lymphocytes, which are immune to paternally derived alloantigens of the fetuses. The present studies were undertaken to determine the nature of the tolerance in radiation-induced chimeras. Nine healthy canine radiation chimeras were studied between 173 days and 2.5 yr after 1200–1500 R of total body irradiation and transplantation of allogenic bone marrow. When donor and recipient were of opposite sex, cytogenetic analysis invariably showed 100% donor type lymphocytes in the peripheral blood, marrow, and lymph nodes. Skin fibroblasts from chimeras and from normal dogs were plated in vitro and tested for colony inhibition by exposure to sera and peripheral lymphocytes from both chimeric and normal dogs. In all cases studied, it was found that lymphocytes from a chimeric dog could inhibit colony formation by plated fibroblasts from the same dog, whereas lymphocytes from other chimeras or from normal dogs did not. Serum from the same chimera, but not from normal dogs or other chimeras, abrogated the inhibitory effect of the lymphocytes. The results of these in vitro studies show that the lymphocytes in the chimera (donor lymphocytes) are capable of inhibiting the host fibroblasts and suggest that the immunological "tolerance" of these chimeric dogs is mediated in vivo by blocking serum factors which may be enhancing antibodies.

135. On the Physiological Mechanism of Sensory Changes Produced with LSD-25. ROBERT HENKIN, Bethesda, Md.

Subjective responses, sensory detection thresholds, and measurements of perceptual capacity for taste, smell, and hearing were obtained in five chronic LSD users, aged 21–33. Each subject was studied on a metabolic ward for two 8 day periods 1 yr apart under several conditions: (1) base line, (2) administration of LSD, 1 μg/kg or 2 μg/kg, (3) 24 hr after LSD, and (4) after administration of 15 mg D-amphetamine or 50 mg nicotinic acid. Median detection and recognition thresholds for taste and smell under base-line conditions were normal; however, mean audiometric thresholds were significantly below normal (−8 db ASO ± 1.8 SE at 1000 Hz) although auditory perception was normal or moderately impaired. Neither thresholds nor auditory perception changed after administration of amphetamine or nicotinic acid. After administration of the higher dose of LSD, taste detection acuity increased while recognition ability decreased. Mean audiometric thresholds decreased farther below base line (−12 db ASO ± 1.3 at 1000 Hz), while thresholds for auditory discomfort were significantly elevated, indicating expansion of the dynamic range of hearing. Several indices of auditory perception became severely impaired on LSD; as compared with base line, word recognition ability decreased significantly (14%), tone localization became impaired, and the ability to judge loudness of tones presented alternately to each ear decreased (mean error >15 db). Many values returned to base line within 24 hr after LSD was discontinued. No significant olfactory changes occurred. These studies indicate that chronic LSD users, like patients with untreated adreno cortical insufficiency, have increased detection acuity for some sensory modalities while exhibiting decreased perceptual ability. These changes, which correlate with subjective responses of users, are exaggerated during acute LSD administration. This suggests that LSD heightens sensory acuity while decreasing ability to integrate and interpret sensory signals.

136. Bone Collagen Metabolism and the Etiology of Osteoporosis. DOROTHY H. HENNEMAN,* CHARLES Y. C. PAK,* MEYER D. LIFSCHITZ,* AND FREDERIC C. BARTTER,** Raritan, N. J., and Bethesda, Md.

Studies of cell metabolism and of the composition and solubility of collagen were carried out in fragments of metaphyseal bone obtained by biopsy from eight patients with severe early-onset "idiopathic" osteoporosis, and at autopsy from eight patients with senile or postmenopausal osteoporosis; biopsy specimens from 23 normal subjects served as controls. In patients with severe early-onset osteoporosis, total collagen of bone per unit dried, defatted, decalcified matrix was markedly below normal ($P < 0.01$), whereas the amount soluble in neutral saline, acid, weak alkali, or EDTA was above normal. Values for

proline per unit decalcified bone were only slightly below normal ($P < 0.05$), and those for mucopolysaccharide moderately below normal; this indicates a hydroxyproline-poor collagen or an unidentified protein. When incubated with glucose and ^{14}C-L-proline, these fragments produced more lactate than normal, but the specific activity of proline and hydroxyproline in the soluble and insoluble collagen fractions was normal or above normal. Cell nitrogen per unit dried, defatted, decalcified matrix was also increased, so that total collagen biosynthesis in the fragments was elevated. In bone from patients with postmenopausal and senile osteoporosis, the percentage of collagen soluble in neutral saline and in acid was increased but total collagen was not decreased. A decrease in total collagen in severe idiopathic osteoporosis in the presence of normal or increased synthesis suggests an increase in degradation; this could reflect failure of newly synthesized, abnormally soluble (poorly polymerized) collagen to calcify. Increased lactate production is also associated with increased bone resorption. These findings in severe idiopathic osteoporosis suggest that abnormalities in collagen metabolism may be primary factors in the etiology of this disorder.

137. Morphology of Contraction of the Human Right Ventricle. MICHAEL V. HERMAN,* CHARLES E. BEMIS,* DOUGLASS F. ADAMS,* EDMUND H. SONNENBLICK, AND RICHARD GORLIN, Boston, Mass.

In animal studies, Rushmer compared the right ventricular (RV) contraction pattern to an "old-fashioned bellows." In contrast, Carlson concluded that systolic contraction of the canine RV resembles a "check-valve pump," where a downward movement of the pulmonic valve and RV outflow tract toward the apex advances the column of blood into the pulmonary artery without significant RV inward motion. In 20 subjects found to be normal at diagnostic cardiac catheterization, biplane cineventriculography (100 frames per sec) was performed in a 30° right anterior oblique (RAO) and a 60° left anterior oblique (LAO) position, using a power injection of 50 ml contrast agent. After life-size calibration, motion analysis was plotted as a function of time for each projection as per cent shortening from end-diastolic length for the long axis, the outflow tract, and RV mid and apical short axes in the RAO position, and the septum to free wall axes in the LAO position. Results showed uniform inward movement of all dimensions along the normal RV inner silhouette during systolic contraction. This was similar to that seen in the normal left ventricle with no time delay between body and outflow tract contraction. Each minor axis shortened 30–50% and the long axes 10–30%, this movement being similar in magnitude again to left ventricular systole. Shape analysis in subjects and models suggested a complicated geometric figure resembling a crescent-shaped triangle. Initial RV volume studies suggest that a simple figure of revolution does not apply and an integrated segmental volume analysis is necessary. Although its geometric shape is complex, the normal human RV exhibits a uniform inward squeezing movement during systolic contraction both in time and in space. This forms a basis for analysis of diseases affecting the right ventricle.

138. Demonstration of Transfer RNA Uptake by Human Lymphocytes and Evidence for Relation with the Cell Cycle. F. HERRERA,* R. C. GALLO,* AND R. H. ADAMSON,* Bethesda, Md. (introduced by D. P. Rall**).

A block in cell differentiation due to changes in protein synthesis secondary to altered tRNAs may be a mechanism for leukemogenesis, and differences in tRNA between normal and leukemic human lymphoblasts have been demonstrated in this laboratory. To study the effects of different tRNAs on normal and leukemic cells, we have shown that exogenous tRNA is taken up by mammalian cells. ^{14}C-tRNA was isolated from log phase *Escherichia coli* grown with 50 μM ^{14}C-uridine. ^{14}C-tRNA (1 μg/10^6 cells) was added at the start of incubation to L1210 leukemic cells and human lymphoblasts in tissue culture (NC-37). Aliquots were taken at intervals, deposited on Millipore filters, and washed, and macromolecules were precipitated with TCA and counted. A rapid uptake of tRNA was demonstrated (0.05 μg/10^6 cells per min) in L1210 cells and human lymphoblasts with equilibrium attained after 1 min of incubation. The uptake was not due to tRNA degradation products. Uptake was confirmed by radioautographic electron microscope studies. Evidence was obtained that cell viability and membrane integrity were not impaired. Studies were also designed to determine whether uptake depended on the cell cycle. Normal human lymphocytes were stimulated with PHA for various times. The tRNA uptake by unstimulated lymphocytes was low ($\frac{1}{3}$ to $\frac{1}{4}$ of L1210 or NC-37), increased after 12 hr of PHA stimulation, and was maximal after 24–48 hr, a time of greatest RNA and DNA synthesis. The uptake at this time approached the level found in L1210 and NC-37 cells. These results show that (1) mammalian cells rapidly take up exogenous tRNA; (2) the uptake may be dependent on the cell cycle, and changes in uptake by human lymphocytes occur after stimulation with PHA.

139. Placental Content and Purification of Human Chorionic Thyrotropin. JEROME M. HERSHMAN* AND WILLARD R. STARNES,* Birmingham, Ala. (introduced by Ben Friedman**).

The human chorionic thyrotropin (HCT) content of 15 placentas was measured by bioassay and radioimmunoassay after extraction of each placenta individually by a procedure described previously. There was marked individual variation, with a range of 3–18,500 mU TSH per 500 g placental tissue. 12 placentas contained less than 70 mU, two were in the range found previously in extraction of batches of placentas (113–2200 mU), and one greatly exceeded this range. Incubation of HCT with a placental enzyme preparation failed to destroy biologic or immunologic activity, indicating that proteolytic degradation of HCT within the placenta was not the explanation for the marked individual variation in placental HCT content. Preliminary data suggest that this variation is mainly attributable to a decline in synthesis of HCT at the end of pregnancy. Gel filtration of the thyrotropic fraction (89 mU/mg protein) from the placenta rich in HCT yielded a material with activity of 1000 mU/mg. The molecular weight of this HCT, based on its mobility on Sephadex, was identical with that of ^{125}I-labeled bovine

thyrotropin (BTSH). The highly purified HCT was tagged with ^{125}I and reacted with an antibody to BTSH; 62% of the labeled HCT was immunoreactive, which implies that the true specific activity of HCT may be 1500–2000 mU/mg. The ^{125}I-labeled HCT formed precipitin bands corresponding to BTSH on radioautographs after immunoelectrophoresis and immunodiffusion against anti-BTSH. Disc gel electrophoresis showed that ^{125}I-labeled HCT was much more electronegative than ^{125}I-BTSH. These data indicate that, although HCT and BTSH share a major immunologic determinant and have a similar molecular weight, there is a significant molecular difference between them.

140. Herpes-Like Virus Infection in Sarcoidosis. YASHAR HIRSHAUT,* PHILIP GLADE,* LUIZ OCTAVIO VIEIRA,* EUGENE AINBENDER,* LOUIS SILTZBACH,* AND KURT HIRSCHHORN, New York, N. Y.

Since its discovery within leukocyte cultures prepared from Burkitt's tumor, the herpes-like virus (HLV) has been implicated in the etiology of infectious mononucleosis and is suspect in the pathogenesis of Burkitt's lymphoma and nasopharyngeal carcinoma (NPCa). The evidence for these associations derives from seroepidemiological data which show that all patients with these diseases have antibody to HLV. The antibody may first appear during infectious mononucleosis, but is present at high titer in sera from patients with Burkitt's tumor and NPCa. We have identified a fourth disease, sarcoidosis, in which all patients have anti-HLV antibody, often at markedly elevated titers. Sera from 131 patients with sarcoidosis were titered against HLV in the Jijoye cell line using an indirect immunofluorescence assay. All sera gave positive reactions at a 1/10 dilution. Whereas 76% of 91 control sera reacted at this low titer, 79% of titers in patients with sarcoidosis were 1/640 or above. Such titers were most frequent in individuals with chronic disease of more than 2 yr duration (77%) and in patients who had apparently recovered from their disease (81%). Patients with disease of recent onset had a 46% incidence of titers greater than 1/640. Immunoglobulin concentrations were determined in 125 sera. High anti-HLV titers were more common in sera with high IgG levels, but many sera with normal IgG concentrations had titers of 1/640 or higher. The association of at least four diseases with infection by the herpes-like virus suggests that conclusions regarding the pathogenic role of HLV in any condition should be made with caution. (Supported by NIH grants CA-08748, AI-02272, AI-07131; SVCP 69-2078.)

141. Uptake of Free Fatty Acids by Human Platelets. JOHN C. HOAK,* ARTHUR A. SPECTOR,* AND GLENNA L. FRY,* Iowa City, Iowa (introduced by Willis M. Fowler**).

In short-term incubation studies, most of the albumin-bound ^{14}C-labeled free fatty acid (FFA) taken up by platelets remained unesterified. At a given FFA:albumin molar ratio ($\bar{\nu}$), uptake of saturated FFA was greater than that of unsaturated FFA. Uptake increased as the $\bar{\nu}$ was raised. Most of the labeled FFA in the platelets could be removed by exposure of the platelets to albumin. This suggested that the labeled FFA was associated chiefly with the platelet surface. Radioautography with electron microscopy was used to localize the radioactivity after albumin-bound ^{3}H-palmitate had been incubated with platelets for 5 min. Analysis of grain counts (mean percentage of total counts ± SEM) indicated 61 ± 2% activity with the platelet membrane, 17 ± 2% in vacuoles and canaliculi, 2 ± 1% in granules, < 1% in mitochondria, and 16 ± 2% in cytoplasm. Background count was 4 ± 1%. These results suggested that most FFA was in the platelet membrane or in contact with the surface. Effects of FFA uptake upon platelet function were also evaluated. Platelet suspensions, containing fibrinogen and glucose, were incubated with albumin-bound FFA before ADP was added. Fatty acid–poor albumin was a control. Unsaturated FFA had little effect, but palmitate and stearate enhanced ADP-induced platelet aggregation progressively as $\bar{\nu}$ increased. Mixtures of FFA, found in human plasma, had an effect similar to that found with individual fatty acids. Enhanced ADP-induced aggregation was observed with platelets suspended in plasma containing high FFA as compared with platelets in plasma with low FFA. These results suggest that FFA uptake by the platelet may alter membrane reactivity and represent an important influence upon platelet function and survival. (Research supported by grants from the NIH.)

142. Hydroxy Fatty Acid: An Apparent Cause of Diarrhea in Patients with Ileal Resection and Steatorrhea. ALAN F. HOFMANN, J. RAINER POLEY,* HAGOP S. MEKHJIAN,* AND SIDNEY F. PHILLIPS,* Rochester, Minn.

We previously described three patients with large ileal resection, interrupted enterohepatic circulation of bile acids, decreased jejunal bile acid concentration, steatorrhea, and diarrhea. Fecal weight, frequency, and Na^+ were decreased by replacement of dietary long-chain fatty acid (LCFA) by medium-chain fatty acid (MCFA) but not by cholestyramine. To explain this apparent cathartic effect of LCFA, we measured its bacterial degradation product, hydroxy fatty acid (OHFA), which is structurally similar to ricinoleic acid, a known cathartic. OHFA excretion was high with LCFA but decreased markedly with MCFA substitution. There was extensive bacterial degradation of bile acids and an acidic fecal pH (6.3–6.5). The predominant bile acids, lithocholic and deoxycholic acids, are insoluble under these conditions; also the concentration of bile acids in the aqueous phase of ultracentrifuged stool was <2 mM, too low to induce water secretion in the perfused human colon. Four control patients with smaller resections and similar diarrhea but little steatorrhea did not excrete OHFA and were unimproved by MCFA substitution but responded to cholestyramine. These patients had much less bacterial degradation of bile acids, higher fecal pH (6.7–7.3), and a concentration of bile acids in solution (3–7 mM) which would induce secretion in the perfused colon. In contrast to those with large resections, these patients are considered to have bile acid diarrhea. Thus, complex bile acid–fatty acid–bacteria interactions determine the cause of diarrhea in patients with ileal resection. In patients with large resection and steatorrhea (>20 g/day), bile acid malabsorption causes fat maldigestion leading to fat malabsorption; bacteria convert

unabsorbed fatty acid to OHFA, which appears to be a major cause of diarrhea. (Research supported by a grant from the NIH.)

143. Coronaviruses and Viral Hepatitis. A. W. HOLMES,* F. DEINHARDT, W. HARRIS,* F. BALL,* AND G. CLINE,* Chicago, Ill., Oak Ridge, Tenn., and Birmingham, Ala.

Zuckerman, Taylor, and Almeida recently have reported the presence of paramyxovirus-like particles resembling coronaviruses in sera of patients with HAA-negative chronic active hepatitis. During the past 3 yr, in our studies on the transmission of viral hepatitis to marmosets, we have examined infectious and control (preinoculation) sera of marmosets and acute-phase sera of patients by density gradient centrifugation, by electron microscopy, and by testing for infectivity in marmosets. The infectious agent in acute-phase marmoset sera banded at a density of 1.21 in cesium chloride, and such infectious fractions (but not noninfectious fractions of different densities) contained intact paramyxovirus-like particles with a diameter of about 1200 A. These were morphologically similar to the coronaviruses reported by Zuckerman and associates. Similar paramyxovirus-like particles were seen at a density of 1.21 from acute-phase sera of two humans with viral hepatitis, and one of these sera induced hepatitis in marmosets. No such particles could be demonstrated in preinoculation marmoset sera, two normal human sera, or the acute-phase serum of a human case of HAA-positive serum hepatitis. This HAA-positive serum and its 1.20–1.25 and 1.30–1.32 density gradient fractions were not infectious for marmosets. The independent observations of paramyxovirus-like particles in sera of chronic hepatitis patients by Zuckerman and associates, and in human and marmoset sera by our own group, do not prove that these particles are the causative agents of human infectious viral hepatitis, but they are sufficiently suggestive to justify further intensive studies of their significance in human viral hepatitis. (Supported by the United States Army Medical Research and Development Command, the National Institute of Allergy and Infectious Diseases, the National Cancer Institute, the National Institute of General Medical Sciences, and the Atomic Energy Commission.)

144. Postnatal Fluid Reabsorption in Single Nephrons of the Dog Kidney. MICHAEL HORSTER,* Hanover, N. H. (introduced by Heinz Valtin).

Postnatal renal function was determined by superficial nephron micropuncture analysis combined with clearance techniques in 24 purebred beagles ranging in age from 2 to 74 days. Glomerular filtration rate (C_{In}; n = 183) and renal plasma flow (C_{PAH}/E_{PAH}; n = 139), which were monitored continuously, increased linearly with age from 0.13 and 0.80 to the adult levels of 1.0 and 3.0 ml/min per g kidney weight (KW), respectively; E_{PAH} (n = 52) increased from 0.22 to 0.83, and filtration fraction doubled during this age span. Since intratubular perfusion of superficial nephrons was not visible before the 3rd week of life, single-nephron analysis was carried out in 14 of the 24 dogs, 21–74 days old. Single-nephron filtration rate (n = 84) increased from 3.2 to 20.5 nl/min, and reached mature values of 1.4 nl/min per g KW at the end of this period. Proximal tubular transit time was constant at all ages (mean 11.6 ± 0.2 sec [SEM]; n = 37), whereas tubular diameter (n = 62) increased 3-fold. Tubular fluid-to-plasma inulin ratio (TF/P_{In}) at the accessible end of superficial convoluted tubules averaged 1.94 ± 0.04 (n = 84), and was independent of age, indicating constant proximal fractional fluid reabsorption (mean 48.5%). Intratubular hydrostatic pressure remained constant with age at 11.9 ± 0.3 mm Hg (n = 76), whereas peritubular capillary pressure (n = 79) increased from 6.1 mm Hg at 2 days to the intratubular value at 40 days. These findings indicate that glomerulotubular balance of sodium and water is achieved with the onset of postnatal intratubular perfusion. Further, this adjustment of proximal reabsorption to load in the maturing nephron occurs in the presence of changing tubular volume, peritubular capillary pressure, and filtration fraction. (Supported by the National Kidney Foundation and USPHS research grant AM-08469.)

145. Forearm Metabolism in Human Experimental Obesity. EDWARD S. HORTON,* CARL F. RUNGE,* AND ETHAN A. H. SIMS,* Burlington, Vt. (introduced by Gilbert H. Mudge**).

To evaluate whether or not peripheral insulin resistance develops in normal subjects who gain weight, forearm metabolism was studied by the technique of Rabinowitz and Zierler in five volunteers at lean weight and after gaining 17–25% above base-line weight by overeating, and in additional control subjects. Blood flow (ml/min per 100 ml forearm), arterial–deep venous (A-DV) and arterial–superficial venous (A-SV) concentration differences (μmoles/ml), and uptake or production rates (Q = μmoles/min per 100 ml forearm) of glucose and lactate were measured before, during, and after arterial insulin infusion (100 μU/kg per min). Forearm blood flow was not significantly different at lean and peak weight. In spite of an increase in mean basal arterial immunoreactive insulin from 6.9 ± 0.3 μU/ml at lean weight to 10.3 ± 0.8 at peak weight, mean basal glucose A-DV was reduced from 0.13 ± 0.03 to 0.06 ± 0.02; A-SV from 0.11 ± 0.02 to 0.07 ± 0.03; and Q from 0.87 ± 0.31 to 0.55 ± 0.28. There was no significant change in basal lactate metabolism. These findings are in contrast to the increased basal glucose uptake observed by others in spontaneous obesity. Four out of five subjects had a significantly decreased response to insulin at peak weight. Mean glucose A-DV during insulin infusion decreased from 0.65 ± 0.13 at lean weight to 0.29 ± 0.15 at peak weight; A-SV from 0.35 ± 0.06 to 0.12 ± 0.03; and Q from 3.92 ± 0.55 to 2.76 ± 1.48. Mean lactate production was unchanged from −0.70 ± 0.08 to −0.72 ± 0.17. In all cases, deep venous insulin concentrations were higher during the peak weight study than during the lean weight study. These results indicate that, as in spontaneous obesity, peripheral insulin resistance may develop in normal lean subjects with experimental obesity of equivalent degree. (Research supported by grants from the USPHS, National Institute of Arthritis and Metabolic Diseases and General Clinical Research Centers.)

146. Variable Representation and Immunogenicity of Histocompatibility Antigens on Different Tissues. ALLAN HOTTI,* SHU-MAN FU,* AND HALSTED HOLMAN, Stanford, Calif.

Major human and mouse transplantation antigens are present on cell membranes. Mouse antigens induce hemagglutinating and cytotoxic antibodies, and rejection sensitization. It has been assumed that the antigens are represented uniformly on all tissues, albeit in different concentrations, and that the same antigen induces both humoral and cellular responses. Membrane antigens of different tissues have been studied, employing, as donor-recipient pairs, mice from congenic strains which differ only at the major histocompatibility chromosome region (H-2). Data will be presented demonstrating that (1) H-2 antigens on membranes of spleen, liver, kidney, and heart exist in qualitatively different arrays: (2) antigens for the hemagglutinating and cytotoxic antibody response exist on liver membranes in tolerogenic form; (3) antigens responsible for the hemagglutination, cytotoxic, and rejection reactions are partially dissimilar; (4) immunogenicity can be modified selectively by chemical means; and (5) humoral and rejection responses in the same animal are separable. The varying representation and immunogenicity of histocompatibility antigens on different tissue may in part explain the variability observed in rejection responses to grafts of different tissues. They may also condition the appearance of hyperacute, normal, or chronic rejection patterns which are observed clinically in different, immunologically altered recipients. The identification of histocompatibility tolerogens, and the ability to modify antigens chemically, underscore the possibility of employing transplantation antigens to induce specific, harmless, clinically meaningful immunological unresponsiveness to donor tissues. Maximal allograft survival may require preparation and assessment of recipients in specific ways for each type of tissue graft. (Research supported by a grant from the NIH.)

147. The Immune Response in Canine and Human Brucellosis As Determined by Antigen Processing. CRAIG W. HOWE,* Minneapolis, Minn. (introduced by Wesley W. Spink**).

The variation in antibody response is an indirect means for assessing the specific antigenic portion of an invading microbe. A *Brucella canis* infection in the beagle, the natural host for this rough, Gram-negative bacterium, which also causes human infection, has provided a model system for studying the immune response to a defined macromolecular complex. Using both purified canine and human antibodies coupled to cyanogen bromide–activated Sepharose, a matrix capable of binding specific antigenic portions of a crude bacterial sonicate was formed. Dogs naturally infected with *Br. canis* produced initially large quantities of both 19S precipitins and agglutinins in the serum. When these IgM antibodies acted as the immunoadsorbent, a lipipolysaccharide (LPS) was eluted as the major antigenic stimulant. Approximately 3 wk later, sera $7S_{\gamma 1}$, $7S_{\gamma 2a}$, and $7S_{\gamma 2b}$ predominated. These three classes reacted weakly with the LPS, but the major antigen now appeared as a protein-nucleic acid complex. Although a small amount of IgA was present in the colostrum of an infected bitch, specific antibody was associated only with a heretofore undescribed mercaptoethanol-sensitive 7S fraction exhibiting IgG electrophoretic mobility. This colostral antibody reacted solely with yet another unique carbohydrate antigen. In the human the early and late responses were characterized by IgG antibodies directed almost exclusively toward the LPS component. In a patient infected with *Brucella suis,* a related organism, cross-reacting antibodies toward *Br. canis* were 7S in nature and again reacted with the LPS. The evidence from the chronically infected canine suggests either a delayed unmasking of the protein–nucleic acid complex or a slow release of the three 7S cell-bound classes, but in either case represents a mechanism unlike the one seen in man. (Supported by NIH training grant 5-TO1-AI-00194-08.)

148. The Secretion and Interconversion of Deoxycorticosterone with Its C-21 Sulfoconjugate. TAH H. HSU* AND TURNER BLEDSOE,* Baltimore, Md. (introduced by Richard J. Johns).

After simultaneous injection of 1,2-^{3}H-DOC-SO_4 and 4-^{14}C-DOC into a human subject, 72 hr urines were collected to study the rates of production (PR), secretion (SR), and interconversion of deoxycorticosterone (DOC) and its C-21 sulfoconjugate (DOC-SO_4). Double-isotope derivative dilution and competitive protein-binding radioassay were employed for the measurement of specific activity (sp. act.) of DOC-SO_4 and tetrahydro-DOC-glucuronide (H_4DOC). The ^{14}C sp. act. for DOC-SO_4 was less than that for H_4DOC, demonstrating that DOC-SO_4 is a secretory product of the human adrenal. The interconversion rates were low: that of DOC to DOC-SO_4 ranged from 0.002 to 0.013; that of DOC-SO_4 to DOC ranged from 0.033 to 0.104. The PR of DOC-SO_4 in normals averaged 95.5 μg/day, in mild Cushing's syndrome 342 μg/day, during metyrapone 841 μg/day, and in the ectopic ACTH syndrome 819 μg/day. These data suggest ACTH stimulation of DOC-SO_4. Analysis of specific activity for both ^{3}H and ^{14}C of DOC-SO_4 and H_4DOC before and during metyrapone, however, showed the following results: Before metyrapone: SR DOC 477 μg/day, SR DOC-SO_4 114 μg/day, PR DOC-SO_4 342 μg/day; during metyrapone, SR DOC 10,577 μg/day, SR DOC-SO_4 148 μg/day, PR DOC-SO_4 841 μg/day. This analysis shows that 47% of the DOC-SO_4 produced during metyrapone is a result of the interconversion of DOC to DOC-SO_4 and that the SR of DOC-SO_4 does not rise significantly. Conclusions: (1) Deoxycorticosterone C-21-sulfate is shown in man to be among those sulfoconjugated steroids secreted preformed by the adrenal gland. (2) Though the interconversion rate between DOC and DOC-SO_4 is low, the disproportionate rise in DOC secretion under conditions of ACTH excess allows DOC to contribute significantly to DOC-SO_4. Therefore all studies of DOC-SO_4 metabolism must include measurement of DOC and DOC-SO_4. (3) ACTH is not a major determinant of DOC-SO_4 biosynthesis, hence this steroid may be associated with the biosynthetic pathways located outside the zona fasciculata. (Research supported by NIH grant 5-RO1-AM-11607.)

149. Impairment and Recovery of Pulmonary Antibacterial Defense Mechanisms after Oxygen Administration. GARY HUBER,* MARC LA FORCE,* AND ROBERT MASON,* Boston, Mass., and Bethesda, Md. (introduced by Edward H. Kass**).

The relation between oxygen administration and the development of pulmonary infection is not well understood. Intrapulmonary bacterial inactivation was evaluated by quantitating bacterial viability and radiotracer activity in lungs of mice 6 hr after exposure to an aerosol of radiolabeled (^{32}P) *Staphylcoccus aureus*. Control animals (P_{O_2} 120 mm Hg) inactivated 92.9 ± 0.5% of the inhaled bacteria. In animals exposed for 48 hr or less and in 42% of the 72 hr animals, administration of 100% oxygen (P_{O_2} 650 mm Hg) induced progressive impairment of bacterial inactivation proportional to duration of oxygen exposure (86.5 ± 1.6% inactivation after 12 hr exposure, 78.5 ± 2.4% after 24 hr, 69.2 ± 3.4% after 48 hr, and 66.0 ± 5.6% after 72 hr). Electron microscope observation of cells recovered by bronchopulmonary lavage demonstrated progressive vacuolization of alveolar macrophages. Correlation of pulmonary phospholipid analyses, histology, and wet-dry lung weights with bacterial inactivation revealed that dose-related alterations in macrophage structure and function occurred before the development of pulmonary edema, congestive atelectasis, or hyaline membrane formation. In 58% of 72 hr and 92% of 96 hr oxygen-exposed animals, bacterial replication exceeded inactivation following the development of biochemical and structural characteristics of pulmonary oxygen toxicity. There was also a dose-response depression of pulmonary antibacterial activity at lower oxygen concentrations, with 74.5 ± 4.3% inactivation in animals exposed to 60% oxygen (P_{O_2} 400 mm Hg) for 72 hr. After exposure to 100% oxygen for 72 hr, an additional 72 hr in ambient air was required for recovery of normal bacterial killing. Thus, excess oxygen is toxic to pulmonary alveolar macrophages and to their antibacterial function in a dose-dependent relation. Bacterial replication exceeds inactivation when pulmonary parenchymal manifestations of oxygen toxicity occur.

150. Barium-Soluble Acid-Labile Carbon Dioxide in Muscle and Plasma Extracts. JAMES B. HUDSON,* Augusta, Ga. (introduced by Alfred J. Bollet).

When muscle HCO_3^- content is calculated from the total acid-labile CO_2 of an alkaline extract, a problem is raised by the apparent failure of $BaCl_2$ to completely remove the acid-labile CO_2 by precipitation. Since this phenomenon is not well understood, and might even reflect CO_2 release by non-HCO_3^- sources, we have attempted to characterize further this barium-soluble fraction. Using the Conway microdiffusion technique, mean barium-soluble CO_2 was 84% of total CO_2 for rat muscle extracts and 9% for plasma. On a gel filtration column (Sephadex G-25) 93–102% of plasma total CO_2 was recovered in a single peak at column volume; barium-soluble CO_2 was eluted in earlier samples, largely in association with protein, and in amounts substantially greater than applied to the column. When alkaline extracts of muscle were acidified with HCl and realkalinized with NaOH, no acid-labile CO_2 could be detected before addition of $BaCl_2$, but barium-soluble CO_2 was still present in amounts twice those found before acidification. Barium-soluble CO_2 alone was also found after similar treatment of alkaline solutions of casein, casein hydrolysate, glutamic acid, and valine. Mean barium-soluble radioactivities after addition of $NaH^{14}CO_3$ to alkaline extracts of muscle, plasma, and $NaHCO_3$ were respectively 44, 4.5, and 0.4% of total radioactivity. These studies suggest that while HCO_3^- in alkaline muscle extracts appears to be partially protected from precipitation by $BaCl_2$, a portion of barium-soluble CO_2 may also be derived from sources which do not contribute to total CO_2. (Research supported by a grant from the Life Insurance Medical Research Fund.)

151. Type X Glycogenosis: Deficient Activity of Cyclic Adenosine-3′,5′-Monophosphate–Dependent Kinase. GEORGE HUG, WILLIAM K. SCHUBERT,* AND GAIL CHUCK,* Cincinnati, Ohio.

A 5-yr-old white girl presented with asymptomatic hepatomegaly. No other physical abnormalities were present. Glycogen concentration in the liver was 9.8% (normal <6%) and in the skeletal muscle 2.1% (normal <1%). Excessive glycogen accumulation was confirmed by electron microscope examination. Phosphorylase activity in the liver was 0.8 μmoles phosphate per g per min (normal mean 25.1 μmoles phosphate per g per min, SD 6.5). In vitro activation of phosphorylase occurred after the addition of phosphorylase kinase to the liver homogenate, thus excluding type VI glycogenosis (liver phosphorylase deficiency). In the muscle, total phosphorylase was normal (54.1 μmoles phosphate per g per min), but it was all in the inactive form (i.e. present as phosphorylase *b*), whereas normally 80% of total human muscle phosphorylase is in the active form (i.e. present as phosphorylase *a*). In vitro activation of muscle phosphorylase occurred at pH 6.8 in the absence of cyclic 3′,5′-AMP, thus excluding phosphorylase kinase deficiency (type IX glycogenosis). In vitro activation of phosphorylase did not occur at pH 6.8 even in the presence of cyclic 3′,5′-AMP. These results may be explained by deficient activity of the cyclic 3′,5′-AMP–dependent kinase that normally activates phosphorylase kinase. This interpretation is further supported by normal activation of muscle phosphorylase at pH 6.8 after the inclusion in the homogenate of purified cyclic AMP–dependent kinase or of phosphorylase kinase–deficient mouse muscle. Since classification of glycogenoses is on the basis of one enzymatic defect for one type, we suggest that deficient activity of cyclic 3′,5′-AMP–dependent kinase be called type X glycogenosis. (Supported by NIH grants AM-13903 and RR-00123.)

152. Fetal Adrenal Growth and Function after Maternal Adrenalectomy. BENJAMIN T. JACKSON,* HELMUT F. J. RAUSCHECKER,* RONALD A. MALT,* AND GEORGE J. PIASECKI,* Boston, Mass. (introduced by William E. Huckabee).

Growth characteristics and quantitative function of fetal adrenals and, indirectly, fetal secretion of ACTH were studied in dog fetuses *in utero*. Experiments were conducted in two groups of pregnant beagles, one normal and one in-

cluding animals adrenalectomized and maintained without hormonal supplement for 10–17 days before fetal study. Cannulas for collection of adrenal effluent were inserted in fetuses at 56 days gestation. Timed fetal adrenal samples were collected before and after injection of 10 mμ ACTH into the fetus; thereafter, fetal and maternal arterial samples were obtained. Concentrations of cortisol were determined by the double-isotope method of Kliman and Peterson; fetal cortisol secretory rates were calculated. Fetuses and fetal adrenals were weighed, and adrenals were analyzed for RNA and DNA according to Munro and Fleck. Ratios of fetal adrenal to body weights (mg/kg) were 126 ± 21 in normals and 175 ± 34 in the adrenalectomy group. Adrenal RNA and DNA (μg/g protein) were 36.7 ± 7.8 and 40.9 ± 10.7 respectively in normals, and 40.8 ± 5.4 and 39.3 ± 7.2 respectively in the adrenalectomy group. Cortisol data: *normals:* concentrations (ng/ml), fetal arterial 94 ± 20, maternal arterial 92 ± 14; fetal secretory rates relative to body weights (ng/min per kg), pre-ACTH 288 ± 279, post-ACTH 532 ± 352; *adrenalectomy group:* concentrations (ng/ml), fetal arterial 47 ± 19, maternal arterial 7 ± 4; fetal secretory rates relative to body weights (ng/min per kg), pre-ACTH 195 ± 64, post-ACTH 379 ± 171. Conclusions: (1) Reduction in cortisol by maternal adrenalectomy causes increased secretion of ACTH by the fetus as shown by fetal adrenal gland enlargement. (2) Increased RNA/DNA ratios in fetal adrenals after maternal adrenalectomy indicate that increased ACTH stimulation causes primarily hypertrophy rather than hyperplasia of the fetal adrenal gland. (3) Increased ACTH stimulation fails to elevate fetal cortisol secretory capacity. (4) The dichotomy between morphologic and functional effects on the fetal adrenal of increased ACTH stimulation is possibly explained by (a) enlargement of adrenal cells without concomitant increase in enzyme capacity, or (b) formation of an intermediate in the fetal synthesis of cortisol exclusively in an extra-adrenal tissue which limits the rate of synthesis of cortisol by the fetal adrenal. (5) Fetal secretion of ACTH is partially but not entirely suppressed by circulating maternal cortisol in normal pregnancy. (This work was supported by NIH grant HD-01272.)

153. A Protein Abnormality in Red Cell Membranes of Hereditary Spherocytosis. HARRY S. JACOB, ALBERT RUBY,* ERIC S. OVERLAND,* AND DANIEL MAZIA,* Minneapolis, Minn., and Berkeley, Calif.

Characteristics of hereditary spherocytes—their increased sodium permeability, tendency to lose membrane fragments, excessive ghost rigidity, and peculiar blistered appearance under scanning electron microscopy—suggest a genetic error of membrane structural material. We extracted lipid-free membrane proteins, with yields of 70–80%, from RBC of nine hereditary spherocytosis (HS) patients representing six families. Others report that one such protein from normal membranes aggregates into actin-like microfilaments when treated with cations. Our normal protein preparations, treated with 1 mM Ca^{++} or 20 mM K^+, increased in S_{20} from 2.5 to 9.0; HS protein remained virtually unchanged at 2.5–3.5. Reflecting the heterozygosity of HS, two protein peaks, aggregated and nonaggregated, were discernible in some HS, but no normal, extracts. Vinblastine precipitates microfilamentous proteins (including actin); it precipitated 40–60% less HS membrane protein than normal. Optimal precipitation required ATP and Ca^{++}. The aggregates dispersed at 4°C, behavior reminiscent of microtubules in cells. RBC in which membrane sulfhydryls are blockaded by paramercuribenzoate mimic hereditary spherocytes in hyperpermeability, rigidity, fragmentation proclivity, osmotic fragility, sphering, and splenic destruction. Membrane protein from paramercuribenzoate-treated RBC was largely unaggregable, and 40% less Vinblastine precipitable. Abnormalities of HS membrane protein correlated with surface characteristics of intact cells. Hereditary spherocytes agglutinated after half the electrolyte-free washes required for normal RBC. Tiny Ca^{++} additions (3×10^{-5} M) disagglutinated normal RBC, whereas spherocytes required 2×10^{-3} M. If preincubated with adenosine to increase cellular ATP, hereditary spherocytes became unagglutinable; concomitantly, Vinblastine precipitability of membrane protein improved. We conclude that HS membrane protein is genetically altered and cannot form normal, possibly contractile, aggregates. This suggests that such a conformation underlies normal RBC biconcavity and deformability, which, if precluded by mutation(s) in membrane protein structure, engenders spherocytosis, rigidity, and premature RBC destruction. Potentially many such mutations are possible, explaining, perhaps, the variable severity of HS in different families.

154. Effect of Saline Infusion on Filtration Rate and Sodium Reabsorption in Single Superficial and Juxtamedullary Nephrons. REX L. JAMISON,* St. Louis, Mo. (introduced by Stanford Wessler**).

The infusion of isotonic saline is known to decrease fractional sodium reabsorption and to increase single-nephron GFR (SGFR) in superficial nephrons. Since recent work has revealed functional differences between superficial and juxtamedullary nephrons, their response to a saline infusion was compared. SGFR and fractional sodium reabsorption were measured in proximal and distal tubules of superficial nephrons and in the bend of Henle's loop of juxtamedullary nephrons of rats infused with bicarbonate saline (expansion). Micropuncture collections were made from the same tubular segment before and after expansion. 17 control-paired collections during continued hydropenia showed a decrease in SGFR ($P < 0.025$) but no change in fractional sodium reabsorption in superficial nephrons and no change in SGFR or fractional sodium reabsorption in juxtamedullary nephrons. After expansion, sodium excretion increased markedly in each of 14 rats. In superficial nephrons, SGFR increased ($P < 0.001$) and fractional sodium reabsorption decreased in both proximal and distal tubular segments ($P < 0.001$) ($n = 26$). In juxtamedullary nephrons, SGFR and fractional sodium reabsorption did not change significantly, although fractional water reabsorption was strikingly reduced ($P < 0.001$) ($n = 14$). The increased excretion of sodium was thus associated with a disproportionate increase in the amount of filtered sodium presented to superficial nephrons and a decrease in the over-all fraction of filtered sodium reabsorbed by the proximal tubule and descending limb of Henle's loop of superficial nephrons, whereas fractional sodium reabsorption by the corresponding segment of juxtamedullary neph-

cluding animals adrenalectomized and maintained without hormonal supplement for 10–17 days before fetal study. Cannulas for collection of adrenal effluent were inserted in fetuses at 56 days gestation. Timed fetal adrenal samples were collected before and after injection of 10 mμ ACTH into the fetus; thereafter, fetal and maternal arterial samples were obtained. Concentrations of cortisol were determined by the double-isotope method of Kliman and Peterson; fetal cortisol secretory rates were calculated. Fetuses and fetal adrenals were weighed, and adrenals were analyzed for RNA and DNA according to Munro and Fleck. Ratios of fetal adrenal to body weights (mg/kg) were 126 ± 21 in normals and 175 ± 34 in the adrenalectomy group. Adrenal RNA and DNA (μg/g protein) were 36.7 ± 7.8 and 40.9 ± 10.7 respectively in normals, and 40.8 ± 5.4 and 39.3 ± 7.2 respectively in the adrenalectomy group. Cortisol data: *normals:* concentrations (ng/ml), fetal arterial 94 ± 20, maternal arterial 92 ± 14; fetal secretory rates relative to body weights (ng/min per kg), pre-ACTH 288 ± 279, post-ACTH 532 ± 352; *adrenalectomy group:* concentrations (ng/ml), fetal arterial 47 ± 19, maternal arterial 7 ± 4; fetal secretory rates relative to body weights (ng/min per kg), pre-ACTH 195 ± 64, post-ACTH 379 ± 171. Conclusions: (1) Reduction in cortisol by maternal adrenalectomy causes increased secretion of ACTH by the fetus as shown by fetal adrenal gland enlargement. (2) Increased RNA/DNA ratios in fetal adrenals after maternal adrenalectomy indicate that increased ACTH stimulation causes primarily hypertrophy rather than hyperplasia of the fetal adrenal gland. (3) Increased ACTH stimulation fails to elevate fetal cortisol secretory capacity. (4) The dichotomy between morphologic and functional effects on the fetal adrenal of increased ACTH stimulation is possibly explained by (a) enlargement of adrenal cells without concomitant increase in enzyme capacity, or (b) formation of an intermediate in the fetal synthesis of cortisol exclusively in an extra-adrenal tissue which limits the rate of synthesis of cortisol by the fetal adrenal. (5) Fetal secretion of ACTH is partially but not entirely suppressed by circulating maternal cortisol in normal pregnancy. (This work was supported by NIH grant HD-01272.)

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154. Effect of Saline Infusion on Filtration Rate and Sodium Reabsorption in Single Superficial and Juxtamedullary Nephrons. REX L. JAMISON,* St. Louis, Mo. (introduced by Stanford Wessler**).

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of type B RBC by polymorphonuclear leukocytes much more effectively than the mother's serum. Mildly reduced (1 or 10 mM dithiothreitol), alkylated (excess iodoacetamide) colostrum opsonized type B RBC as efficiently as untreated colostrum despite some decrease in anti-B hemagglutination titers. Vigorously reduced (100 mM dithiothreitol), alkylated colostrum neither agglutinated nor opsonized type B RBC, but rendered them agglutinable by specific IgA antiserum. Clarified colostrum was absorbed with an anti-IgM-bromacetylcellulose immunoabsorbent and filtered through Sephadex G-200 (0.4 M NaCl, Tris-HCl buffer, pH 7.4). The excluded peak, containing immunoelectrophoretically pure IgA, agglutinated and opsonized type B RBC in the absence of complement. Addition of rabbit anti-IgA, previously absorbed with type B RBC, completely neutralized these activities. These studies clearly demonstrate that colostral IgA is capable of opsonizing particulate antigens without mediation of complement, and suggest that this may be an important mechanism in the defense of mucosal surfaces against foreign antigens. (Research supported by the Veterans Administration and NIH grant AM-13717.)

159. Membrane Lipids and Leukocyte Glucose Metabolism. SANDRA S. KAPLAN,* YALE NEMERSON, AND STUART C. FINCH,** New Haven, Conn.

Polymorphonuclear (PMN) leukocytes react to phagocytosis or treatment with surface-active agents with increased oxygen consumption and augmented oxidation of glucose via the hexose monophosphate shunt (HMPS). Though the mechanism for this stimulation is unknown, its requirement for an intact cell suggests that it may be initiated by changes in the cell membrane. To test this hypothesis, the effects of phospholipases C and A on human PMN leukocyte HMPS activity, Krebs cycle activity, and phagocytosis were studied. Peripheral blood leukocytes, washed and suspended in Krebs-Ringer phosphate buffer, were incubated alone or with phospholipase C (*Clostridium welchii*) or phospholipase A (*Crotalus adamenteus*) for 20–30 min at 37°C, at which time 1-^{14}C- or 6-^{14}C-glucose was added to each flask. Phagocytosis was initiated with latex particles in two of four flasks with and without phospholipase. HMPS and Krebs cycle activities were evaluated by radioassay of evolved $^{14}CO_2$. Phagocytosis was determined from stained smears. Resting HMPS activities in comparison with control values were 382% with phospholipase C and 359% with phospholipase A. Resting Krebs cycle activities were 270% and 190% of control values, respectively, with phospholipases C and A. Phagocytosis was not impaired, but after phagocytosis and treatment with either phospholipase both HMPS and Krebs cycle activities were reduced to values ranging from 54% to 86% of normal. The increased glucose flow through the HMPS and Krebs cycle with phospholipase probably is the result of stimulation of lipid synthesis following membrane phospholipid hydrolysis. Phospholipase interference with phospholipid-dependent enzymes probably was responsible for inhibition of phagocytizing metabolism. Since lipid metabolism is increased during phagocytosis, the related generation of NADP may be the primary factor for early HMPS activation. (Supported by NIH grant 11106-07.)

160. The Effect of Water Diuresis on Spread of Bacteria through the Urinary Tract. DONALD KAYE, Philadelphia, Pa.

After injection of 2–4 × 10^7 *Escherichia coli, Staphylococcus aureus,* or enterococci into the left renal medulla of rats, the bacterial titers gradually decreased. However, more than 10^5 bacteria could usually be recovered from the left kidney for at least 1 wk. With *Staph. aureus* or enterococcal infection, the titers in the right kidneys rapidly increased, and by 1 wk after injection, there were usually more than 5 × 10^4 bacteria in the right kidney. In contrast, with *E. coli* infection the titers in the right kidney remained low and were usually 10^3 per kidney or lower. Diuresis was produced by offering rats 5% glucose in water starting 4 days before injection of bacteria and continuing for 2 wk after injection. Titers of enterococci in both kidneys were not affected by diuresis. Titers of staphylococci in both kidneys decreased at a more rapid rate in diuresing rats than in nondiuresing rats. In diuresing rats infected with *E. coli,* the bacterial titers in the right kidneys increased to equal the numbers in the left kidneys (10^7 per kidney) by 2 days after injection. Thereafter the titers in both kidneys of diuresing rats decreased at a more rapid rate than the titers in left kidneys of nondiuresing rats but at a less rapid rate than titers in right kidneys of nondiuresing rats. Histological studies demonstrated marked pyelonephritis of all left kidneys. The right kidneys demonstrated histological changes only in rats with titers of 10^5 or more per kidney. Many of these kidneys showed marked infiltration of polymorphonuclear leukocytes in the medulla just beneath the epithelium lining the papilla. These studies demonstrate that the effect of water diuresis on rats with renal infection produced by different bacteria inoculated by the same method is variable, depending on the bacteria used. (Research supported by grant AI-09489 from the NIH.)

161. Biosynthesis of Intestinal Glycoprotein. YOUNG S. KIM,* JOSE PERDOMO,* AND MARVIN H. SLEISENGER, San Francisco, Calif.

Two hypotheses have been suggested for glycosylation of intestinal glycoprotein (IGP): (1) single-site localization at Golgi cisternae; (2) multiple-site localization at both rough (RER) and smooth (SER) endoplasmic reticulum. In the present study, subcellular fractions from rat small intestinal mucosal homogenate (IMH) were examined for incorporation of ^{14}C-glucosamine and ^{3}H-leucine into protein and for the localization of glycosyl transferases. Pulse labeling of precursors showed significant incorporation into both RER and SER as early as 10 min after the injection. The specific activity of protein-bound ^{14}C-glucosamine in the SER was greater than that in the RER at all times up to 120 min after the injection (6 × at 20 min and 2 × at 70 min). To examine further the biosynthetic steps of IGP, rat IMH was studied for subcellular localization of glycosyl transferases. Glycosyl transferases were most active in microsomal fractions. Both SER and RER showed transfer of *N*-acetylglucosamine, *N*-acetylgalactosamine, and galactose to endogenous protein acceptors from corresponding sugar nucleotides. The SER was more active than the RER for three glycosyl

transferases studied: *N*-acetylglucosaminyl (3×), *N*-acetylgalactosaminyl (4×), and galactosyl (7×) transferases. Antiserum prepared against the purified soluble glycoprotein (SGP) isolated from rat IMH gave a single precipitin arc of immunological identity against SGP and SER, whereas RER failed to give an immunoprecipitin reaction. In conclusion, these results indicate that (1) carbohydrates of IGP are attached to protein backbones at both RER and SER during their transport through the vesicles of RER and SER by the action of specific glycosyl transferases; (2) the immunological specificity of the SGP may be formed within the SER.

162. Inefficacy of Vitamin K in Stimulating the Production of Factors II, VII, and X in Protein Calorie Malnutrition in Thai Children. ROGER K. KIPFER,* DONALD M. ALLEN, AND ROBERT E. OLSON,** Chiang Mai, Thailand, and St. Louis, Mo.

The study employed 20 children with protein calorie malnutrition 6 months to 4 yr of age. They were 60 ± 14% of expected weight and 91 ± 9% of expected height for age. Kwashiorkor was diagnosed in 26%, combined kwashiorkor marasmus in 40%, and marasmus in 34%. Other admission findings included mean values for hemoglobin of 8.8 ± 1.3 g, serum protein of 5.0 ± 1.3 g, serum albumin of 1.8 ± 0.6 g, serum cholesterol of 98 ± 46 mg/100 ml, vitamin A of 18 ± 20 μg/100 ml, and vitamin E of 0.27 ± 0.21 mg/100 ml. Vitamin K-dependent clotting plasma factors II, VII, and X were assayed by one-stage methods. All factors varied widely in children on admission, from 5 to 100% of normal. The administration of vitamin K_1 (as Aquamephyton) in intravenous doses ranging from 0.1 to 10.0 mg per day gave a negligible to marginal response in 50% of the patients, presumably because of protein lack and consequent lack of a responsive ribosome system in the liver. After 10 days of nutritious diet and fluid therapy, 20% of the patients still exhibited subnormal levels for the K-dependent protein. This antianabolic response to vitamin K_1 can be duplicated in the isolated perfused vitamin K–deficient rat liver given antibiotic inhibitors of protein synthesis such as puromycin or cycloheximide, but not actinomycin D. The data support the view that the action of vitamin K is dependent upon a functional ribosomal system with an adequate supply of amino acids. (Supported in part by NIH grant AM-09992.)

163. Nonuniform Contractility across the Heart Wall Caused by Redistribution of Coronary Flow. EDWARD S. KIRK,* MARVIN E. TURBOW,* CHARLES W. URSCHEL,* AND EDMUND H. SONNENBLICK, Boston, Mass.

Although local coronary occlusion leads to segmental loss of contraction, differences in function across the wall have not been shown. Orientation of strain gauge arches in relation to fiber direction in the heart was used to separate the contractile responses across the wall, since fiber direction changes progressively, rotating about 120° from epicardium to endocardium. Gauges aligned parallel to epicardial fibers and attached with shallow sutures responded to changes in contractility of superficial fibers caused by cooling the epicardial surface beneath the transducer. By contrast, gauges oriented perpendicular to epicardial fibers did not respond. However, deep sutures were required for unambiguous responses from deep fibers. Left coronary arteries were isolated and perfused in dogs, using a Gregg cannula and a cannulating electromagnetic flowmeter. Responses of the two gauges were examined during ischemia induced by lowering coronary perfusion pressure, which causes a steep gradient of blood flow across the left ventricular wall. Marked autoregulation of coronary flow occurred when perfusion pressure was reduced by clamping, but as flow decreased, signs of failure occurred first and were more pronounced in deep fibers than in superficial ones. Contractility appeared to be impaired in proportion to the degree of ischemia of each layer. In contrast, uniform ischemia caused by total occlusion resulted in more equal responses from the two transducers. With coronary occlusion set at threshold for impairment of contractility, nitroglycerine (150 μg i.v. or 12 μg intracoronary) could selectively depress contractility in deeper layers, probably owing to redistribution of blood flow between layers of the heart. Thus coronary stenosis creates inhomogeneity of blood flow, which causes the heart to function as concentric shells of varying contractility. (Research supported by grants from the NIH.)

164. A Specific Assay for the Detection of Intravascular Coagulation. C. THOMAS KISKER* AND RUTH RUSH,* Cincinnati, Ohio (introduced by Edward L. Pratt**).

Diagnosis and management of intravascular coagulation are based on often inconsistent and confusing secondary changes in various clotting factors. Therefore a method was developed for detecting fibrin monomer (FM) (fibrinogen after alpha peptide removal), thus directly assaying the effect of thrombin activity. Enzymatic incorporation of ^{14}C-glycine ethyl ester (GEE) into FM by factor XIII was measured. In human plasma incubated for 7 min with 0.02 U thrombin per ml, ^{14}C-GEE incorporation increased from 190 to 340 dpm/mg clottable protein (CP) over blank values. No increased ^{14}C-GEE incorporation occurred in human plasma incubated with 200 U streptokinase per ml for 15 min. In six dogs given thrombin intravenously, ^{14}C-GEE incorporation into fibrinogen and changes in other coagulation factors were measured. ^{14}C-GEE incorporation into CP increased 2- to 6-fold, and predictable alterations of other factors occurred. In two dogs a 5-fold increase in ^{14}C-GEE incorporation persisted 1 hr after the infusion was stopped. Three patients with intravascular coagulation were studied to relate ^{14}C-GEE incorporation into CP with changes in other coagulation factors. Increased incorporation was found in the presence and absence of fibrinogen split products in the serum, with a negative or positive gel test, and with increased or decreased factor VIII activity. Furthermore, ^{14}C-GEE incorporation returned to normal values at times when fibrinogen split products and a positive gel test could still be found. Therefore the method appears to be specific and sensitive. It is useful in confirming diagnoses, judging effectiveness of therapy, and studying relations of coagulation factors during episodes of intravascular coagulation. (Research supported by grant CA-05196 from the NIH.)

165. Isolated Adrenal Cells: A New Tool for Direct Study of ACTH. ABBAS E. KITABCHI,* SUSAN Y. THOMPSON,* LOUISE C. KITCHELL,* AND WILLIAM C. DUCKWORTH,* Memphis, Tenn. (introduced by Gene H. Stollerman**).

The currently used bioassay for ACTH lacks sensitivity in that it is unable to detect levels of less than 40 μU. The immunoassay at present is also somewhat unsatisfactory. These difficulties are eliminated by our modification of the isolated adrenal cell (IAC) preparation of Swallow and Sayers. In this procedure a relatively homogeneous and highly sensitive cell suspension is obtained which responds to as little as 0.5 μU of ACTH with production of corticosterone, and so may be used for ACTH assay in biologic tissues. In addition to ACTH, the stimulatory effects of dibutyryl cyclic 3',5'-AMP (D-cAMP) and β1–24-corticotropin (Syn) on corticosterone production have been examined. A sigmoid dose-response curve is obtained for each of the above stimulators with half-maximal dose-response values of 0.03 μmole/ml of incubation mixture for D-cAMP, 30 pg/ml for Syn, and 5 μU/ml for ACTH. Maximal stimulation with 10–20 μU of ACTH per ml resulted in a 10- to 30-fold increase in steroidogenesis. Insulin, proinsulin, glucagon, growth hormone, or catecholamines had no stimulatory effects on steroidogenesis. ACTH values obtained by the IAC method on plasma and tumor tissue from a patient with ectopic ACTH syndrome agreed closely with the values obtained by another bioassay and by the radioimmunoassay. Administration of metyrapone to four normal subjects increased ACTH levels measured by our method from 2.8 $\pm$ 0.9 to 15.9 $\pm$ 4.5 μU/ml of plasma ($P < 0.02$). Conclusion: IAC is a new tool for studying the mechanism of action of ACTH, and could be utilized in a new bioassay for ACTH, 100-fold more sensitive than the currently used bioassay procedure. (Supported by grants from the NIH and the Veterans Administration.)

166. Studies of the Mechanism of Diphenylhydantoin-Induced Inhibition of Insulin Secretion. STEPHEN KIZER,* MARIO VARGAS-CORDON,* KLAUS BRENDEL,* AND RUBIN BRESSLER, Durham, N. C.

Diphenylhydantoin (DPH), a potent anticonvulsant, reduces the excitability of neural tissues by decreasing intracellular Na^+ concentrations, apparently by virtue of a specific stimulatory effect on the neuronal Na^+-K^+-Mg^{++} ATPase. Recently, attention has been directed to the occurrence of hyperglycemia, nonketotic hyperosmolar coma, and hypoinsulinemia following DPH overdosage. We therefore undertook to study the effect of DPH on insulin secretion in vitro, using both pancreas pieces and isolated islets of Langerhans. In both pieces and isolated islets, DPH (7 μg/ml) markedly reduced the insulinogenic effect of 150 mg/100 cc of glucose (50–70%) without altering the rate of glucose oxidation, and also reduces the intracellular Na^+ concentration as measured by $^{22}Na^+$ accumulation. Because of these observations, attempts were made to reverse the DPH-induced inhibition of insulin secretion by various agents which are known to cause inhibition of the Na^+-K^+-Mg^{++} ATPase in other systems. Ouabain, 10^{-4} M, and 0.1 mM K^+ each reverses the DPH-induced insulinopenia. Hydroxydiphenylhydantoin, a metabolite of DPH without anticonvulsant activity, does not inhibit insulin secretion, whereas mephenytoin, a related hydantoin anticonvulsant, is inhibitory. Pentobarbital, a barbiturate anticonvulsant, does not diminish insulin secretion. From these data, it is concluded that the hydantoin anticonvulsants are potent inhibitors of insulin secretion by virtue of their ability to diminish intracellular Na^+ concentrations, thereby hyperpolarizing the pancreatic β-cell and reducing its excitability. Agents which increase intracellular Na^+ by decreasing Na^+-K^+-Mg^{++} ATPase activity readily reverse hydantoin-induced inhibition of insulin secretion. Though not directly supporting the postulate that the depressant effect of DPH on cellular excitability is mediated by a stimulatory effect of the hydantoins on the membrane Na^+-K^+-Mg^{++} ATPase, these observations nevertheless provide indirect evidence for an important role of the Na^+-K^+-Mg^{++} ATPase in the regulation of insulin secretion. (Research supported by grants from the USPHS, the NIH [HE-07061, AM-12706], and the American Heart Association [G67-904].)

167. Distribution and Specificity of Antibodies to Double-Stranded RNA in Human Sera. D. KOFFLER,* R. THOBURN,* V. AGNELLO,* AND H. G. KUNKEL,** New York, N. Y.

The recent finding of antibodies to double-stranded RNA in the sera of patients with systemic lupus erythematosus (SLE) has aroused considerable interest, as reported by Schur and associates and by Koffler and associates. The present study was undertaken to define the specificity of these antibodies and their distribution in various disorders. Antibodies reactive with polyribonucleotides were analyzed by a hemagglutination test. Sixty sera from patients with SLE showed an incidence of antibodies to poly A-U of 55%, to poly I-C of 22%, and to poly A of 23%. Sera from patients with random hospital diseases, rheumatoid arthritis, chronic active hepatitis, infectious mononucleosis, and procainamide-induced SLE showed a definite but low incidence of antibodies to these polynucleotides. Several studies in individual patients with SLE of RNA antibodies in comparison with DNA antibodies during exacerbations of disease proved of special interest. Most peaks of antibody activity directed against poly A-U were accompanied by a concomitant rise in the titer of antibodies to native DNA. In contrast, peaks of antibody to single-stranded DNA appeared frequently in the absence of antibodies to native DNA. The specificity of antibodies reactive with poly A-U was determined by hemagglutination inhibition test. Cross-reactions with several polynucleotides were assessed as follows: poly I-C, strong; poly A, moderate; single-stranded DNA, weak; and native DNA, absent. Rabbit antisera prepared to poly A-U and poly I-C methylated bovine serum albumin conjugates showed predominant specificity for the homologous polyribonucleotide. Studies of human sera indicate that antibodies to a double-stranded polyribonucleotide poly A-U have a distribution similar to that of antibodies for native DNA. Although the significance of these antibodies in SLE has not been ascertained, the presence of the antibodies suggests that viral nucleotides or breakdown products of tissue RNA may serve

as immunogens in patients with active SLE. (Supported by USPHS grants AM-04761 and AM-11115.)

168. Sodium Chloride and Water Transport in the Descending Limb of Henle. JUHA P. KOKKO,* Dallas, Texas (introduced by Floyd C. Rector, Jr.).

The unique membrane characteristics of the thin descending limb of Henle play an integral part in the operation of the countercurrent system. We examined these properties in vitro by perfusing isolated thin descending limbs of Henle in rabbits. Active transport of NaCl was ruled out by perfusing with isosmolal ultrafiltrate of the same rabbit serum as the bath; net fluid transport was -0.07 ± 0.06 nl mm^{-1} min^{-1}, and transmembrane potential was zero. Passive permeability coefficient for Na (P_{Na}) was determined from the disappearance rate of ^{22}Na from isosmolal perfusion solution. P_{Na} was $1.69 \pm 0.16 \times 10^{-5}$ cm sec^{-1}, which is significantly less than P_{Na} in the proximal convoluted tubule (PCT). Reflection coefficient for NaCl (σ NaCl) was measured by perfusing the tubule with Na-free raffinose solution in a bath of rabbit serum to which sufficient NaCl was added to obtain conditions of zero net fluid movement. The measured σ NaCl of 0.96 ± 0.01 is significantly greater than σ NaCl in the PCT. Water permeability to osmotic gradients (Lp) was determined by perfusing with ultrafiltrate of rabbit serum in a bath made hyperosmotic by addition of either 100 mOsm raffinose or NaCl. Lp with raffinose was $1.71 \pm 0.15 \times 10^{-4}$ ml cm^{-2} sec^{-1} atm^{-1} and with NaCl $1.68 \pm 0.08 \times 10^{-1}$ ml cm^{-2} sec^{-1} atm^{-1}, indicating much greater water permeability than in the PCT. In each case the measured increase in osmolality of the collected fluid was secondary to efflux of water. This combination of permeability characteristics shows that medullary interstitial Na is able to generate high effective osmotic forces which allow the concentration of the descending loop fluid to occur primarily by abstraction of water without significant net entry of NaCl. (Research supported by grants from the NIH and the Dallas Heart Association.)

169. The *Macaca irus* Monkey: An Experimental Model for the Study of Coronary Artery Disease and Its Sequelae. DIETER M. KRAMSCH,* ANDREW HUVOS,* AND WILLIAM HOLLANDER, Boston, Mass.

40 control *Macaca irus* monkeys fed a standard diet and 40 experimental monkeys fed a 2% cholesterol diet for 12–18 months were studied. The coronary arteries of the control monkeys were normal. All experimental monkeys developed severe coronary atherosclerosis resulting in more than 60% narrowing of the arterial lumen. The lesions resembled human coronary artery disease both in distribution and in microscopic appearance. During the atherogenic diet the mean serum cholesterol rose from 123 to 349 mg/100 ml without changes in the serum triglycerides. These lipid changes, as revealed by electrophoretic and ultracentrifugal analyses, were associated with increases in β-lipoproteins without alterations in pre-β- or α_1-lipoproteins (type II hyperlipoproteinemia). During the atherogenic diet 25 of the 40 experimental monkeys developed abnormal ECG. The abnormalities included marked left axis deviation (in 7), deep Q waves (in 5), and ST-T wave changes (in 18). 10 control and 10 experimental monkeys were infused with isoproterenol (1 μg/min). Isoproterenol produced marked ST-T depressions in all the experimental monkeys but not in the control monkeys. Comparable changes in heart rate and blood pressure occurred during the infusion in both groups. Nine of the experimental monkeys died suddenly—three at rest, four during exertion, and two after isoproterenol infusion. At autopsy the hearts of the experimental monkeys with coronary artery disease revealed extensive myocardial necrosis, endocardial thickening, and subendocardial fibrosis. Selective coronary arteriography showed a close correlation with the postmortem findings in the coronary arteries. In conclusion: A primate model has been established for controlled prospective studies of coronary (atherosclerotic) heart disease. (Supported by NIH grants HE-01536 and HE-10739.)

170. Effect of Ethanol on Lactate Metabolism. ROBERT A. KREISBERG,* W. CRAWFORD OWEN,* AND ALAN M. SIEGAL,* Birmingham, Ala. (introduced by T. Joseph Reeves**).

To define the mechanism of ethanol-induced hyperlacticacidemia, lactate metabolism was studied in human subjects before and during the administration of ethanol. U-^{14}C-lactate was administered by a primed-constant infusion technique, and lactate turnover, oxidation, and conversion to glucose were determined by isotopic dilution and precursor-product specific activity ratios. Before administration of ethanol, blood lactate concentrations were constant and lactate production and removal rates were equal. In subjects fasted for 12 hr, ethanol (10 g orally per hr) produced a prompt and sustained increase in blood lactate concentrations which reached values 2–2½ times those of the control period. During the period in which blood lactate levels were rising, lactate production declined slightly or was unchanged, while lactate removal was markedly reduced. Lactate oxidation was unchanged by alcohol. Lactate incorporation into glucose was markedly reduced, although there were only minor changes in the blood glucose concentrations. Despite continued administration of ethanol, lactate removal increased toward control values and within 60–90 min was again equal to lactate production. Coincidently with return to the steady state, the blood lactate concentration became constant. Conclusion: Hyperlacticacidemia induced by alcohol is due to underutilization of lactate rather than to overproduction, as has been generally thought. Decreased incorporation of lactate into glucose is at least partially responsible for the elevation in lactate concentrations that occur following alcohol. (This research was supported by NIH grants AM-09722, 5-MO1-RR-32, and T1-AM-5053.)

171. The Relative Excess of Norepinephrine over Dopamine and Renin Hyperresponsiveness in Hyperkinetic Circulation: A Possibly Hereditary Disorder. OTTO KUCHEL,* JEAN L. CUCHE,* ANDRÉ BARBEAU,* MICHAEL BRECHT,* ROGER BOUCHER,* WOJCIECH NOWACZYNSKI,* AND JACQUES GENEST,* Montreal, Canada (introduced by Maurice McGregor**).

Hyperkinetic circulation (hyperresponsiveness of β-adrenergic receptors) is characterized by a sympathetic over-

response to various stimuli (posture, cold, emotion). Since the renin release is probably mediated through adrenergic receptors and since we have demonstrated an indirect correlation between plasma renin and urinary dopamine: norepinephrine ratio, we studied those parameters in seven subjects with hyperkinetic circulation (five of them hypertensives), with stimulation by upright posture and sodium depletion. Hyperreactors with an increased sympathetic outflow had a lower urinary dopamine and higher norepinephrine excretion (dopamine:norepinephrine ratio lower, $P < 0.02$) and higher plasma norepinephrine and renin activity than control subjects. With upright posture these differences are even more evident (higher renin, $P < 0.02$). With sodium depletion there is an additional fall in dopamine and rise in norepinephrine, with a very high response in renin. If the stimulus of upright posture is added, in three out of six patients a decrease in renin occurs instead of a further rise, pointing to a depletion of the system. The hereditary basis of the disorder is suggested by its incidence in three sisters, two of them hypertensives, the third labile hypertensive, but all with the same underlying hormonal anomaly. Hyperkinetic circulation may be due to a qualitatively different sympathetic discharge, yielding less dopamine and more norepinephrine. Dopamine is known to oppose some biological effects of norepinephrine on receptor sites. Less dopamine and more norepinephrine on the renal adrenergic receptors may be related to renin hyperresponsiveness. Higher renin and aldosterone may initiate the development of hypertension. The tracing of this anomaly from childhood by these hormonal parameters may be a way to detect hyperreactors.

172. Mediation of Digitalis-Induced Peripheral Vasoconstriction in Dogs by Alpha Adrenergic Mechanisms; Parasympathetic Modulation in the Intact, Conscious State. RAJ KUMAR,* WILLIAM B. HOOD, JR.,* AND WALTER H. ABELMANN,** Boston, Mass.

Digitalis-induced augmentation of systemic vascular resistance and afterload to contraction has been generally observed both in animal studies and in patients in the presence of anesthesia or heart failure, states presumably associated with altered autonomic tone. In contrast, these effects have not been observed in conscious animals and humans without heart disease. The effect of acetyl strophanthidin (AS) infusion (3 μg/kg per min) on mean aortic pressure (AO) and systemic resistance (SR) was investigated during pharmacological denervation with atropine sulfate (0.2 mg/kg), propranolol (0.3 mg/kg), and dibenzylene (3 mg/kg). Serial studies, at least 24 hr apart, were carried out in six intact conscious dogs. Acetyl strophanthidin did not significantly change AO or SR in the control state. Maximal vasconstriction from AS was observed in atropinized animals (SR increased $34 \pm 13\%$ [SEM], $P < 0.05$; AO, 108 ± 2 to 188 ± 12 mm Hg, $P < 0.001$). Dibenzylene blocked this atropine- and AS-induced vasoconstriction (SR increased $8 \pm 9\%$, NS; AO, 92 ± 8 to 108 ± 10 mm Hg, $P < 0.05$). AS-induced vasoconstriction was also noted in atropinized animals with beta blockade (SR increased $25 \pm 11\%$, $0.05 < P < 0.10$; AO, 111 ± 6 to 153 ± 14 mm Hg, $P < 0.02$); however, this increase was less than with parasympathetic blockade alone. Digitalis-induced peripheral vasoconstriction is primarily mediated through alpha receptors. Intact parasympathetic tone completely compensates for this vasoconstriction; however, it can be unmasked by decrease in parasympathetic tone. The role of beta receptors is limited. Clinically observed digitalis-induced hypertension in patients with cardiovascular disease has thus been reproduced in the conscious animal model and its pathogenesis delineated; its prevention and treatment with alpha blockade merits investigation. (Supported by the NIH and the Massachusetts Heart Association.)

173. Cellular Binding of Polymyxin and Aminoglycoside Antibiotics: A Clue to Their Pharmacologic Properties? CALVIN M. KUNIN, Charlottesville, Va.

Studies in this laboratory have revealed the presence of nonenzymatic, insoluble, heat-stable inhibitors of the polymyxin and aminoglycoside antibiotics. Other antibiotics, such as penicillins, tetracycline, and erythromycin, are not inhibited, and the effect is not related to serum protein binding. Liver and kidney from various species have more activity than other tissues. The polymyxin inhibitor appears to be an acid phospholipid; aminoglycoside inhibitors are nonlipid. Polymyxin B and colistin sulfate are much more sensitive to tissue inhibitors than is colistinmethanesulfonate. Among the aminoglycoside antibiotics, neomycin followed by kanamycin is most inhibited, streptomycin is least, and gentamycin is intermediate. These findings correlate well with the relative toxicity of the drugs and the abundance of free amino groups in each molecule. More recent studies reveal that uptake of the drugs by whole cells differs markedly from that of homogenates. Polymyxin B is removed by freshly dispersed rabbit kidney and liver cells, but not by human erythrocytes or tissue culture lines, or dispersed or cultured chick embryo cells. Aminoglycosides are taken up only by disrupted kidney and liver cells. Thus, the polymyxin B inhibitor appears to be on the surface of the cell, whereas the aminoglycoside factor is intracellular. Polymyxin B can be completely dissociated from its binding site in the liver and kidney and released into the aqueous phase by treatment with 1 N H_2SO_4 and lipid solvents. Thus, the drug is not destroyed, but probably bound to a cellular component. Precipitin lines can be obtained in gel diffusion studies between polymyxin B and acid phospholipids, supporting the notion that these are the binding sites in membrane surfaces. Binding of polymyxin B to kidney cells in vivo may explain the relatively delayed renal clearance and greater nephrotoxicity of this drug as compared with colistinmethanesulfonate. (This study was supported by grant AI-008972-01-A1 from the USPHS.)

174. Low Oxygen Pressure: A Cause of Erythrocyte Membrane Rigidity. P. L. LACELLE* AND R. I. WEED, Rochester, N. Y.

Increased intracellular Ca/ATP ratio is accompanied by erythrocyte membrane rigidity, which is reversible by regeneration of critical levels of ATP in the depleted cell. Because ATP binds to deoxyhemoglobin, the effects of deoxygenation on deformability of human normal and hereditary spherocytic (HS) erythrocytes was studied to evaluate whether low P_{O_2} might influence the ability of erythrocytes

to survive in the microcirculation. In fresh normal cells, deformability measured as the negative pressure P_t (6.8 mm H_2O) sufficient to cause the erythrocyte to enter a standard 2.9 μ micropipette did not change as P_{O_2} decreased to 30 mm Hg. However, between 30 and 20 mm Hg P_{O_2}, P_t increased significantly to 45 mm H_2O, and as P_{O_2} approached zero, P_t rose to 500 mm H_2O, a value similar to that observed in ATP-depleted cells and in ghosts containing 10^{-4} M Ca but no ATP. Reversion to normal deformability occurred when P_{O_2} was restored to >30 mm Hg. HS cells, having $P_t = 48$ mm H_2O at 100 mm Hg P_{O_2}, manifested marked increase in rigidity beginning at $P_{O_2} < 40$ mm Hg. Although normal and HS cells have increased passive and active Na flux at $P_{O_2} < 30$ mm Hg, no significant volume (<5%) or shape changes occur. It is suggested that effective removal of ATP by binding to deoxyhemoglobin adversely affects membrane rigidity by altering the Ca/ATP ratio. Since P_{O_2} approaches low levels under physiologic circumstances (e.g. $P_{O_2} = 25$ mm Hg in coronary sinus blood and 15–30 mm Hg in maternal, placental venous blood), any pathologic decrease in tissue P_{O_2} may have significant effects on red cell deformability. In the stagnant circulation of the splenic pulp, predisposition of HS cells toward rigidity at low P_{O_2} may be a major factor enhancing sequestration. (Supported by NIH grant HE-06241.)

175. Synchronization and Recruitment in Acute Leukemia. BEATRICE LAMPKIN,* TAKESHI NAGAO,* ANN LICHTENBERG,* AND ALVIN MAUER, Cincinnati, Ohio.

The following study was made to determine the effect of synchronization of leukemic cells in a segment of the mitotic cycle (MC) by cytosine arabinoside (CA) on the subsequent administration of another drug. Ten patients were given CA, 5 mg/kg rapidly. Effect on marrow blasts was followed by serial measurements of ^{3}H-thymidine labeling and mitotic indices. When synchronization in DNA synthesis occurred, seven patients (three lymphoblastic [ALL], three myeloblastic [AML], one lymphosarcoma with leukemic transformation) were given Vincristine, and three patients (two ALL, one lymphosarcoma with leukemic transformation) were given methotrexate, 1 mg/kg intravenously. An additional patient (ALL) was given methotrexate only. Marrow drug effect was determined, and for methotrexate studies, ^{3}H-deoxyuridine labeling was also measured. Partial synchronization by CA was achieved in nine patients. In two, clear evidence for recruitment of resting cells into MC was found. In four patients (three ALL, one lymphosarcoma) Vincristine arrest in mitosis occurred in a larger number of cells than would be expected with the drug alone. Vincristine had little effect on MC of cells in AML. Methotrexate alone caused partial synchronization of MC, and partial synchronization also occurred in one ALL with methotrexate after CA. Resting cell recruitment was found in lymphosarcoma after CA and methotrexate. The results indicate that both synchronization and recruitment can be obtained with CA and methotrexate. A greater yield of leukemic cell death can be achieved by primary synchronization of MC for subsequent administration of a secondary MC-dependent drug. (Research supported by grants CA-04826 and CA-05196 from the NIH.)

176. Evidence for Two Triglyceride Lipases in Post-Heparin Plasma. JOHN C. LA ROSA,* ROBERT I. LEVY,* H. G. WINDMUELLER,* AND DONALD S. FREDRICKSON, Bethesda, Md.

Familial type I hyperlipoproteinemia is believed due to deficient activity of triglyceride lipase (TGL) that appears in plasma after heparin. However, lipolysis of artificial glyceride emulsions by post-heparin plasma (PHP) in type I is sometimes quantitatively normal. Using ^{14}C-triolein as substrate and high density lipoprotein (HDL) at one-thirtieth the normal plasma concentration, we have obtained an explanation for this paradox. A "TGL_1" released by heparin from rat adipose tissue, heart, lungs, and kidney optimally active at low ionic strengths (0.15 M NaCl or KBr) and inhibited by higher salt concentrations, protamine, and pyrophosphate, has been differentiated from a "TGL_2," released by heparin from rat liver. TGL_2 is resistant to salt inactivation and not inhibited by protamine or pyrophosphate. Liver TGL_2, having activity against artificial emulsions equal to that of TGL_1 from adipose tissue, was only one-eighth as active against chylomicrons or very low density lipoproteins. The TGL in PHP from normal subjects had characteristics similar to those of TGL_2. When HDL concentrations were increased 30 times, (1) TGL_1 activity increased 4-fold but TGL_2 was reduced by 60% or more; (2) the TGL activity in PHP from normal subjects was enhanced at low and inhibited at high ionic strengths; (3) the TGL in type I PHP was markedly decreased at all salt concentrations. It is concluded that PHP normally contains lipases similar to adipose tissue TGL_1 and the liver TGL_2. The latter is relatively inactive against lipoprotein glyceride, and assays using artificial emulsions tend to exaggerate the effective capacity for lipolysis. This is especially pertinent in type I, in which most plasma lipase activity is like TGL_2. It is inferred that the defect in type I is specifically a deficiency in TGL_1 activity.

177. The Physiological Significance of Ceruloplasmin (Ferroxidase) in Iron Metabolism. G. R. LEE,* H. P. ROESER,* S. NACHT,* AND G. E. CARTWRIGHT,** Salt Lake City, Utah.

In previous investigations, we observed that cell-to-plasma iron transfer was impaired in copper-deficient swine. That this abnormality might be a consequence of ceruloplasmin (Cp) lack was suggested by the observations of Osaki, Frieden, and associates, who demonstrated that Cp has iron-oxidizing (ferroxidase) activity in vitro. The present study was designed to test this hypothesis. Distinct episodes of hypoferremia were observed in 10 of 12 copper-deficient pigs with normal iron stores. These episodes occurred after plasma ceruloplasmin decreased to less than 1% of normal (p-phenylenediamine oxidase activity, mean $\pm$ SD 0.0034 ± 0.003; control 0.59 ± 0.26). When Cp was administered intravenously, an immediate increase in plasma iron was observed, and the rate of increase was related to the logarithm of the induced plasma Cp level [ΔFe (μg/min) $= 4 + 1.2$ log (pPD oxidase activity)]. When $CuSO_4$ was administered, plasma iron increased only after Cp appeared in the plasma. To determine whether Cp is necessary for plasma iron binding in vivo,

ferrous or ferric iron was administered intravenously in a dose of 200 μg per 100 ml estimated plasma volume. The observed increases in plasma iron (mean ± SD, μg/100 ml) were as follows: control: ferrous 182 ± 52, ferric 145 ± 35; copper deficient: ferrous 91 ± 23, ferric 140 ± 30. Thus, retention of ferrous iron in plasma was impaired in Cp-deficient animals. These observations suggest (1) that Cp is essential to the normal transfer of iron from cells to plasma in vivo, and (2) that Cp acts by speeding the oxidation of ferrous iron to ferric, thereby facilitating binding to plasma transferrin. (Research supported by NIH grant AM-04489.)

178. Regulation of the Fuel of Respiration of Outer Renal Medulla by Sodium-Potassium ATPase. JAMES B. LEE,* JOHN C. ALEXANDER,* AND DANIEL A. ABODEELY,* St. Louis, Mo. (introduced by Goronwy O. Broun, Sr.**).

The source and regulation of substrate oxidation necessary for the vigorous hyperosmotic sodium reabsorption occurring in the thick ascending limb of the outer renal medulla are unknown. To study this, slices of outer rabbit renal medulla were incubated in Krebs-Ringer-Tris buffer, 37°C, 90 min, ^{14}C-glucose 10 mM and ^{14}C-palmitate 2 mM at medium [Na^+] between 0 and 350 mM. Measurements were made of oxygen consumption (Q_{O_2}), glucose (G), and palmitate (P) incorporation into CO_2, and ATPase activity in the 10,000 *g* slice homogenate supernatant. Q_{O_2} at 0 [Na^+] was 714 ± 59 μl rising to 1756 ± 78 μl/g wet weight on increasing [Na^+] to 350 mM. There were corresponding increases in G → CO_2 from 3.19 ± 0.43 to 5.53 ± 0.23 μl/g wet weight (44% of Q_{O_2}), P → CO_2 from 0.11 ± 0.01 to 0.63 ± 0.04 μmole/g wet weight (19% of Q_{O_2}), and endogenous respiration from 235 to 684 μl/g wet weight (37% of Q_{O_2}). Na-K ATPase increased from 7.7 ± 1.7 at 0 [Na^+] to 17.4 ± 2.0 μmoles P_i/mg protein per hr at 350 mM [Na^+]. Ouabain (10^{-3} M) completely inhibited sodium-induced acceleration of Q_{O_2}, G → CO_2, P → CO_2, endogenous respiration, and Na-K ATPase activity; the K_i for metabolic and enzymatic inhibition was similar (10^{-5} M). These results suggest that (1) Na-K ATPase activity is a major determinant of the rate of outer medullary substrate oxidation, (2) extracellular [Na^+] is of paramount importance in the regulation of this activity, and (3) glucose is the principal exogenous fuel of respiration of the outer renal medulla yielding energy applied to hyperosmotic active sodium transport by the thick ascending limb. (Supported by NIH grant AM-13036-02.)

179. Reciprocity of Plasma Low and Very Low Density Lipoprotein Concentrations in Lipemia. ROBERT S. LEES* AND DANA E. WILSON,* Cambridge, Mass. (introduced by Richard J. Wurtman).

Quantitation of individual plasma lipoproteins is essential in assessing dietary and drug treatment of hyperglyceridemia, since changes in lipoprotein levels may not be reflected by plasma lipid values. We present here data which show a reciprocal relation between plasma low density lipoprotein (LDL) and very low density lipoprotein (VLDL) concentrations with spontaneous and induced changes in plasma lipoprotein concentrations. We measured concentration by cholesterol content of individual lipoproteins in plasma from eight lipemic subjects (two type III, four type IV, two type V); six were maintained on formula diets in a metabolic ward for at least 6 months. In addition, two normal volunteers maintained on formula feedings were studied on normal and high-carbohydrate diets. Reciprocal changes in LDL cholesterol accompanied changes in VLDL produced by dietary change and by Clofibrate therapy. LDL cholesterol doubled in four of five subjects with types IV and V treated with Clofibrate, while VLDL fell by 56–84%. In two subjects, LDL reached abnormally high concentrations. LDL did not rise in two type III hyperlipoproteinemic patients, despite a comparable decrement in VLDL. The results suggest that reciprocity between LDL and VLDL occurs regularly in types IV and V, but does not occur in type III hyperlipoproteinemia. This reciprocity may be due to competition at sites of lipoprotein synthesis between LDL and VLDL for available cholesterol and protein. Alternatively, it may reflect increased formation of LDL from VLDL degradation. If plasma LDL concentration is related to atherogenesis, drug treatment of lipemic patients may increase atherogenic risk. (Supported by NIH grants RR-88 and HE-12621 and American Heart Association grant 69-714.)

180. "Radio Receptor Assay" of ACTH: A New Assay for Polypeptide Hormones. ROBERT LEFKOWITZ,* JESSE ROTH, AND IRA PASTAN, Bethesda, Md.

ACTH binding and adenyl cyclase activation are early essential steps in ACTH-induced steroidogenesis. The ACTH receptors with the ACTH-sensitive cyclase from ACTH-sensitive steroid-producing adrenal tumors were extracted by disrupting 4000 *g* particles in a French Press with phosphatidylethanolamine and fluoride; the receptor–adenyl cyclase unit was recovered in the 100,000 *g* supernatant. Pure ^{125}I-monoiodo-ACTH, freed of unlabeled ACTH by CM-cellulose chromatography, had almost full biological activity. For assay of ACTH, ^{125}I-ACTH (0.5 pg/ml) and unlabeled ACTH were mixed with ACTH receptors. After 1 hr, bound and free ^{125}I-ACTH were separated by filtration on G-75 Sephadex or by adsorption of free ^{125}I-ACTH to microfine silica. Unlabeled ACTH at 1 pg/ml (2×10^{-13} M; 0.1 μU/ml) produced significant displacement of ^{125}I-ACTH. With excess ACTH, displacement was complete. ACTH and five ACTH derivatives, differing widely in biological potency, displaced ^{125}I-ACTH in direct proportion to their capacity to activate adenyl cyclase. Nonspecific binding or displacement was uniformly absent. Albumin and insulin failed to alter ^{125}I-ACTH binding; ^{131}I-FSH, ^{131}I-HCS, ^{125}I-vasopressin, and ^{131}I-insulin failed to bind. Extracts from kidney, muscle, liver, and heart did not bind ^{125}I-ACTH. Unextracted plasma from two patients with pituitary Cushing's syndrome had ACTH at >4000 pg/ml (7000 by immunoassay) and 350 pg/ml; one patient with ectopic ACTH syndrome had 200 pg/ml (230–360 bioassay; 80–260 immunoassay), and one adrenalectomized patient had >1000 pg/ml. One steroid-suppressed and two hypopituitary patients had undetectable ACTH. Thus, using a specific hormone receptor and pure

biologically active labeled hormone we have devised a rapid assay for minute amounts of hormone with a biological rather than an immunological specificity.

181. Complement-Requiring Neutralizing Antibodies to Herpesvirus Hominis in Man and Rabbit. A. MARTIN LERNER, MARY JANE SHIPPEY,* AND ELIZABETH JANE BAILEY,* Detroit, Mich.

Recent data indicate that herpesvirus hominis is the commonest cause of fatal encephalitis in the United States. When seizures and coma ensue, mortality or severe and lasting neurologic residuals occur in 60–80% of the afflicted patients. To judge from our experience in Michigan, there are at least a thousand cases in the United States each year. At present a definitive early diagnosis can be made only by brain biopsy and isolation of herpesvirus. This is successful in half the cases of herpesvirus hominis encephalitis in which biopsy is done. We have reported that intravenous idoxuridine may alter the prognosis. As a possible means of facilitating early diagnosis, we continue to study specific complement-requiring neutralizing antibodies which (1) peak during the acute phase of infection, and (2) disappear during convalescence. Prototype, type 1 (oral), or type 2 (genital) strains of herpesvirus hominis were repeatedly injected intravenously into rabbits. Sera were obtained every several weeks for 6 months. Similar sera were studied from patients with herpesvirus encephalitis. Homotypic and heterotypic complement-requiring neutralizing (CRN) and conventional neutralizing (N) antibodies were measured by a liquid overlay plaque reduction method. Complement-requiring neutralizing antibodies appeared after infection with type 1 herpesviruses, but did not appear with type 2 infections. Complement-requiring neutralizing antibodies were completely resistant to mercaptoethanol reduction, indicating that they are 7S immunoglobulins. Conventional neutralizing antibodies of the acute phase were sensitive to mercaptoethanol. Titers of CRN and N were not augmented by the addition of antiglobulin. Isolates of herpesvirus hominis obtained at brain biopsy could be readily typed by unitypic rabbit hyperimmune serum. All encephalitogenic strains belonged to type 1. (Research supported by grants AI-00261 and AI-05721 from the NIH.)

182. Diminished Skin Blood Flow in Scleroderma. E. C. LE ROY,* P. J. CANNON,* AND J. A. DOWNEY,* New York, N. Y. (introduced by H. L. Nossel).

In an attempt to define scleroderma (systemic sclerosis) at a stage prior to the irreversible, "hidebound" fibrosis, skin blood flow (^{133}Xe) was measured in nine normal and eight scleroderma subjects in a temperature- and humidity-controlled room during cooling (18°C, 1 hr) and after warming by reflex heat vasodilatation (opposite arm in water bath, 44°C). Sublingual, skin, and ambient temperatures were monitored each minute. ^{133}Xe in saline (0.01–0.03 ml) was injected intracutaneously into the dorsal skin of the proximal phalanx of the third finger. Clearance of isotope was monitored by scintillation detector coupled to ratemeter and plotted semilogarithmically; cutaneous clearance, obtained by graphic resolution of observed washout and shown to be blood-flow dependent by arterial occlusion and by the studies of Sejrsen, was expressed as clearance constant ($K = 0.693/t_{\frac{1}{2}}$) and flow ($F = K \cdot \lambda \cdot 100$), in ml per 100 g tissue per min. At 18°C cutaneous clearance was totally obliterated in eight of nine scleroderma studies. The data are as follows (mean ± SD): clearance constant (K), normal 0.24 ± 0.15, scleroderma 0.04 ± 0.07, $P < 0.005$; flow, normal 18.6 ± 11.0 ml/100 g per min, scleroderma 2.73 ± 5.2, $P < 0.005$; skin temperature at injection site, normal 21.8 ± 0.6°C, scleroderma 21.6 ± 0.3, $P > 0.9$. After warming, all scleroderma subjects increased skin blood flow into the range of normal subjects, suggesting functional rather than structural vascular disease. Also, forearm skin flows at 24°C were similar in normal ($F \pm SD = 24.7 \pm 4.1$) and scleroderma (24.0 ± 6.1) subjects. During warming, surface skin temperature in scleroderma subjects remained well below that of normal subjects (25.9 ± 0.9 vs. 33.8 ± 0.5°C), despite sublingual temperature rises greater than normal. This dichotomy between skin temperature and blood flow suggests an abnormal thermal insulatory capacity of scleroderma skin. Oral pretreatment with guanethedine (40–75 mg/day) blocked the vasoconstriction during cooling in four of five scleroderma subjects. The intense cold-induced interruption of the microcirculation of the skin demonstrated in this study may be important in the pathogenesis of scleroderma. This microcirculatory defect would appear to be more approachable therapeutically than the now irreversible fibrosis. (Supported by the RGK Foundation and a Research Career Development award, USPHS.)

183. Solubilization of Myocardial Adenyl Cyclase with Loss of Hormone Responsiveness. GERALD S. LEVEY,* Bethesda, Md. (introduced by G. D. Aurbach).

Adenyl cyclase is a membrane-bound enzyme thought to mediate the effects of a variety of hormones on their respective target tissues. The purification of this enzyme has been impaired because of its association with particulate cellular components and the instability of the enzyme in broken cell preparations. In this investigation 90–100% of the fluoride-stimulatable adenyl cyclase in cat left ventricle was solubilized by homogenization of the tissue in Lubrol-PX. Without Lubrol most of the adenyl cyclase activity was precipitated by centrifugation at 12,000 *g* for 10 min, whereas almost all the activity was found in the 12,000 *g* supernatant of homogenates made with the detergent. The activity in the 12,000 *g* supernatant was not sedimented at centrifugal speeds as great as 250,000 *g* for 2 hr. Electron microscope observation of the 105,000 *g* and 250,000 *g* supernatant preparations showed almost no particulate material, in contrast to the 12,000 *g* supernatant, which contained abundant particles. Adenyl cyclase activity was not filterable by a 0.22 μ Millipore filter. Results of Sephadex chromatography indicated a molecular weight of 100,000 to 200,000. The solubilized enzyme was activated by sodium fluoride but not by norepinephrine, glucagon, or thyroxine, the hormones that activate particulate myocardial adenyl cyclase. These hormones were similarly without effect with preparations freed of detergent by chromatography on DEAE-cellulose. These results suggest that hormone receptor sites are a physical component of the cell membrane and not an inherent part of the enzyme.