148. Cholesterol Solubility in Bile: Retardation of Precipitation and Dissolution by Liquid Crystalline Mesophase. R. T. HOLZBACH,* M. F. OLSZEWSKI,* AND M. MARSH,* Cleveland, Ohio (introduced by Daniel L. Horrigan**).

That cholesterol supersaturation is frequent in healthy man has been recently shown. Thus this factor alone can no longer be considered the central issue in gallstone pathogenesis. Identification of those factors that promote its nucleation and precipitation from abnormal supersaturated biles and those which inhibit precipitation from equally supersaturated normal biles is now required. We have demonstrated that high lecithin concentrations retard the rate of cholesterol precipitation from supersaturated in vitro solutions compositionally patterned after bile. In addition, the gallstone-dissolving effect of chenodeoxycholic acid may be limited by the kinetics of cholesterol dissolution. Other recent work has shown that this too may be correlated with relative lecithin concentration. Using a slow-flow self-diffusion technique, our recent studies support this claim of retarded cholesterol dissolution into undersaturated solutions of high relative lecithin concentrations. Using periodic polarizing microscopy, a previously undescribed spontaneous liquid → solid phase transition was observed in supersaturated model solutions. Conditions included sterile equilibration at 37°C extending at times up to 3 wk. In these systems phase transition phenomena and transition rates were strong functions of lecithin concentrations. Liquid crystalline mesophases of 3–30 μ diameter were observed in both crystallite nucleation and dissolution studies with solutions containing appropriate lecithin concentrations. Identical spontaneous phase transitions were observed in supersaturated normal human bile specimens. These findings indicate that rapid nucleation of the mesophase can explain the rate-retarding effects on cholesterol nucleation and precipitation, as well as upon its dissolution in model solutions containing high lecithin concentrations. Finally, the observed spontaneous liquid → solid crystalline phase transition may have in vivo relevance for the earliest stages of cholelithiasis.

149. Human Erythrocyte Redox States: Effects of P_{O_2} and pH on NAD^+/NADH and $NADP^+$/NADPH Systems. B. R. HORN,* J. THEODORE,* AND E. D. ROBIN,** Stanford, Calif.

The state of oxygenation of hemoglobin appears to influence erythrocyte metabolic processes. The cellular redox state is intimately linked to such processes. Free NAD^+/NADH and $NADP^+$/NADPH ratios may be used to measure the redox state of cells. These are calculated by determining the ratio of the concentrations of oxidized and reduced metabolites of suitable nucleotide-linked reactions, using the known Keq of the reaction. These ratios were measured in normal human red cells at P_{O_2}'s of 150 (AIR) and 15(N_2) torr, and at extracellular pH's of 7.4 and 7.05. The NAD^+/NADH ratio was determined from the ratio of lactate to pyruvate. The $NADP^+$/NADPH system was investigated by utilizing the polyol pathway: GLUCOSE + NADPH + $H^+ \rightleftarrows$ SORBITOL + $NADP^+$. The glucose/sorbitol ratio is reported as an index of the $NADP^+$/NADPH ratio since the Keq for this reaction is not known.

	NAD^+/NADH			Glucose/sorbitol		
	Air	N_2	P	Air	N_2	P
pH 7.4	12.8	8.6	<0.05	392	460	=0.05
pH 7.05	1151	742	<0.005	620	840	<0.05

These findings suggest that (*a*) significant changes in the redox state of red cells occur as a function of hemoglobin oxygenation; (*b*) the NAD^+/NADH and glucose/sorbitol ratios vary inversely with deoxygenation indicating that the polyol pathway may act as an electron shuttle between NADPH and NADH; and (*c*) there is a marked change in the redox state of red cells with alteration in pH, especially the NAD^+/NADH ratio, which can only partly be accounted for by mass action.

150. Selective T-Cell Killing of Human Lymphocytes by Ultraviolet Radiation. SHELDON HOROWITZ,* DEREK CRIPPS,* AND RICHARD HONG, Madison, Wis.

The effects of ultraviolet radiation (UV) on human B- and T-lymphocytes were studied. Peripheral blood lymphocytes from (12) normal adults were irradiated with a germicidal lamp (GL) in graded doses (1–20 min) and with monochromatic UV of 254, 280, and 296 nm. B-lymphocytes were defined as those bearing surface immunoglobulin markers detected by immunofluorescence and T-lymphocytes were defined by formation of rosettes with sheep red blood cells. Functional activity was assessed in vitro by mitogenic stimulation: PHA for T-cells and pokeweed mitogen (PWM) for B-cells. Viability was determined by eosin-dye exclusion. Low intensities of the GL had a minimal effect on the viability of B- and T-lymphocytes and mitogen responsiveness. Longer exposures (0.19–0.67 W·s/cm^2) killed T-cells (50–100%) without significant effects on B-cells. For each individual, a UV intensity was found which resulted in loss of rosette-forming capacity and ability to respond to PHA but preservation of normal numbers of surface immunoglobulin-bearing cells and response to PWM. At higher intensities (0.67–2.0 W • s/cm^2), in addition to 100% T-cell killing, there was significant B-cell killing (50–100%). The most effective monochromatic UV for T-cell killing was at 254 nm with less effect at 280 and 296 nm. It is concluded that graded doses of UV can selectively destroy human peripheral blood T-lymphocytes, while allowing functional B-lymphocytes to survive. (Research supported by NIH grants 5-TO1-00317-07, AM-15086, and AM-09995.)

151. Effect of Beta-Adrenergic Blockade on Left Ventricular Function in Exercise. LAWRENCE D. HORWITZ,* JAMES M. ATKINS,* AND STEPHEN J. LESHIN,* Dallas, Tex. (introduced by Robert L. Johnson, Jr.**).

The hemodynamic effects of propranolol (1 mg/kg intravenously) were evaluated in 10 dogs running a graded exercise regimen on a treadmill. Measurements were made of left ventricular internal diameter with a sonocardiometer, left ventricular pressure with a solid-state intracardiac gauge, ascending aorta flow with an electromagnetic flowmeter, and arteriovenous oxygen difference with pulmonary artery and aorta blood. Peak work loads were reduced by propranolol. At the same levels of mild and moderate exercise mean total oxygen consumptions were 31.3 and 35.5 ml/min per kg during exercise without blockade and 27.6 and 28.1 with propranolol. Propranolol significantly reduced heart rate, stroke volume, left ventricular systolic pressure, and left ventricular dp/dt max, and increased left ventricular end-systolic diameter at all levels of exercise. During mild exercise left ventricular end-diastolic diameter and arteriovenous oxygen difference were increased with propranolol. During moderate and severe exercise these differences were no longer present. It is concluded that beta-block limits exercise capacity by attenuating the heart rate response and the rate and extent of muscle fiber shortening. At comparable levels of submaximal exercise, propranolol resulted in reduced total oxy-

gen consumption, and the exercise loads were accomplished with lower cardiac outputs and developed tensions. (Research supported by a grant from the NHLI.)

152. The Role of Pulmonary Surfactant in the Bactericidal Activity of Alveolar Macrophages During Oxygen Toxicity. GARY HUBER,* DENISE O'CONNELL,* AND MARC LAFORCE,* Boston, Mass. (introduced by Edward Kass **).

Although the alveolar macrophage (AM) is the key cell in antibacterial defenses in the lung, the bactericidal role of pulmonary surfactant is controversial. Prolonged exposure to 100% oxygen resulted in progressive depression in vivo of the intrapulmonary inactivation of an aerosolized challenge of radiolabeled (^{32}P) *Staphylococcus aureus*. Oxygen exposure also induced progressive increases in alveolar surface tension forces (quantified by pressure-volume relationships and correlation of alveolar bubble stability indices with ultrastructural morphometry) and decreases in surface activity and phospholipid phosphorus recoverable by branchopulmonary lavage. However, AM from control and oxygen-toxic rats were comparable in number, viability, and phagocytic uptake of staphylococci in vitro. Bacterial precoating with an acellular surfactant fraction (SF), recovered by centrifugation of lavage fluid (40,000 *g*), was required for intracellular killing by AM when evaluated in vitro with lysostaphin, in spite of the presence of excess strain-specificity antibody and fresh complement. SF by itself had no bactericidal activity. Incorporation of SF from control animals provided equivalent bactericidal activity for both control and oxygen-toxic AM, whereas incorporation of SF from oxygen-toxic animals resulted in no intracellular killing by either control or oxygen-toxic AM. In vivo adaptive tolerance to oxygen toxicity, induced by intermittent exposure to oxygen and characterized morphometrically by proliferation of granular pneumocytes (cellular origin of surfactant), attenuated the impairment in bactericidal activity and in recoverable surfactant. These experiments imply that bacterial coating with normal SF stimulates bactericidal activity of both control and oxygen-toxic AM and that the depressed bactericidal activity in the oxygen-toxic lung is secondary to alterations in SF rather than to a direct impairment in the AM.

153. Isolation and Characterization of DNA, RNA, and Immune Complexes from Systemic Lupus Erythematosus (SLE) and Normal Plasma. DICK HUNTER,* JEANETTE DILLEY,* AND HALSTED HOLMAN,** Stanford, Calif.

Soluble complexes of DNA and antibody to DNA appear to cause SLE nephritis. Antibody to RNA is often associated with other clinical forms of rheumatic disease. Antigenic plasma nucleic acids and soluble complexes of those nucleic acids with antibody have not hitherto been isolated or characterized. A chromatographic method will be reported for isolation of DNA and RNA from plasma. Both nucleic acids are present in a single peak trailing behind the plasma proteins. Approximately $\frac{2}{3}$ of the peak is DNA. This elution position is quite different from that of DNA or RNA chromatographed alone. The DNA is very antigenic. By chemical and immunological criteria it appears to be native DNA. The buoyant density (ca. 1.720) of the isolated, purified DNA free of RNA is different from known mammalian DNA. The RNA forms unusual orcinol color reactions and is poorly reactive immunologically. DNA and RNA are present in normal plasma at a total concentration approximately 5 mg/100 ml. They appear increased in SLE, especially during disease activity, reaching levels between 10 and 20 mg/100 ml. At times, isolated DNA and RNA are accompanied by small amounts of IgG with high specificity for native DNA. This IgG is presumably a component of a complex. The plasma nucleic acids may be previously unknown human types or may be derived from either altered normal human nucleic acids or microorganisms. Their participation in tissue damage may depend upon the type of antibody produced in the disease. (Research supported by grant from NIH.)

154. Sodium Chloride, Urea, and Water Transport in the Thin Ascending Limb of Henle: Generation of Osmotic Gradients by Passive Diffusion of Solutes. MASASHI IMAI* AND JUHA KOKKO,* Dallas, Tex. (introduced by Donald W. Seldin **).

Studies were designed to examine whether the thin ascending limb of Henle (TALH) decreases its luminal solute concentration by an active or a passive transport process. In all experiments isolated segments of rabbit TALH were perfused in vitro. When tubules were perfused with solutions identical with the bath, active transport of NaCl was excluded by the following: (*a*) collected fluid osmolality remained unchanged and same as the bath; (*b*) net water reabsorption could not be demonstrated; and (*c*) transtubular potential difference was zero. Isotopic permeability coefficients ($\times 10^{-5}$ cm/s) were calculated from the disappearance rate of the respective isotope added to the perfusate. These values indicate that TALH is moderately permeable to [^{14}C] urea (6.74±1.04) while having a higher permeability to ^{22}Na (24.9±1.9) and ^{36}Cl (116±9.8) than any other segment similarly studied. Osmotic water permeability was zero. When TALH were perfused with a 600 mOsm/liter solution predominantly of NaCl against a 600 mOsm/liter bath in which 50% of osmolality was NaCl and 50% urea (to simulate in vivo papillary interstitium), the collected fluid osmolality was decreased significantly below that of the bath (300 mOsm/liter per mm of tubule). The decrease in osmolality was due to greater efflux of NaCl as compared to influx of urea. We conclude that (*a*) TALH is not capable of active salt transport and (*b*) the unique membrane characteristics of TALH allows for generation of osmotic gradients (lumen less concentrated than adjacent surroundings) on purely passive mechanisms when perfused with isosmolal salt solutions in a bath with appropriate salt and urea concentrations. These findings are consistent with the passive countercurrent model previously proposed. (Research supported by grants from NIH.)

155. On the Apparent Increase in Production of Natriuretic Factor in Chronic Uremia. E. IPAKCHI,* JACQUES BOURGOIGNIE,* K. HWANG,* AND NEAL S. BRICKER, Bronx, N. Y.

Serum of patients and dogs with chronic uremia contains a natriuretic factor which is not detectable in normal serum. That the inhibitor is a physiologic mediator of the adaptive natriuresis per nephron is suggested by its absence in sodium-retaining uremic patients with nephrotic syndrome and uremic dogs in which the natriuresis is obviated by "proportional reduction" of sodium. The present studies represent an effort to detect the inhibitor in urine from normal and uremic subjects. If an inhibitor accumulates in uremia because of failure of excretion, it should be less detectable in uremic than in normal urine. However, if production is increased or degradation decreased, excretion might be greater despite low glomerular filtration rates (GFR's). Quantitative urine samples, collected from chronically uremic patients and normal subjects, were lyophilized, fractionated by gel filtration, and bioassayed in rats. The total fraction excreted in 2 h was infused in 1 ml of Ringer's. A single fraction

from uremic patients produced natriuresis ($\Delta U_{Na}V = 126$ μEq/h); the same fraction from normal subjects was non-natriuretic ($\Delta U_{Na}V = 24$ μEq/h ($P < 0.05$ uremic vs. normal). The natriuretic fractions from uremic urine and uremic serum have similar characteristics: (*a*) both appear in Sephadex G-25 eluates immediately after the salt and urea peaks, (*b*) both resist freezing and boiling, (*c*) both are inactivated by leucine-aminopeptidase, and (*d*) neither increases GFR. If the inhibitors in urine and serum are the same, elevated serum levels are not due to diminished excretion. Rather, the rate of synthesis must be increased and/or the rate of inactivation decreased. In either event, feedback suppression is not evident. The cumulative data support the concept that adaptation in sodium excretion in chronic uremia is associated with elaboration of a humoral natriuretic agent.

156. PGE_2 Acts As an Intrarenal Hormone Regulating Medullary Vascular Resistance. HAROLD D. ITSKOVITZ,* NORBERTO A. TERRAGNO,* AND JOHN C. MCGIFF, Milwaukee, Wis.

The role of PGE_2 as a local hormone in the regulation of intrarenal hemodynamics was assessed in isolated blood perfused canine kidneys. Solvent extraction and thin-layer chromatography, coupled with parallel bioassay, were used to measure "PGE," expressed as PGE_2 equivalents. Perfusate concentrations of PGE in six experiments increased from 0.04±0.02 (SEM) ng/ml during the initial 100 min to 0.80± 0.40 ng/ml by 200 min and to 2.23±0.66 ng/ml by 400 min. Renal blood flow increased *pari passu*. In four experiments, indomethacin (5 mg) reduced perfusate concentrations of PGE within 30 min from 1.37±0.58 ng/ml to 0.31±0.23 ng/ml. These results suggest a high synthetic rate for PGE in isolated kidneys which could be inhibited by indomethacin. Before indomethacin the intrarenal distribution of blood flow, as measured by radioactive microspheres, changed from a ratio of 79:21 (outer half; inner half of renal cortex) to 64:36 ($P < 0.01$). Inhibition of prostaglandin synthesis by indomethacin reversed this trend with a resultant increased fractional blood flow to the outer cortex (85:15; $P < 0.025$); simultaneously, renal blood flow decreased from 221±16 to 147±15 ml/min ($P < 0.01$), in spite of keeping renal perfusion pressure constant. Infusion of PGE_2 at rates as great as 2 μg/min did not prevent a redistribution of blood flow to the outer cortex after giving indomethacin, pointing to the importance of tissue synthesis rather than circulating levels of PGE_2 as the critical factor responsible for these hemodynamic responses. PGE_2 presumably acts as a local hormone at its site of synthesis within the renal medulla to affect medullary vascular resistance and thereby blood flow, both to inner cortical and medullary areas. (Research supported by grants HL 12677 and HL 13624 from NIH.)

157. Synthesis of Antihemophilic Factor (AHF) by Cultured Human Endothelial Cells. ERIC A. JAFFE,* LEON W. HOYER,* AND RALPH L. NACHMAN, Farmington, Conn., and New York.

Previous studies have identified AHF antigen exclusively in endothelial cells in human tissue sections by immunofluorescence using a monospecific rabbit antibody to human AHF (1972. *Fed. Proc.* **31**: 262, Abstr.). Evidence for synthesis was sought using cultured endothelial cells derived from human umbilical veins. These cells were differentiated from blood vessel medial smooth muscle cells and fibroblasts by cell morphology and growth pattern, and the presence of Weibel-Palade bodies, smooth muscle actomyosin, and ABH antigens. AHF antigen was identified in cultured endothelial cells by immunofluorescence. Soluble AHF antigen was also detected in culture media from endothelial cells by specific radioimmunoassay using ^{125}I-labeled anti-AHF. Culture medium not previously exposed to cells was devoid of antigen as were control media obtained from cultured smooth muscle cells and fibroblasts. To determine whether the AHF antigen in endothelial cell culture fluid represented release of previously stored or newly synthesized protein, the cultured cells were pulse labeled with radioactive amino acids. High molecular weight, AHF antigen-rich protein was separated from the radioactively labeled culture medium by agarose-gel chromatography. In three separate experiments, monospecific anti-AHF precipitated 8.0±2.1% of the radioactivity in this fraction, whereas normal rabbit serum precipitated 1.7±0.8%. Incorporation of radioactive amino acids into the immune precipitates was inhibited by culturing endothelial cells in the presence of cycloheximide. No AHF clotting activity was recovered in the tissue culture media, suggesting either that the endothelial cells synthesize an inactive molecule or that the functional activity is destroyed by a thrombin-like enzyme which we identified in the culture medium fetal calf serum.

158. Sulfodibromophthalein Excretion in Man. NORMAN B. JAVITT, DANIEL DHUMEAUX,* AND PIERRE BERTHELOT,* New York and Creteil, France.

We have found that sulfodibromophthalein (DBSP, pheno 3,6-dibromophthalein disulfonate) is not metabolized by man and therefore provides new insights into the critical determinants of hepatic transport function particularly when compared to sulfobromophthalein (BSP), which is conjugated mostly with glutathione. Eight individuals without liver disease and nine with cirrhosis were given equimolar amounts of BSP and DBSP in random sequence within 36 h. Each study consisted of three different infusions of 30 min duration. Storage (S, μmole/μmole per 100 ml) and maximum excretion rate (T_m, μmole/min) were calculated from simultaneous equations (Wheeler method). In the normal group, S as measured by DBSP was 125±57 (mean±SD), more than double that estimated by BSP (58±34). In cirrhosis, the reduction in S measured by DBSP (49±34) was proportionate to the reduction estimated using BSP (21±15). An excellent correlation ($r = 0.96$) was found between the estimation of S by both BSP and DBSP. T_m for BSP was 9.0±1.2 (mean±SD), not significantly different ($P > 0.10$) from the T_m for DBSP (10.9±1.6) in the normal group. Comparable reductions occurred in cirrhosis (BSP=4.0±1.2; DBSP = 5.5±1.2). These studies demonstrate that (*a*) DBSP can be used safely in man, (*b*) DBSP is distributed in man as a single species in plasma, liver, and bile, thus providing a more accurate estimation of S and (*c*) regardless of the possible role of glutathione as a determinant of BSP excretion, a profound defect in membrane-mediated transport must occur to account for the reduction in DBSP T_m and S in liver disease. (Research supported by grant AM-13094 from the NIH.)

159. Normal Plasma Renin Activity in Malignant Hypertension. JAMES G. JOHNSON,* SANDRA LEE,* SERGIO ACCHIARDO,* CESAR CUESTAS,* LEONARD SHARE,* AND FRED E. HATCH,* Memphis, Tenn. (introduced by E. E. Muirhead**).

The malignant phase of hypertension, defined as severe hypertension, renal insufficiency, and hypertensive retinopathy including papilledema, is observed in all hypertensive settings with the possible exception of coarctation of the aorta. Recent investigations have emphasized the importance of renin-angiotensin-aldosterone system in the pathogenesis of the

malignant phase. To evaluate this concept, 21 black patients with malignant hypertension were studied by measuring plasma renin activity (PRA) by radioimmunoassay of angiotensin I before treatment with diazoxide and furosemide. The age range of the 21 patients was 23–50 yr. There were 9 females and 12 males. Blood was obtained immediately after admission and PRA was determined in two separate laboratories using different antibodies to angiotensin I. Of the 21 patients, 10 had PRA clearly within the normal range for sodium-loaded upright patients, and 7 had PRA within the normal range for sodium-loaded recumbent patients. The range of PRA for the "normal reninemic" group was 0.2–4.4 ng/ml per h, whereas the range for the "high reninemic" group was 5.6–58 ng/mg per h. Of the normal reninemic group, 3 were admitted with oliguric renal failure and 2 others subsequently died of cerebral vascular accidents (CVA). One other patient in this group experienced two CVA's. Of the 11 patients in the high reninemic group, 3 were admitted with oliguric renal failure and one suffered a CVA. These data fail to support the concept that an elevated PRA is mandatory for the development and maintenance of the malignant phase of hypertension. (Research supported by a grant from NHLI.)

160. Inhibition of Phagocytic Bactericidal Activity by Superoxide Dismutase: A Possible Role for Superoxide Anion in the Killing of Phagocytized Bacteria. RICHARD B. JOHNSTON, JR.,* BERNARD KEELE,* LAWRENCE WEBB,* DALE KESSLER,* AND K. V. RAJAGOPALAN,* Birmingham, Ala., and Durham, N. C. (introduced by Max D. Cooper).

Enzyme systems capable of generating superoxide anion (O_2^-), a highly reactive free radical of oxygen, have been identified in many mammalian tissues. The physiological control of O_2^- has been attributed to superoxide dismutase (SOD), which catalyzes the removal of superoxide anion ($O_2^- + O_2^- + 2H^{+SOD} \rightarrow H_2O_2 + O_2$). The product of this reaction, hydrogen peroxide (H_2O_2), has been implicated in the killing of phagocytized bacteria; it has not been clear whether O_2^- itself serves a useful biological purpose. We have found SOD in leukocytes from normal humans and patients with chronic granulomatous disease and have demonstrated antibacterial activity of an O_2^--generating system. In an attempt to determine if O_2^- might be involved in phagocytic bactericidal activity, we studied the killing of *E. coli* and *S. aureus* by normal leukocytes in the presence of SOD. When the enzyme was fixed to latex particles which could enter the phagocytic vacuole along with bacteria, there was almost complete inhibition of phagocytic killing by SOD compared to albumin or heat-denatured SOD as controls. Similar inhibition of killing was effected by catalase, which catalyzes removal of H_2O_2. SOD did not inhibit ingestion, as measured by uptake of ^{14}C-labeled *E. coli*, by counting numbers of ingested bacteria and yeasts on smear, or by measurement of the respiratory burst which accompanies phagocytosis. These findings are compatible with the concept that both O_2^- and H_2O_2 are required for the optimal killing of ingested bacteria by phagocytes, perhaps through generation of highly oxidative hydroxyl radicals ($OH\cdot$) in the reaction $O_2^- + H_2O_2 \rightarrow OH\cdot + OH^- + O_2$.

161. Lecithin-Cholesterol Acyl Transferase Deficiency in Acute Pancreatitis. DON PAUL JONES,* Detroit, Mich. (introduced by Benjamin Lewis**).

Patients with pancreatitis may have hyperlipemia with decreased percentages of serum cholesterol esters. Decreased cholesterol esters correlate with decreased serum cholesterol esterifying activity (CEA) in patients with liver disease. We have studied CEA in pancreatitis and also have determined whether decreased CEA reflects lecithin cholesterol acyl transferase (LCAT) deficiency or an opposing serum cholesterol ester hydrolase (CEH). CEA, LCAT, and CEH were evaluated in 13 patients with liver disease and in 17 patients with pancreatitis without liver disease. CEA assay quantitated the decrease in concentration of serum unesterified cholesterol during 5 h incubation. LCAT assay determined the rate of [H^3] cholesterol ester synthesis in serum containing [H^3] cholesterol-labeled lipoproteins. CEH was assayed (*a*) under incubation conditions for hepatic CEH assay, and (*b*) by measuring [H^3] cholesterol formation from a unique substrate containing [H^3] cholesterol esters in natural serum lipoproteins. 90% of [H^3] cholesterol esters were hydrolyzed when substrate was incubated with dog plasma containing known pancreatic CEH. Mean CEA and LCAT activity were decreased in both patient groups (pancreatitis: CEA, 48%, LCAT, 66%; liver disease: CEA, 72%, LCAT, 65% of normal). In both, CEA and LCAT correlated positively with each other (pancreatitis: $r = 0.819$ [$P < 0.05$]; liver disease: $r = 0.869$ [$P < 0.01$]) and with the percent of cholesterol esters. No CEH was found. Sera from four patients with pancreatitis and LCAT deficiency did not inhibit LCAT in normal sera. Patients with pancreatitis acquire LCAT deficiency which may be related etiologically to pancreatic hyperlipemia. Low CEA associated with both pancreatitis and liver disease represents LCAT deficiency rather than the presence of CEH. No serum LCAT inhibitor was found. LCAT deficiency in pancreatitis merits study of mechanism, diagnostic and prognostic value, and relationships to hyperlipemia.

162. A Possible Bifunctional Role for Cystathionine Synthase. OLIVER W. JONES* AND MARY A. GRISHAVER,* La Jolla, Calif. (introduced by William L. Nyhan).

Patients with homocystinuria due to cystathionine synthase deficiency fall into two major categories regarding biochemical responsiveness to pyridoxal phosphate: those who respond by reversal of methioninemia and homocystinuria and those who have no change in the specific aminoacidemia/aminoaciduria after pyridoxal phosphate therapy. We find an enzyme in human fetal tissue which catalyzes the reaction: $H_2S + \text{serine} \xrightarrow[\text{sulfhydrase}]{\text{serine}} \text{cysteine}$, bypassing the reaction requiring cystathionine synthase. Serine sulfhydrase is present in human fetal skin, liver, and brain and has been purified 100- to 150-fold. There is an absolute requirement for serine and pyridoxal phosphate. Serine sulfhydrase is also demonstrable in human adult liver. K_m values for pyridoxal phosphate with fetal serine sulfhydrase are 5- to 6-fold higher than adult serine sulfhydrase suggesting that more available pyridoxal phosphate is required to achieve maximal catalytic activity with fetal enzyme. Serine sulfhydrase and cystathionine synthase have the following similarities: (*a*) identical purification through six steps including elution at the same point by DEAE chromatography; (*b*) similar temperature sensitivity; and (*c*) the same mobility on gel electrophoresis and identical response to serine and sulfhydryl analogs. Our results suggest that in human fetal tissue certain enzyme protein may have bifunctional catalytic properties. If cysteine biosynthesis is crucial to normal development, alternate routes for a specific biochemical reaction might be advantageous. In contrast, a point mutation on the enzyme polypeptide chain might render it incapable of catalyzing one reaction but capable of catalyzing another. It is possible that some homocystinuric mutants might still have serine sulfhydrase activity and cysteine biosynthesis in response to

pyridoxal phosphate, while the catalytic site for cystathionine synthase is rendered inactive by mutation. (Supported by grants from NIH and The National Foundation.)

163. Modulation of the Immunologic Release of Chemical Mediators from Human Lung and Nasal Polyps. MICHAEL KALINER* AND K. FRANK AUSTEN, Boston, Mass.

Fragments of human lung and nasal polyps passively sensitized with human IgE release histamine, slow reacting substance of anaphylaxis (SRS-A), and eosinophil chemotactic factor of anaphylaxis (ECF-A) upon antigen challenge. The immunologically activated secretion of these mediators from both tissues is suppressed by beta-adrenergic or prostaglandin stimulation and enhanced by alpha-adrenergic and cholinergic stimulation. In human lung tissue, the evidence implicating hormonally induced alterations in the tissue concentrations of cyclic AMP in modulation of mediator release includes: (*a*) the kinetic relationship between increases in cyclic AMP and inhibition of mediator release after stimulation with isoproterenol or the prostaglandins; (*b*) the parallel rank order of potency (ID_{50}) of isoproterenol (7.5×10^{-8}M) $\simeq$ epinephrine (5×10^{-8}M) > norepinephrine (10^{-6}M) > PGE_1 (5×10^{-5}M) > $PGR_{2\alpha}$ (5×10^{-4}M) in terms of suppressing mediator release and increasing cyclic AMP; (*c*) the synergism expressed between stimulators of adenylate cyclase and inhibitors of phosphodiesterase upon both phenomena; and (*d*) the association of depletions of cyclic AMP levels with enhancement of mediator release produced by alpha-adrenergic and a low dose prostaglandin (5×10^{-8}M) stimulation or by the nonhormonal agent imidazole. Cholinergic enhancement (Carbachol 10^{-9}–10^{-12}M) of mediator release, presumably acting through increases in cyclic GMP levels, is independent of measurable changes in cyclic AMP. Whereas the requirements for divalent cations and energy in the antigen-activated secretory process conforms to the usual requirements for secretion, the inhibitory effect of cyclic AMP is an exception. One could argue that the immunologic release of chemical mediators is antihomeostatic and that the inhibitory actions of cyclic AMP on mediator release favor homeostasis. (Supported by grants AI-07722 and RR-05669 from the NIH.)

164. Collagen-Induced Platelet Aggregation: Inhibition by Benorylate. ANDREW H. KANG,* EDWIN H. BEACHEY,* AND RICHARD L. KATZMAN,* Memphis, Tenn. (introduced by Lester Van Middlesworth**).

It is well established that native collagen both in solution and native-type fibril form induces platelet aggregation, and it has been suggested that the intact secondary and tertiary structures of collagen are essential in the process. In view of published evidence suggesting the involvement of platelet membrane-bound glycosyltransferases in the process, we have investigated the possible role of the primary structure of collagen. Contrary to several previous reports, our data clearly indicate that denatured, purified polypeptide chain, α1, is capable of inducing platelet aggregation in vitro using standard techniques. Furthermore, of the nine CNBr peptides of α1, only one peptide, α1-CB5, containing the only residue of Glu-Gal-Hyl in α1, is active. In addition, purified Glu-Gal-Hyl inhibited collagen-induced aggregation, indicating that the primary structure of collagen also plays a role in platelet aggregation. Because aspirin is a known inhibitor of native collagen-induced platelet aggregation, it was of interest to test if it also interfered with α1-induced aggregation. In addition, benorylate, a new lipid-soluble ester of acetylsalicylic acid and *N*-acetyl *p*-aminophenol, was also tested, since the latter had been reported to possess anti-inflammatory and antirheumatic properties comparable to aspirin but to cause significantly less gastrointestinal bleeding. Benorylate or aspirin in 100- or 200-mg doses was administered to prefasted (24 h) New Zealand White rabbits (2 kg). 2 h after ingestion, aggregation times of platelets obtained from aspirin or benorylate-fed rabbits were (*a*) aspirin 100 mg, 8.5 min; 200 mg, no aggregation after 12 min and (*b*) benorylate, 100 mg, 3.5 min; 200 mg, 9.0 min. Control platelets aggregated within 3 min. These results show that, on a molar basis, the inhibitory effect of benorylate upon platelet aggregation is identical with that of aspirin, and that factors other than interference with platelet aggregation must account for the reportedly greater amounts of gastrointestinal bleeding observed with aspirin than with benorylate. (Supported by grants from NIH and VA.)

165. Crystalline IgMλ Cytoplasmic Inclusions in Chronic Lymphocytic Leukemic Lymphocytes: a New Syndrome? MANUEL E. KAPLAN, ROBERT E. RYDELL,* AND CONNIE CLARK,* Minneapolis, Minn.

Cytoplasmic crystalline inclusions have been observed very infrequently in peripheral blood lymphocytes (PBL) from apparently normal individuals and from patients with chronic lymphocytic leukemia (CLL). The purpose of this report is to describe, in 4 of 29 CLL patients, cytoplasmic crystalline material made up, in each case, of IgMλ. Isolated PBL were routinely stained for membrane-bound immunoglobulin (M-Ig) and cytoplasmic immunoglobulin (C-Ig) with fluorochrome-conjugated (FC) antisera specific for μ-, γ-, α-, κ-, and λ-determinants. Patients were categorized on the basis of large populations of PBL containing monoclonal M-Ig:

	M-Ig type						
No. of patients	$\mu\kappa$	$\mu\lambda$	$\gamma\kappa$	$\alpha\kappa$	μ	Mixed	None
29	8	9	3	1	1	1	6

In four of nine M-IgMλ ($\mu\lambda$) patients, crystalline cytoplasmic inclusions staining specifically with FC anti-μ and anti-λ were detected. Electron microscopy showed the crystals within the rough endoplasmic reticulum. Similar crystalline inclusions were found in none of the remaining 25 patients. The statistical probability that this finding would occur solely by chance is 0.005. Double fluorescent staining of M-Ig and C-Ig, utilizing fluorescein- and rhodamine-conjugated antisera, revealed that essentially all PBL containing crystals exhibited M-IgMλ, whereas a considerably larger population of cells had M-Igλ but lacked crystals. In none of the patients with crystals studied was a paraprotein demonstrable in serum or urine. These studies indicate that (*a*) functionally different populations of neoplastic lymphocytes may exist in a given CLL patient; and (*b*) the mechanism(s) resulting in membrane insertion and/or extracellular secretion of IgMλ by CLL lymphocytes is frequently abnormal and may result in its intracellular crystallization. (Research was supported by grant AM 13717 from the NIH and grant 01/4828.1 from the VA.)

166. Platelet Adenine Nucleotide Metabolism During Adenosine Diphosphate (ADP)-Induced Aggregation. HERMAN E. KATTLOVE,* Torrance, Calif. (introduced by Kouichi R. Tanaka**).

Inhibition of platelet metabolism leads to decreased aggregation in response to ADP. This is thought to be related to decreased platelet adenosine triphosphate (ATP) content. The purpose of these studies was to determine the role of ATP in ADP-induced platelet aggregation. [^{3}H] adenine-labeled human platelet-rich plasma (PRP) was incubated

with 5 mM 2 deoxy-D-glucose (2 DG), together with either 2 mM KCN or 20 μg/ml oligomycin, and tested simultaneously for extent of ADP-induced aggregation and platelet ATP content. Aggregation declined and correlated closely ($r = +0.93$, $P < 0.001$) with the decrease in platelet ATP as measured by either chemical techniques or by radioactive techniques after separation by high voltage electrophoresis. This correlation suggests an important role for ATP in aggregation. This role was demonstrated when [^{3}H] adenine-labeled PRP was exposed to ADP in the presence of metabolic inhibitors such as 3.5 mM KCN. After 30–60 s, 10–20% of the ATP was converted to inosine monophosphate (IMP). This occurred whether or not the PRP was stirred. This did not occur in PRP exposed to ADP in the absence of metabolic inhibitors. Further studies demonstrated that within the first 5 s after ADP addition, 10–20% of the platelet ATP was first converted to ADP which was subsequently converted to IMP. The presumed effect of the KCN in these studies is to prevent the regeneration of ATP. Other inhibitors, such as 2 DG alone or oligomycin alone, were also effective. The ADP-induced breakdown of ATP was not prevented by 1 mM ouabain. We conclude that ATP is utilized during ADP-induced platelet aggregation. This is consistent with the hypothesis that ADP causes aggregation by stimulating the platelet contractile protein, thrombosthenin, and we would postulate that the ATP breakdown we observed is due to the ATPase activity of this protein. (Supported by NIH grant HE 14344.)

167. Epinephrine-Induced Enhancement of Myocardial Contractility: Possible Mediation by β-Receptor:Adenylate Cyclase:Adenosine 3′, 5′-Monophosphate-Dependent Protein Kinase:Calcium Transport System Located on the Sarcoplasmic Reticulum. ARNOLD M. KATZ, MADELEINE A. KIRCHBERGER,* MICHIHIKO TADA,* AND DORIS I. REPKE,* New York.

The positive inotropic effect of epinephrine may result from stimulation of adenylate cyclase (ACase), which catalyzes adenosine-3′,5′-monophosphate (cAMP) formation. cAMP has recently been shown to stimulate cAMP-dependent protein kinase (PK) to phosphorylate cardiac microsomes that are rich in sarcoplasmic reticulum (SR) membranes, concurrently enhancing both Ca uptake and Ca-activated ATPase. This more rapid Ca transport into SR could effect the classical epinephrine effects on the heart: inotropy and abbreviation of systole, the former due to increased intracellular retention of activator Ca^{++}. In the present study, we examined the possibility that all components of this control system are located on the SR. Cardiac microsomes prepared in dilute bicarbonate buffer represent *enriched SR,* viz. 45 nmoles/mg Ca-binding sites, and have minimal contamination by plasma membrane fragments, viz. (Na^++K^+)-activated ATPase < 1 μmoles/mg per h. ACase activity (111±9 [SEM] pmoles/mg per min), which was stimulated by epinephrine ($K_m \sim 5\times10^{-6}$ M epinephrine), was significantly greater than ACase of microsomes prepared in sucrose (90±4 pmoles/mg per min) which have fewer Ca-binding sites (13 nmoles/mg) and are rich in plasma membrane markers, viz. (Na^++K^+)-activated ATPase > 4 μmoles/mg per hr. This indicates that SR possesses both β-receptors and ACase. *Enriched SR* also contain active PK, viz. histone phosphorylation is at least doubled by added cAMP and is phosphorylated by endogenous PK (0.3 nmoles P per mg per 10 min) with twofold stimulation by added cAMP ($K_m \sim 10^7$ M cAMP). Furthermore, these endogenous systems can enhance Ca uptake after epinephrine. Epinephrine-induced inotropy thus may result from sequential stimulation of β-receptor, ACase, PK, and a Ca transport system all located on the SR, and need not require increased cytoplasmic cAMP. (Supported by NHLI and NYHA.)

168. Control of Aldosterone Secretion in Supine Man. FRED H. KATZ,* GARY O. ZERBE,* AND STROTHER H. WALKER,* Denver, Colo. (introduced by Karl E. Sussman).

Striking coincidence between peak plasma levels of aldosterone and cortisol in the early morning in two normal supine subjects was recently reported from this laboratory. Eight additional supine normal men and women have now been studied on the metabolic ward and the relationship between plasma aldosterone, renin activity, and cortisol in the 10 experiments investigated by regression analysis. Two of the subjects received 10-meq sodium diets, and the rest 110-meq sodium diets. One of the four women on normal sodium was taking oral contraceptives. Plasma was sampled every 30 min for 24 h or every 10 min from midnight to 8:00 a.m. Peak plasma aldosterone in all was seen in the early morning hours. The regression analyses revealed that of the eight subjects on normal sodium only four showed a statistically significant ($P = 0.05$ or less) correlation between renin and aldosterone levels. The correlation was also significant in the two on sodium restriction. The correlation of cortisol and aldosterone however was significant in all 10 subjects. Two other men were given dexamethasone., 0.75 mg every 12 h for 5 days, and subjected to a 24 h study while receiving a normal sodium diet. Although their plasma cortisol concentrations were constantly below the normal range, the early morning peak aldosterone levels, like those in untreated subjects, persisted on dexamethasone, and were correlated with renin. These results suggest that aldosterone secretion is under the control of the same central nervous system bio-rhythm which governs ACTH-induced cortisol secretion, although suppression of ACTH by exogenous steroid does not affect the bio-rhythmicity of aldosterone secretion. (Supported by grants from The Population Council, NIH, and the VA.)

169. The Low Renin State: Definition and Implications. DAVID C. KEM,* NORMAN J. KRAMER,* CELSO GOMEZ-SANCHEZ,* MARTIN WHITE,* AND NORMAN M. KAPLAN, Dallas, Tex.

Low plasma renin activity (PRA) has been used as a marker for determining diagnosis and prognosis in the hypertensive population. We have (*a*) simplified the screening technique for recognition of low PRA, (*b*) compared white and black normotensives and hypertensives, and (*c*) examined the relation between low PRA and hypertensive vascular complications. (*a*) PRA was performed by radioimmunoassay after generation at pH 5.5. PRA 30 min after 40 mg Lasix intravenously plus upright posture (Lasix test) was highly correlated ($P < 0.01$) to PRA after 2 ($r = +0.79$) and 4 h ($r = +0.58$) upright and PRA after 3 days low sodium diet plus 2 h upright ($r = +0.81$, $P < 0.01$). Lasix values were higher than upright alone but lower than low salt plus upright. (*b*) Lasix test PRA's were similar in 30 normotensive whites (mean 5.3±0.57 SE) and in 18 hypertensive whites (4.3±0.67) but significantly higher ($P < 0.02$) than in 23 normotensive blacks (3.4±0.60) who had, in turn, significantly higher values ($P < 0.02$) than did 27 hypertensive blacks (1.6±0.39). PRA's were lower in blacks despite higher renin substrates. (*c*) Lasix PRA's were as low (1.6±0.59) in 13 black hypertensives who had experienced a stroke or myocardial infarction as in the 27 without complications. Thus (*a*) the PRA state may be defined by a fairly simple procedure, (*b*) blacks have lower PRA than

whites so that published comparisons between normotensive whites and hypertensive blacks may be invalid; in addition to other factors, race must be considered in comparing groups, and (*c*) low PRA does not seem to protect against hypertensive complications. (Supported by NIH grant I-ROI-HL-14863-01.)

170. Demonstration of Fatty Acid-Binding Proteins Associated with the Brush Borders of Hamster Intestinal Epithelium. J. I. KESSLER AND S. MISHKIN,* Montreal, Quebec, Canada.

We have shown that the uptake of fatty acids (FA) by the small intestine is accomplished through a reversible association of FA to the epithelium. Analogous results were obtained with brush borders suggesting that the FA binding may be the property of the microvillous membrane. This study was undertaken in an attempt to define further the FA-binding properties of hamster brush borders. Micellar [^{14}C] FA or [^{14}C] FA dissolved in 50 μl dioxane:propylene glycol (2:1 v/v) was mixed with brush borders and kept for 1 h at 4°C. After three washings the brush borders were disrupted and five fractions (A, B, C, C^1, and D) were obtained by discontinuous density gradient centrifugation (1965. *J. Cell. Biol.* **26**: 687). The microvillous membrane fractions (C and C^1) contained 50–75% of the radioactivity and over 98% of it was in the form of free FA. These fractions were sonnicated and the supernatant was chromatographed on Sephadex G-75. The radioactivity was associated with the void volume peak and with a low molecular weight (10–12,000) protein. On addition of unlabeled FA, part of the radioactive FA from the low molecular weight protein was displaced to the void volume peak. Fractions A, B, and D and preparations of basal and lateral cell membranes (1969. *Biochim. Biophys. Acta.* **173**: 456 and 1972. *Biochim. Biophys Acta.* **274**: 336) exhibited only minimal FA binding. These results indicate that the FA binding by the intestinal epithelium may be related to FA-binding proteins in the microvillous membrane.

171. Specificities of Peptide Hydrolases in the Brush Border and Cytosol Fractions of the Rat Small Intestine. YOUNG S. KIM, YONG W. KIM,* AND MARVIN H. SLEISENGER,** San Francisco, Calif.

Peptide hydrolases have been shown to be localized in the brush border and cytosol fractions of the small intestine. In the present study, the specificities of peptide hydrolases from the brush border and the cytosol fractions of the rat small intestinal mucosa were examined with respect to the length of the peptide substrates and the amino acid residues hydrolyzed. Partially purified enzymes were prepared as described previously (1972. *J. Clin. Invest.* **51**: 1135). The quantitation and identification of the products of enzymatic hydrolysis was carried out using an amino acid analyzer. When heteropeptides and homopeptides up to six amino acids in length were examined as substrates, the cytosol fraction was found to be capable of hydrolyzing only the dipeptides and tripeptides; the brush border fraction hydrolyzed these peptides and those of greater length. The cytosol preparation had an apparent K_m for L-ala-gly of 2.35 mM, and for L-ala-(gly)$_2$, 0.95 mM. The brush border fraction had the following apparent K_m values: L-ala-gly, 0.65 mM; L-ala-(gly)$_2$ 1.29 mM; L-ala-(gly)$_3$, 1.01 mM; and L-ala-(gly)$_4$, 0.84 mM. Timed incubation studies using the above and other heteropeptides revealed that both brush border and cytosol peptide hydrolases cleaved amino acid residues sequentially from the amino termini of the peptides. Using dipeptide through hexapeptide substrates, no carboxypeptidase or endopeptidase activity was observed in either fraction. These results, in addition to the zymogram studies reported previously, suggest that the brush border and cytosol peptide hydrolases may serve two different cellular functions. The cytosol hydrolase activity is probably only involved in the final breakdown of peptides, while the brush border has the capability of hydrolyzing peptides of larger size which may be the primary products of dietary protein digestion. (Supported by VA research grant and VA RE TR-48.)

172. Increased Glucose-Induced Proinsulin Levels with Increasing Age. ABBAS E. KITABCHI AND WILLIAM C. DUCKWORTH,* Memphis, Tenn.

Although deterioration of glucose tolerance with age has been associated with increasing age, the contributory role of the circulating insulin and/or the less active precursor, proinsulin, has not been elucidated. The total immunoreactive insulin (TIR) and proinsulin-like material (PLM) in response to oral glucose was studied in 70 nonobese subjects of varying ages with normal glucose tolerance tests. Plasma for glucose, TIR, and PLM was obtained before and at 30-min intervals after a 100 g oral glucose load. Although each individual subject had a normal glucose response, the mean glucose levels increased with increasing age. In the three older groups (ages 45–54, 55–64, and 65–74) the integrated glucose areas above fasting were all significantly greater ($P < 0.05$) than in the youngest group (ages 15–24). No significant differences in TIR responses were seen, but plasma PLM was significantly higher in the older-age subjects. All values except fasting were significantly greater in subjects aged 45–74 years, as compared with subjects 15–44 years of age ($P < 0.01$). The total areas under the PLM curves were also significantly greater ($P < 0.01$) in the three older groups, as compared with the youngest group. A significant correlation ($P < 0.001$) between the amount of proinsulin and the age of the subject was seen while no significant correlation between TIR and age could be demonstrated. These findings may reflect a decreased conversion of proinsulin to insulin in the aging pancreas which could play a role in the glucose intolerance associated with aging. (Supported by grants from VA and NIH.)

173. Erythrocyte Membrane Lipid Abnormalities in Hypophosphatemic Hemolysis. JOHN C. KLOCK AND STEPHEN B. SHOHET, San Francisco, Calif. (introduced by Louis K. Diamond).

To evaluate possible distortions in lipid membrane composition or renewal in hypophosphatemic erythrocytes, we have measured erythrocyte phospholipids from two severely hypophosphatemic patients with hemolytic anemia (serum phosphate 0.4 and 0.2 mg/100 ml). Neither patient was jaundiced or hyperlipemic. The blood smear showed marked anisocytosis with many spherocytes, some echinocytes, and approximately 20% normal discocytes (probably secondary to transfusions). Total erythrocyte lipids were markedly elevated (187, 165 γP/cc cells vs. normal 125γ). This was mostly due to an increase in phosphatidylcholine (PC) (76; 71γP/cc cells vs. normal 35$\gamma\pm$5). Erythrocyte cholesterol was unremarkable. The ratios of phosphatidylcholine to phosphatidylethanolamine (PC:PE) were increased (2.54; 2.60 vs. normal 0.95±0.08). Erythrocyte ATP levels were also reduced (0.91; 0.76 μm/cc cells vs. normal 1.68±0.12) and osmotic resistance was increased, consistent with elevated membrane lipid (50% hemolysis at 0.36% saline). After 24 h incubation in autologous plasma, considerable loss of membrane lipid was observed (20% vs. normal <7%). In contrast to similar incubations of hereditary spherocytosis cells,

this loss was not wholly symmetric (PC:PE of 2.54 → 2.04). After parenteral phosphate therapy, all abnormalities were gradually corrected. Hemolysis in hypophosphatemic hemolytic anemia has been demonstrated by others to be associated with severe ATP depletion. Cell stiffness secondary to loss of CA^{++} chelating effect of ATP has been suggested as one mechanism for this hemolysis. Hemolysis has also been demonstrated in a kindred of patients who have abnormalities similar to those noted here (high erythrocyte PC, abnormal PC:PE, and increased osmotic resistance). A possible additional mechanism for hemolysis in patients with severe hypophosphatemia may be that low levels of ATP affect the balance and stability of membrane lipids. (Supported by NIH grants AM-16095 and AM-37337.)

174. On the Relationship of Hyperuricemia and Hyperuricosuria to Muscle Injury and Their Possible Role in Heat Stress Nephropathy. J. P. KNOCHEL, Dallas, Tex. (introduced by Floyd Rector, Jr).

Myoglobinuria and hyperuricemia may be prominent factors in the pathogenesis of acute renal failure during physical training in hot climates. Indeed, hyperuricemia may attain values observed in patients with urate nephropathy associated with other conditions. Myoglobinuria may be an associated phenomenon and conditions favoring precipitation of uric acid and myoglobin, such as dehydration and excretion of a concentrated, acid urine, commonly prevail under such circumstances. Studies were designed to determine if hyperuricemia was due to decreased excretion or increased production of uric acid (UA) and if related to increased production, whether it was possibly associated with skeletal muscle injury. Weekly observations were made in hot and cool weather on 22 healthy subjects during 35 days of stereotyped physical training under metabolic balance conditions. Dietary intake was constant (nitrogen = 23.4 g/day). The most striking changes were noted during the second week of training. Mean uric acid concentration [UA] in serum rose from 5.8(3.9–6.8) to 8.3(6.1–12.2) mg/100 ml ($P < 0.001$). Mean uric acid excretion (UurV) rose from 637(454–855) to 1031(792–1555) mg/day ($P < 0.001$). Uric acid clearance (Cur) did not change and at no time could serum [UA] be ascribed to a fall of creatinine clearance. Mean CPK activity rose from 7(0–16) to 58(9–304) U/ml ($P < 0.001$). Mean creatine excretion rose from 52(32–73) to 92(37–124) mg/24 h ($P < 0.001$). While there was no effect of climatic temperature on the foregoing, those training in hot weather had a mean [UA] in urine of 62(32–102) mg/100 ml; mean urine volume 1311(755–1842) ml/day and mean urine osmolality of 828(521–1128) mOsm/Kg H_2O. In contrast, mean values for subjects in cool weather were [UA] in urine, 35(26–42); urine volume 2787(2080–3655) and urine osmolality 449(367–525). All values were significantly different ($P < 0.001$). We conclude that (*a*) hyperuricemia during physical conditioning is due to overproduction of uric acid and not to a net decrease of uric acid clearance; (*b*) overproduction of uric acid can be correlated with muscle injury; and (*c*) hyperuricemia and hyperuricemia and hyperuricosuria may play a role in heat stress nephropathy.

175. Lipoprotein Changes in Pregnancy: a Distinct Endogenous Hypertriglyceridemia. ROBERT H. KNOPP* AND MARIA R. WARTH,* Boston, Mass. (introduced by Ronald A. Arky).

Plasma triglyceride and cholesterol rise threefold and a quarterfold in normal pregnancy. To determine a mechanism for these changes, triglyceride, cholesterol, and phospholipid were measured in the very low density, low density, and high density lipoprotein fractions (VLDL, LDL, and HDL, respectively). Lipoproteins were separated by ultracentrifugation and precipitation with $MnCl_2$ and heparin. VLDL protein was determined by the Lowry method. In 10 nonpregnant subjects, triglycerides in VLDL, LDL, and HDL, respectively, were: (mean±SEM) 23.7±3.6, 11.5±1.8, and 25.1±2.5 mg/100 ml. In 16 nondiabetic third trimester subjects triglycerides were significantly elevated in each fraction: 84.6±7.1 (VLDL), 67.5±4.4 (LDL), and 37.7±2.6 (HDL) mg/100 ml ($P < 0.01$ in each instance). These data point to lipoproteins with larger neutral lipid cores. Phospholipid was measured to determine if the polar surface coats were correspondingly increased. Phospholipid increased significantly (72%) only in the VLDL ($P < 0.01$), as did cholesterol ($P < 0.001$). Similarly, VLDL protein increased 80% ($P < 0.001$). Thus total VLDL rose from 81.5±6.5 to 154.3±13.8 mg/100 ml in pregnancy. The increased levels of VLDL in pregnancy are best explained by a balanced increase in the formation of all VLDL constituents. By contrast only triglycerides increased in the LDL, suggesting an accumulation of light LDL particles seen in the S_f 12–20 fraction (LDL intermediate). Paper electrophoresis of the VLDL fraction disclosed no beta mobility, excluding defective LDL catabolism and over-flow into the VLDL. The data support an increased conversion of VLDL into LDL that exceeds the capacity for triglyceride removal from the LDL intermediate. These adaptations can make triglyceride fuel more readily available to lipid utilizing tissues in pregnancy. (Supported by NIH.)

176. Stem Cell Kinetics in RFM Leukemic Mice. W. H. KNOSPE,* S. HUSSEINI,* F. E. TROBAUGH, JR.,* AND W. FRIED, Chicago, Ill.

RFM mice spontaneously develop myeloid leukemia which is transplantable by a cellular mechanism into nonleukemic RFM mice. The leukemic process progresses to death within 3–4 wk, and leukemic cells can be identified by a chromosome number of 39 as contrasted to 40 in nonleukemic cells. Colony-forming units (CFU) from marrow, spleen, and blood were studied by the spleen colony technique (suspensions of cells from these organs were injected into lethally irradiated mice and the number of resulting macroscopic spleen colonies was counted as per McCulloch and Till) before and after injection of nonleukemic RFM's with 10^6 leukemic cells. The karyotype and histologic type of cells in the resultant spleen colonies was studied. Marrow and spleen CFU increased 3- to 20-fold 2 wk after injection of leukemic cells. 3 wk after injection, the number of marrow CFU dropped to near normal levels while splenic and blood CFU remained high. A normal distribution of erythroid, granulocytic, and megakaryocytic colonies resulted from cells obtained 1 wk after injection of leukemic cells, whereas a marked increase in granulocytic colonies and a marked decrease in erythroid colonies resulted from cells obtained 2 and 3 wk after injection of leukemic cells. Cytogenic studies showed all spleen colonies from cells 1 wk after injection contained cells with 40 chromosomes, whereas nearly all colonies from cells obtained after 3 wk had 39 chromosomes. The latter were all of granulocytic type. No cells in erythroid colonies contained 39 chromosomes. We conclude that the leukemic RFM stem cells are capable only of differentiating into the granulocytic series. As these cells increase in number, growth of nonleukemic stem cells is inhibited. (Research supported by grants from Leukemia Research Foundation and NIH.)

177. Sleep EEG Stages and Growth Hormone (GH) Levels in Endogenous and Exogenous Hypercortisolemia. DOROTHY T. KRIEGER, MARK FINGERHUT,* AND SEYMOUR M. GLICK, New York.

Studies of nocturnal sleep EEG stages, plasma GH, and cortisol levels were performed on the following subjects: (A) six with active Cushing's disease (CD); (B) five with CD in remission; (C) one with Cushing's syndrome (adrenal adenoma) pre- and postadrenalectomy; (D) one with CS (parasellar tumor) pre- and postradiotherapy; (E) three with Nelson's syndrome; (F) three with hypothalamic tumors (normal basal endocrine status); (G) five receiving chronic high dose prednisone treatment; and (H) four normal volunteers during a constant hydrocortisone infusion, after a loading dose 2 h before sleep onset. Compared to normal subjects, stage II sleep was increased and slow wave sleep (SWS: stage III, IV) was decreased in five of six group A patients; three also showed a decrease in REM sleep. Similar stage I and SWS changes were noted in four of five group B patients; patient C pre- and postadrenalectomy; patient D pre- and postirradiation and all three group F patients. In contrast, percent times for all sleep stages were essentially within normal limits in group E, G, and H patients, save for a decrease in REM sleep in the latter. Analysis of GH data has been completed in groups A, B, C, D, E, H. A marked decrease in the nocturnal increment ($\Delta < 5$ ng/ml) was observed in all except patient C postadrenalectomy. Thus, abnormalities in sleep EEG stages II and SWS and lack of nocturnal GH elevation in patients with CD are not solely related to the hypercortisolemia, since such changes are absent in groups F and G and present in CD in remission, as well as in normocortisolemic patients with hypothalamic tumors. Normal sleep stage percentages in group E patients may be related to decreased plasma corticosteroid and/or markedly elevated plasma ACTH levels, whereas the significance of altered sleep stages in patient C is unclear. A normal nocturnal GH rise was absent in group E patients, indicating a dissociation between normal SWS and such a rise. (Research supported by NIH grants NB-02893, FR-71, and AM-09219.)

178. Familial Hyperbetalipoproteinemia (Type II Hyperlipoproteinemia) in Children. P. O. KWITEROVICH,* R. I. LEVY, AND D. S. FREDRICKSON,** Bethesda, Md.

236 children of 90 matings, hyperbetalipoproteinemia (II) × normal (N), were studied. They represented all but four of the living offspring, age 1–19, from these matings (two were not ascertained; two, being propositi, were excluded). Two log normal distributions were obtained for concentrations of both low density (C_{LDL}) and plasma (C) cholesterol. Likelihood ratio test favored a bimodal distribution of C_{LDL} ($\chi^2 = 18.41$, $P < 0.0005$) and C ($\chi^2 = 7.81$, $P < 0.025$) over a unimodal distribution. The derived normal cutoff for C_{LDL} of 164 mg/100 ml was used to calculate a segregation ratio of 45:55 (II:N), consistent with a monogenic trait with dominant phenotype. If a frequency for II in the general population of 5% was assumed, a polygenic model was highly unlikely ($\chi^2 = 65.80$, $P < 10^{-6}$) (Edward's test: expected 22%, observed 45%). The percentage of abnormal children in the first decade (52) significantly exceeded that in the second (39) ($P < 0.01$). The mean triglyceride was higher in the hyperbetalipoproteinemics ($P < 0.0025$). The children from 35 matings: type IIB (hyperbetalipoproteinemia and hyperglyceridemia) × non-type II included 39 IIA, 8 IIB, 1 with type IV, and 52 N. From 55 matings: type IIA × non-type II, there were 56 IIA, 2 IIb, 2 IV, and 76 N. A child of a parent with type IIB was more likely to have IIB than a child of a IIA parent ($\chi^2 = 5.55$, $P < 0.05$). The data strongly support early expression of hyperbetalipoproteinemia inherited by a monogenic model in these children. More than one genetic defect within the group is not excluded.

179. Epidemiologic Studies of Cytomegalovirus (CMV) in Semen. DAVID J. LANG,* RICHARD B. FROST,* LAWRENCE J. D'ANGELO,* AND JOHN F. KUMMER,* Durham, N. C. (introduced by Jerome S. Harris).

We have previously demonstrated the presence of CMV in human semen and suggested that this virus may in some instances be venereally transmitted. The index patient was recuperating from a heterophile-negative mononucleosis and had a history of recent gonococcal infection. We present here the results of our initial epidemiologic studies of CMV in semen. CMV was not recovered from any of the semen specimens of 19 healthy men. Samples of semen were obtained from 94 male members of couples seeking a fertility evaluation; one semen was CMV positive. 16 university students with a history of mononucleosis contributed specimens. Two yielded CMV on culture. 10 men with active bacterial venereal infections were also studied. One of these 10 semen specimens contained infectious CMV. A medical history was available from three of the four individuals with positive semen cultures. All three had multiple sexual partners in the recent past. Two had a history of documented bacterial venereal infections. Two of the three are homosexuals. These preliminary results support the thesis that CMV may be transmitted by venereal contact and that exposure to multiple sexual partners increases the risk of acquiring this virus infection as it does the defined venereal diseases. Infections with CMV may be subclinical and, even if symptomatic, the infected patient may continue to shed virus long after the illness has resolved. We have reported elsewhere that CMV has remained demonstrable in the semen of the asymptomatic index case for more than 10 months. The full importance of the presence of CMV in the male and female genital tracts, beyond its transmission to the fetus and neonate, remains to be determined. (Research supported by NIH NHLI-72-2910B and NIH 2 ROL-HD-AI-04183-02.)

180. Cytomegalovirus (CMV) in Human Semen. DAVID J. LANG,* JOHN F. KUMMER,* AND DAVID P. HARTLEY,* Durham, N. C. (introduced by Samuel L. Katz).

In 1972 we reported the isolation of CMV from the semen of a young man recuperating from heterophile-negative mononucleosis. We report here further studies of CMV in semen and document the persistence of virus for 10 months. Semen containing $10^{7 \cdot 7}$ tissue culture infectious doses ($TCID_{50}$) per ml was studied. The cellular and fluid elements of the semen were separated and the CMV present in each quantitated. 99% of the infectious CMV in semen was found in the supernatant. Samples of semen were examined by electron microscopy. Herpesvirus particles were demonstrable in large extracellular aggregates. No virus was seen within or adsorbed to spermatozoa or inflammatory cells. During early convalescence total sperm counts and the motility of spermatozoa were depressed, and the pH of the semen was abnormally high. Subsequently, these values returned to within the normal range while CMV persisted in high titer. 10 months after its initial demonstration, CMV was still present in semen although the titer was reduced to 10^2 $TCID_{50}$/ml. The patient has been asymptomatic throughout this time and has remained sexually active. Cytomegalovirus was recovered from the cervix of a sexual contact. To

determine whether multiplication of this virus can occur in spermatozoa or associated cells, semen from uninfected individuals was inoculated with CMV in vitro. There was no evidence of virus adsorption or replication. Our studies indicate that CMV may persist in semen, primarily in extracellular fluids, while not replicating in spermatozoa nor necessarily associated with recognizable impairment of sperm function. However, the presence of CMV in semen may be an important factor in the frequent demonstration of this virus in the female genital tract. (Research supported by NIH NHLI-72-2910B and NIH 2 ROL-HD-AI-04183-02.)

181. Clinical and Pharmacologic Studies of Adenine Arabinoside (ara-A) in Serious Herpesvirus Infections. CARL B. LAUTER,* ELIZABETH J. BAILEY,* FRANCIS M. WILSON,* AND A. MARTIN LERNER, Detroit, Mich.

On account of in vitro and in vivo (animal model) activity of ara-A vs. herpesviruses of man and its relative lack of toxicity in these systems, clinical trials are clearly warranted. Ara-A was administered parenterally in schedules of 2.5–20 mg/kg per day to nine patients with serious virologically confirmed herpes simplex (HSV), varicellazoster (V-Z), or cytomegalovirus (CMV) infections. Seven of nine patients were debilitated or immunologically depressed. Noninterferon, nonantibody antiviral activity (AVA) due to ara-A or its active metabolite, ara-hypoxanthine, was assayed by a specific microbiologic method with a sensitivity of 50 μg/0.4 ml. AVA in sera, urines, and vesicular fluids ranged from 50 to 200 μg/0.4 ml, and was found only in four adults receiving 20 mg/kg per day of ara-A. Sporadic AVA was found in urine when intravenous ara-A was infused at a rate of 60 mg/hr, but was constant when delivered more rapidly. Urinary excretion accounted for 17–38% of the daily dose. Improvement and resolution of clinical findings in four patients with disseminated V-Z occurred in 5.7 and 8.7 days, respectively. Similar means for two patients with generalized HSV were 2.5 and 6 days. A patient with herpes progenitalis relapsed after ara-A was stopped, and a newborn with CMV did not respond. Toxicities were (*a*) a transient extrapyramidal syndrome in two patients with Hodgkin's disease receiving the highest dosage of ara-A, (*b*) transient falls in hemoglobin (>2 g/100 ml) in seven of the ten treatment courses, and (*c*) transient transaminase rise in a single patient. There were no white blood cell, platelet, or renal or cardiac side effects. In generalized HSV or V-Z infections ara-A appeared strikingly effective, and a clear advance over available agents (idoxuridine or cytosine arabinoside) which have limited usefulness in immunologically depressed, thrombocytopenic, or leukopenic patients. (Research supported by grants from the NIH and Parke-Davis Co.)

182. Hereditary Deficiency of Sixth Component of Complement (C6) in Man. J. P. LEDDY, M. M. FRANK,* T. GAITHER,* R. S. HEUSKINVELD,* R. T. BRECKENRIDGE,* AND M. R. KLEMPERER,* Rochester, N. Y., and Bethesda, Md.

A young black female (D. B.) in good general health was found to lack serum hemolytic complement (C) activity. All C components were normal except C6 which was undetectable by both functional and immunoprecipitin assays. These findings could not be accounted for by a C6 inhibitor. C6 was also absent in D. B. plasma. Hemolytic C (CH50) titers on family members were normal; however, both parents and five of six available siblings had approximately half-normal C6 levels by functional assay. The other sibling was normal. Biologic properties of D. B. serum include: (*a*) absent bactericidal activity against *S. typhi 0 901* with or without added rabbit antibody; (*b*) normal generation of chemotactic activity for human neutrophils in the presence of endotoxin or aggregated IgG; (*c*) ability to sensitize appropriate cells for immune adherence or agglutination by anti-C3 Coombs serum; and (*d*) inability to lyse PNH red cells in either acid hemolysis or "sugar water" tests. An extensive coagulation workup was normal although clotting time (25°C) and prothrombin consumption (37°C) in plastic tubes were, respectively, at the upper and lower limits of the normal range. Addition of endotoxin (40 μg/ml) or inulin (1–2 mg/ml) to blood from D. B. and a panel of normal donors had no consistent effect on clotting time or prothrombin consumption. These endotoxin and inulin preparations were shown to activate the alternate C pathway by several criteria. These studies document for the first time a human kindred with C6 deficiency. This defect exhibits a classic Mendelian autosomal inheritance, with all three genotypes being recognizable. Heterozygotes would apparently escape detection by common screening methods such as CH50 titration. Unlike the C6-deficient rabbits studied by others, the homozygous C6-deficient human exhibits chemotactic and coagulation functions within the range of normal. (Supported by NIH research grants.)

183. Circulating Prostaglandin A in Human Essential Hypertension. J. B. LEE, A. ATTALLAH,* V. K. VANCE,* C. ELLWOOD,* AND A. PREZYNA,* Buffalo, N. Y.

We have hypothesized that essential hypertension may be the result of intrarenal cortical vasoconstriction secondary to a deficiency of renomedullary PGA whose normal role may be to promote cortical vasodilation by circulating from medulla to cortex by either systemic or intrarenal circulations. Since little is known of endogenous human renal antihypertensive prostaglandins, the present study was designed to test this hypothesis by determining the presence of PGA in human kidney and in normotensive and hypertensive subjects. PGA was determined by radioimmunossay after silicic acid chromatographic separation of PGA from PGE, and $PGF_{2\alpha}$ (sensitivity: 100 pg/ml). Results are expressed as picograms per milliliter plasma or milligram tissue±SEM. In fresh surgical specimens from five normotensive humans [PGA] in cortex was 26±13; outer medulla, 309±163; and papilla 1683±34 ($n = 5$; $P = < 0.005$). Peripheral plasma [PGA] in 14 patients with essential hypertension was 461±49, which was significantly lower ($P = < 0.01$) than in 15 normotensive subjects (724±81). There was a significant negative correlation between PGA and 24 h urinary sodium (212±23 mEq; range 85–364 mEq). In six essential hypertensive patients there was no difference between right (RRV) and left (LRV) renal venous blood although RRV + LRV was significantly higher than the inferior vena cava (IVC) so that RRV + LRV contributed 299 pg PGA to IVC. These results show that (*a*) PGA is present in human renal papilla in high concentration; (*b*) the kidney contributes to the pool of circulatory PGA in patients with essential hypertension; and (*c*) despite this contribution, it appears that a deficiency of circulating PGA in essential hypertension may be a major etiological factor underlying the genesis of this disorder.

184. Interaction of [^{3}H] Norepinephrine with Specific Adrenergic Binding Sites in Cell Membranes of Cultured Myocardial Cells. ROBERT LEFKOWITZ,* DONALD O'HARA,* AND JOSEPH WARSHAW,* Boston, Mass. (introduced by Edgar Haber).

Chronotropic and inotropic effects of catecholamines on myocardium are probably mediated by binding to specific adrenergic receptors. To examine the initial interaction of norepinephrine with intact target tissue, in an environment free of vascular and neural elements, the binding of [^{3}H] norepinephrine to cultured chick embryo myocardial cells was studied. These cells contract rhythmically and their rate of beating is increased by catecholamines. [^{3}H] norepinephrine ($3–5 \times 10^{-9}$ M) was incubated with myocardial cells (5×10^5/ml) at 37°C for 1 h and the [^{3}H] norepinephrine bound to the cells was quantified by Millipore filtration and liquid scintillation counting. Catecholamines and related drugs displaced [^{3}H] norepinephrine from these binding sites. Half-maximal displacement of binding occurred at $1–5 \times 10^{-7}$ M for isoproterenol, epinephrine, and norepinephrine and 10^{-6} M for dopamine. α-Active amines such as phenylephrine and metaraminol competed only at concentrations above 10^{-5} M [cf. Ertel et al. 1971. *J. Pharmacol. Exp. Ther.* **178**: 73; half-maximal stimulation of rate of beating of these cells by norepinephrine at 3×10^{-7} M, comparable effect by phenylephrine at 1.2×10^{-5} M]. Catecholamine metabolites, cholinergic agonists and antogonists, and glucagon did not compete for binding sites. Propranolol inhibited binding by 40% at 10^{-4} M; phentolamine was inert. 2.5×10^6 sites were present per cell. Norepinephrine covalently bound to agarose beads displaced 70% of [^{3}H] norepinephrine when present at 5×10^{-5} M. Trypsin destroyed the binding sites as did trypsin bound to agarose. These findings suggest that the cell surface is the predominant location of these sites. Phospholipase digestion of the cells increased binding, presumably by exposing previously inaccessible sites. Parachloromercuribenzoate, EDTA, and EGTA at 1 mM markedly inhibited binding, suggesting that the binding site contains crucial -SH groups and that small amounts of divalent cations are necessary for the binding reaction.

185. Hemodynamic Basis of Renal Failure in Alcoholic Hepatitis. OSOTH LEKAGUL,* ARIE KEYNAN,* BERNARDO KOTELANSKI,* AND JAY N. COHN, Washington, D. C.

Oliguria and azotemia complicating the course of alcoholic hepatitis (AH) superimposed on cirrhosis is almost invariably fatal. The renal failure has been attributed to undescribed hemodynamic factors. Splanchnic and systemic hemodynamic studies therefore were performed in 28 patients with alcoholic hepatitis, including 13 with (group I) and 15 without (group II) renal failure. Prothrombin time was similar in the two groups, but bilirubin was higher, albumin lower, and ascites greater in group I patients. Group I had significantly lower mean arterial pressure (71.1 mm Hg compared to 79.8 mm Hg) ($P < 0.05$) and higher cardiac output (11.0 liter/min vs. 8.9 liter/min) ($P < 0.05$) than in group II. Hepatic blood flow (isotope dilution method) was comparably high in both groups (2002 ml/min vs. 2208 ml/min), but hepatic oxygen consumption tended to be lower in group I (45.2 ml/min vs. 55.4 ml/min). Hepatic fraction of cardiac output averaged 24.7% in group II but only 19.1% in group I ($P < 0.02$). 12 of 13 patients in group I had complete porta-systemic shunting of mesenteric inflow compared to only 6 of 15 in group II. Hepatic vein wedge-free pressure gradient was lower in group I (14.3 mm Hg vs. 19.3 mm Hg) ($P < 0.02$), indicative of more effective portal decompression in the oliguric group. These data indicate that renal failure in AH occurs in patients with higher cardiac output, lower systemic vascular resistance, and more extensive porta-systematic shunting. Hepatic anoxia could contribute to the hepatic failure. The pattern is consistent with functional arteriovenous shunting, probably largely in the portal bed. (Supported in part by NIH Grant HL 11533.)

186. The Limits of Renal Calcium Conservation in Patients with Idiopathic Hypercalciuria. JACOB LEMANN, JR., AND EDWARD J. LENNON, Milwaukee, Wis.

Renal calcium wasting has been proposed as a mechanism of hypercalciuria among patients with idiopathic hypercalciuria and stones. The patterns and limits of renal calcium conservation were compared in 6 idiopathic hypercalciurics (S; 80±8.6 SEM kg) and 15 adults without a personal or family history of urinary stones (N; 70.4±2.8 kg). All ate similar normal diets for 3 days providing an average of 23.6±2.3 mM calcium per day and then an otherwise comparable liquid diet providing only 1.2±0.1 mM calcium per day. Urines were collected every 6 h. Control $U_{Ca}V$ averaged N 3.54±0.45 and S 8.77±0.68 mM/day, $P < 0.001$. The nadir of $U_{Ca}V$ occurred 18–24 h after starting the low Ca diet in both groups and averaged N 0.06±0.01 and S 0.36±0.08 mM every 6 h, $P < 0.001$. On the first day of dietary Ca deprivation $U_{Ca}V$ declined to N 1.22±0.18 and S 3.60±0.50 mM/day, $P < 0.001$, or to N 35±4 and S 42±6 per cent of control (NS). $U_{Ca}V$ did not fall further, despite continuation of the low Ca diet for 7 days in four N and for 3 days in all other subjects. Control serum total [Ca] was comparable in both groups and fell significantly on the first day of dietary Ca deprivation while serum [PO_4] did not change, serum [Mg] and $U_{PO4}V$ rose, and $U_{Mg}V$ and $U_{Na}V$ fell. For both diets N $U_{Ca}V$ mM/day $= 1 + 0.1$ diet Ca mM/day ($r = 0.89$) and S $U_{Ca}V$ mM/day $= 3.1 + 0.3$ diet Ca mM/day ($r = 0.92$). Idiopathic hypercalciurics cannot achieve quantitatively normal renal calcium conservation during maximal dietary calcium deprivation because of a renal leak and/or greater fractional reabsorption of calcium from gastrointestinal secretions. (Supported by NIH AM 15089 and RR00058.)

187. Increased Collagen Synthesis by Scleroderma Skin Fibroblasts In Vitro. E. CARWILE LEROY, New York.

Events leading to the irreversible fibrosis of skin and viscera which characterizes scleroderma (systemic sclerosis) are not understood. To study the mechanism of this fibrosis, skin fibroblasts from 20 subjects with scleroderma (histologically confirmed) were paired with fibroblasts from normal subjects matched for age, sex, and biopsy site. Diploid fibroblasts propagated from explants were compared in early monolayer subcultures for soluble and insoluble collagen content, rate of collagen synthesis ([^{14}C] proline incorporation), effect of ascorbic acid, glycoprotein, and proteoglycan content, and cell doublings. Media obtained at days 1, 6, and 12 were analyzed; cells were estimated at day 12 by number, DNA, and protein content Scleroderma cultures consistently contained more soluble collagen (nondialyzable hydroxyproline, 86 μg/flask±14, mean±SEM) compared with matched normal cultures (30 μg/flask±11, $P < 0.001$). Insoluble collagen was similar in scleroderma and normal cultures. In 14 experiments, [^{14}C] proline incorporation into collagen hydroxyproline was increased in scleroderma cultures, expressed both as mean dpm/flask (26,414±5,408 vs. 17,743±3,886, $P < 0.001$), and as mean dpm/10^6 cells, (5,042±584 vs. 3,425±291, $P < 0.01$), indicating increased collagen synthesis by scleroderma fibroblasts. Ascorbic acid stimulated scleroderma and normal cultures without affecting differences in collagen synthesis. Glycoprotein content (sialic acid) was slightly higher in scleroderma cultures; proteoglycan (uronic acid) content was similar. The cell doublings in 12 days were slightly

higher in scleroderma cultures (3.1±0.2 vs. 2.5±0.2, $P < 0.02$). Collagen from scleroderma cultures, fibroblasts from uninvolved scleroderma skin, and culture-mixing experiments are under study. The data suggest that fibroblasts from scleroderma skin synthesize collagen more rapidly than fibroblasts from normal skin. The mechanism of this increased collagen synthesis in scleroderma is unclear. (Supported by the Lifschultz and RGK Foundations and the USPHS.)

188. Histidine-Mediated Decreases in Total Body Zinc and in Urinary Porphyrins and Porphyrin Precursors in Two Types of Hepatic Porphyria. J. B. LEVINE,* R. AAMODT,* D. P. TSCHUDY, AND R. HENKIN, Bethesda, Md.

Zinc and porphyrin metabolism were studied in three patients with hepatic porphyria (one with cutanea tarda [PCT] and two with acute intermittent porphyria [AIP] on a metabolic ward at NIH while ingesting a constant diet. Measurements of urinary uroporphyrin and coproporphyrin in PCT, serum and urinary porphobilinogen (PBG) and δ-aminolevulinic acid (δ-ALA) in AIP, serum and urinary zinc and copper and total body ^{65}Zn were made during the following 12–16 day sequential periods: initial control, oral administration of ^{65}Zn, $ZnSO_4$ (300–400 mg/day), and L-histidine (8–32 g/day), and final control. $ZnSO_4$ significantly increased serum and urinary zinc, but had no consistent effect on copper, porphyrins, or porphyrin precursors. L-histidine (24–32 g/day) significantly lowered urinary uroporphyrin and coproporphyrin in PCT and significantly lowered serum and urinary PBG and δ-ALA in AIP; concurrently, significant decreases in biological $t_{\frac{1}{2}}$ of ^{65}Zn, increases in urinary zinc excretion, decreases in serum zinc without significant change in fecal ^{65}Zn, occurred in all patients compared to the $ZnSO_4$ period or to the previous control period. Withdrawal of L-histidine resulted in the rapid onset of significant increases in urine and serum δ-ALA and PBG in AIP. In PCT, urinary uroporphyrin and coproporphyrin remained significantly decreased after L-histidine withdrawal for the remainder of the study. Simultaneous decreases in serum and urinary porphyrin precursors in AIP suggest that L-histidine is not acting via renal mechanisms. Thus, the observed decreases in porphyrins and porphyrin precursors may be related to some direct or indirect effect of L-histidine on porphyrin metabolism or to an L-histidine-mediated mobilization and excretion of a tightly bound tissue zinc pool.

189. Erythrocytes in Chronic Renal Disease: Defective Adaptation to Anemia. MARSHALL A. LICHTMAN,* MARION S. MURPHY,* AND APRIL A. WHITBECK,* Rochester, N. Y. (introduced by Lawrence E. Young **).

Red cells in patients with hypoproliferative anemia without azotemia increase their content of 2,3-diphosphoglycerate (2,3-DPG) and hydrogen ion and thereby increase the efficiency of hemoglobin (hgb) function. We have studied this adaptive mechanism in anemic subjects with and without chronic renal disease (CRD). In 10, nonacidotic (pH = 7.38±0.018), anemic (hgb = 7.22±0.36 g/100 ml) subjects with CRD on maintainance hemodialysis, red cell 2,3-DPG (16.2±0.92 μmoles/g hgb) and P_{50} (25.6±0.31 mm Hg) were significantly less than in 14 subjects with other hypoproliferative anemias (hgb = 7.49±0.46 g/100 ml, 2,3 = DPG = 27.2± 1.3 μmoles/g hgb; P_{50} = 28.3±0.45 mm Hg), but were similar to values in 10 healthy subjects (hgb = 14.6±0.22 g/100 ml; 2,3-DPG = 14.6±0.39 μmoles/g hgb; P_{50} = 26.4±0.26 mm Hg). P_{50} was highly correlated ($r = 0.76$) with 2,3-DPG/hgb molar ratio in the 34 subjects, although patients with CRD have a slightly lower P_{50} at a given 2,3-DPG/hgb ratio. In vivo P_{50} based on cellular pH, 2,3-DPG, and hgb concentration was 26.5±0.62, 25.7±0.87, and 30.8±0.91 mm Hg in healthy subjects, patients with CRD and hypoproliferative, anemic subjects, respectively. However, red cells from patients with CRD increased their 2,3-DPG and P_{50} when incubated with inosine (I), pyruvate (P), and phosphate (P_i) indicating responsive metabolic pathways and hemoglobin. Moreover, red cell adenosine triphosphate (ATP) was elevated in CRD (6.30±0.50 μmoles/g hgb) as compared to healthy subjects (3.48±0.68 μmoles/g hgb). This did not affect P_{50}, since red cell magnesium was also elevated (3.2±1.8 μmoles/ml RBC; healthy subjects = 2.2± 0.08), preventing ATP-hgb interaction in vivo. An inverse correlation of ATP and 2,3-DPG in all anemic subjects, and a fall in ATP as 2,3-DPG rose in response to IPP_i in vitro, suggested that red cell 1,3-DPG is preferentially metabolized by its kinase in an azotemic environment, favoring ATP production, whereas, by its mutase in other hypoproliferative anemias, favoring 2,3-DPG production. This study identifies a defect in the ability of red cells in CRD to adapt so as to facilitate oxygen transport. Although such patients may compensate by increasing cardiac output, the heightened need for transfusion therapy so as to reduce cardiac work and enhance patient performance should be considered.

190. Hemoglobin Brigham ($\alpha_2\beta_2^{100\ pro \rightarrow leu}$): a New Hemoglobin Variant Associated with Familial Erythrocytosis. JACOB J. LOKICH,* WILLIAM C. MALONEY,* H. FRANKLIN BUNN, SALLY M. BRUCKHEIMER,* AND HELEN M. RANNEY,** Boston, Mass., and Buffalo, N. Y.

Members of a large family were found to have erythrocytosis due to the presence of a functionally abnormal hemoglobin. The propositus had increased whole blood oxygen affinity with a P_{50} of 19 torr at pH 7.40, 37°C (normal = 26 torr). 18 other family members, in 9 of whom decreased P_{50} values were demonstrated, had erythrocytosis. Red cell 2,3-DPG and urinary erythropoietin levels were normal. No Hb abnormality was detected by electrophoresis on starch gels (pH 8.6), agar gels (pH 6.5), isoelectric focusing, or ion-exchange column chromatography. No abnormal subunits were demonstrated in the hemoglobin after treatment with *p*-chloromercuribenzoate or 8 M urea. Peptide maps of the β-chains of the hemolysate revealed normal Tp 11 and an additional spot of the same electrophoretic mobility, but a greater R_f. The substitution of leucine for proline at position 100 (G-2) was established by amino acid analysis and by automated Edman sequencing of the total β Tp 11. In this "new" variant, designated Hemoglobin Brigham, the substitution of a bulky leucine residue for proline at G-2 1 may impair the participation of G-1 aspartate in hydrogen bonding with tyrosine C-7 α 1, thus decreasing the stability of the deoxy derivative. Oxygen equilibria of phosphate-free hemolysates of the propositus showed curvilinear Hill plots with increased oxygen affinity, normal Bohr effect and normal reactivity to 2,3-DPG. Heme:globin ratio and the Soret absorbance of the deoxy derivative were normal. Of the 12 known hemoglobin variants associated with familial erythrocytosis, Hemoglobin Brigham and Hemoglobin Olympia ($\alpha_2\beta_2^{20\ val \rightarrow met}$) (1972. *J. Clin. Invest.* **51**: 70a) are electrophoretically silent. Measurement of oxygen affinity is important in the diagnostic evaluation of unexplained erythrocytosis. (Supported by grant C6516 from the NCI and AM 15234 from NIH.)

191. The Relationship Between Cytomegalovirus and Hepatic Function Abnormalities Occurring in the Post-transplant Period. JAMES P. LUBY,* WILLIAM BURNETT,*

ALAN HULL,* AND ATHOL WARE,* Dallas, Tex. (introduced by Jay P. Sanford).

45 consecutive renal transplant recipients have been studied for 3–18 months with an average study time of 9.5 months. Sera were collected before transplant and at 1, 2, 3, 6, 9, 12, 15, and 18 months and tested for complement fixation antibody titers against cytomegalovirus (CMV) and herpes simplex virus, type 1 (HSV_1). Before transplantation, 34.5% had CMV titers $\geq 1:4$, while the comparable figure for HSV_1 was 42.9%. After transplantation, 73.4% had fourfold or greater CMV titer rises, while only 18.4% had such titer rises to HSV_1. 46.5% of the patients (20/44) developed chemical evidence of hepatitis (2 consecutive SGOT's > 65 International Technicon units [95% confidence limit]) after transplantation. 5 of the 20 were HAA+. 12 of the remaining 15 patients had the onset of hepatitis at a time when they were undergoing CMV seroconversion. The HAA− hepatitis attack rate in persons with CMV titer rises 2^0-2^3 was 16.7% (3/18) as contrasted with 60% (12/20) in those with titer rises 2^4-2^8 ($P < 0.01$) 13 of the 20 cases, including 7 associated temporally with CMV seroconversion, had evidence of continuing hepatic dysfunction at and beyond 120 days. The study indicates comparability of initial CMV and HSV_1 antibody rates despite transfusion of an average of 7.7 U per person (predominantly leukocyte-poor blood) ≤ 3 months before transplant. It suggests that CMV may be an important cause of hepatic function abnormalities in the posttransplant period and that some of the changes induced may be chronic. (Research supported by NIH grant 5 T01 AI 00030-13).

192. Altered Vitamin B_6 Metabolism and a Mechanism of Conditioned Deficiency in Chronic Alcohol Abuse. LAWRENCE LUMENG * AND TINK-KAI LI,* Indianapolis, Ind. (introduced by Walter J. Daly).

Vitamin B_6 deficiency occurs in patients with alcoholic liver disease, but its existence in alcoholics without liver disease remains unexplored. Using the plasma concentration of pyridoxal phosphate (PLP), the biologically active form of B_6, as indicator, we have compared alcoholic patients free of clinical liver disease with age and sex-matched controls. Vitamin users were excluded. Plasma [PLP], measured by tyrosine decarboxylase apoenzyme, of alcoholics in the age groups 20–35 yr ($n = 20$), 36–49 yr ($n = 40$), and 50–69 yr ($n = 6$) were 6.0±1.0 (mean±SEM), 5.8±0.4, and 5.2±0.5 ng/ml, respectively, and were significantly ($P < 0.001$) lower than the values for the corresponding control groups (11.9± 1.0, 11.0±0.5, and 8.2±0.6 ng/ml). Although diet may partly be responsible, alcohol ingestion has been shown to impair PLP synthesis. To define the mechanisms of this interference, the PLP synthesizing capacity of erythrocytes and the effects of alcohol and acetaldehyde were examined in vitro. Alcoholics with lowered [PLP] exhibited normal B_6-kinase and B_6-phosphate oxidase activities. Acetaldehyde, 0.05–5 mM, but not alcohol, inhibited the net synthesis of PLP from B_6 and pyridoxol phosphate by 20–40%. This inhibition was abolished by 80 mM phosphate. Studies with disrupted cell systems indicate that acetaldehyde does not alter kinase and oxidase activities, both present in soluble fractions, but activates a membrane associated, phosphate-sensitive phosphatase which hydrolyzes phosphorylated B_6 derivatives. These data provide a mechanism for conditioned vitamin B_6 deficiency in alcohol abuse. (Supported by grants from USAMRDC and Licensed Beverage Industries.)

193. The Use of Derivatized Nylon Catheters for Selective Immunoadsorption In Vivo. LEON R. LYLE,* CHARLES W. PARKER, AND BRENT M. PARKER,* (assisted by Joan Poll *), St. Louis, Mo.

Clear nylon tubing (type 6, 1.34 mm outside diameter) was partially hydrolyzed with acid and reacted with human serum albumin (HSA) or ovalbumin (OA) in the presence of a water-soluble carbodiimide. The ability of the antigen-substituted tubing to react specifically with antibody was demonstrated by incubation with a mixture of ^{125}I-labeled rabbit anti-HSA and ^{131}I-labeled rabbit anti-OA antibodies. The capacity of the HSA-nylon for anti-HSA antibody was 0.5 μg/cm with little nonspecific binding as indicated by a high $^{125}I/^{131}I$ radioactivity ratio and inhibition of ^{125}I binding by soluble HSA. The specificity and capacity of the OA-tubing for anti-OA antibody was comparable. The ability of the derivatized nylon to adsorb antibody in vivo was demonstrated in heparinized dogs. Lengths of HSA- and OA-nylon were passed into the inferior vena cava through the femoral vein and shown to selectively bind ^{125}I- and ^{131}I-labeled antibody, respectively. Direct kinetic measurements of antibody adsorption in vivo indicated that if a reel type arrangement were used (in which fresh conjugated nylon was slowly passed through the venous system) milligram amounts of antibody could easily be removed in a 24 h period. Since the nylon conjugation is applicable to a variety of proteins, haptens, and hapten-protein conjugates, the nylon catheter system could provide a simple means of selective removed of unwanted antigens, antibodies, and substrates in man. As such it has potential applicability in the treatment of drug toxicity, allergy, autoimmune disease, and malignancy. (Supported by grants from NIH).

194. The Multiple Mixed Lymphocyte Reaction: a Universal Antigen for Testing Cellular Immunocompetence? RICHARD J. MANGI * AND FRED S. KANTOR, New Haven, Conn.

Cellular immunity is assessed in humans by skin tests and by two in vitro correlates: lymphocyte stimulation and lymphokine production. The use of specific antigen stimulants is not always possible because identification of specific immunity in humans is variable. The present study attempts to use irradiated human lymphocytes as a universal antigen to test cellular immunocompetence. Conventional one-way mixed lymphocyte reactions (MLR) will produce a response which varies depending upon the histocompatibility of the donor and the responding cells. To obtain maximal responses we have varied the number of stimulating cells, keeping the reactant cells constant, and have also varied the number of donors contributing to a constant number of stimulating cells. MLR's were performed with 1×10^6 reactant lymphocytes. The total number of donor lymphocytes contained equal parts of cells from one to five different unrelated individuals. Maximum stimulation occurred when the ratio of donor to reactant cells was 2 to 4:1. At each concentration of donor cells, maximal response occurred with irradiated lymphocytes from three different donors. More than three different donor populations resulted in no increase or a decrease in response. At each total concentration of irradiated cells, the response elicited by multiple donors was equal to or greater than the response to any one donor alone. We conclude that MLR maximum response is obtained with donor cells from three unrelated individuals cultured with reactant cells in a ratio of 2 to 4:1. The multiple MLR is being assessed as a universal antigen in studies of lymphocyte competence in anergic states and neoplasia and during immunosuppressive therapy. (Research supported in part by NIH grants AI53886-01 and AI 06760-06.)

195. Familial Thrombosis Due to Antithrombin III Deficiency. EWA MARCINIAK * AND CLAUDE FARLEY,* Lexington, Ky. (introduced by J. William Hollingsworth).

At the present time antithrombin III (AIII) is recognized as a blood antiproteinase capable of inactivating both thrombin and factor Xa and is presumably identical with the so-called heparin cofactor. Only one family with hereditary deficiency of AIII has been reported. A second large kindred unduly susceptible to venous thrombosis is the subject of the present study. For evaluation of AIII a procedure involving gel filtration of plasma or serum on Sephadex G-150 with subsequent analysis of antithrombin and antifactor Xa activities in separated fractions was utilized. Low AIII titres ranging from 26 to 50% of normal values were found in plasma of eight members in the four generations, and another six members were suspected of having the deficiency. In serum of the affected patients AIII was virtually absent which indicates its eminent involvement in thrombin neutralization. There was no decrease in antithrombin or antifactor Xa activities residing in the macroglobulin region. The responsiveness to heparin in vivo measured in three deficient cases was only slightly below normal when the intensity and duration of the hypocoagulable state was estimated by conventional assay procedures. However, when the post-heparin AIII activity was evaluated, very significant discrepancy between normal and deficient plasmas was found, reflecting the biological stimulation of AIII by heparin. In five affected members the therapy with oral anticoagulants increased the level of AIII in plasma by 15 to 45% and gave a marked increase of residual AIII in serum. These findings substantiate the outstanding biological role of AIII in the support of blood fluidity and suggest that a decrease in vitamin K-dependent coagulation factors abates the utilization of AIII in vitro and in vivo. (Supported by grant from AHA.)

196. The Role of Phospholipids in Thyroid-Stimulating Hormone (TSH) Stimulation of Adenylate Cyclase in Thyroid Plasma Membranes. KEITH MASHITER,* KAMEJIRO YAMASHITA,* AND JAMES B. FIELD,** Pittsburgh, Pa.

The observation that treatment of thyroid slices with phospholipase C inhibited TSH stimulation of cyclic AMP, glucose oxidation, and ^{32}P incorporation into phospholipids indicated the importance of phospholipids in TSH action. Pretreatment of bovine thyroid plasma membranes with phospholipase A (5 U/ml) or C (1 U/ml) abolished TSH stimulation of adenylate cyclase activity but had no effect on basal or NaF stimulation. Phosphatidylcholine (25 μg) and phosphatidylserine (200 μg), but not phosphatidylethanolamine (200 μg), partially restored TSH responsiveness of bovine thyroid plasma membranes which had been treated with phospholipase A. Phosphatidylcholine produced a greater effect than phosphatidylserine and smaller amounts were effective. Both TSH and NaF stimulation of adenylate cyclase activity in thyroid plasma membranes was diminished by 0.02% Lubrol Px treatment. The effect on TSH was considerably greater. Phosphatidylcholine (200 μg) partially restored TSH stimulation but not NaF activation of adenylate cyclase. These results indicate that phospholipids, especially phosphatidylcholine, are probably essential components in the system by which TSH stimulates adenylate cyclase activity in thyroid plasma membranes. The effects do not seem to involve the catalytic activity of adenylate cyclase, but the data do not permit a distinction between decreased binding of TSH to its receptor or impairment of the signal from the bound hormone to the enzyme activity. (Research supported by grant from NIH.)

197. Adaption to Hyperoxia: Influence on Protein Synthesis by Lung and on Granular Pneumocyte Ultrastructure. DONALD MASSARO AND GLORIA D. MASSARO,* Washington, D. C.

We have previously shown that in vivo exposure of rats to 98% O_2 for 48 h decreases total protein synthesis by lung slices. The decrease in synthesis of protein in a surface-active lung fraction (SAF) is greater than that of total protein. There is a concomitant decrease in the size of lamellar bodies (currently thought to be surfactant storage granules) in granular pneumocytes of O_2 animals. The present study was designed to examine the influence of adaption to O_2 on these processes. We exposed rats to 98% O_2 or compressed air at ambient pressure for 96 h. Lung slices were incubated with [^{14}C] leucine and radioactivity measured in acid-insoluble protein. The SAF was obtained by ultracentrifugation of homogenized lung. We measured free leucine, DNA, and protein and examined granular pneumocytes using ultrastructural stereological methods. After 96 h exposure there was more total acid-insoluble radioactivity in the O_2 group per mg DNA, 12,409±836 cpm/mg DNA, (mean±SEM) than in the air group, 6492±850 cpm/mg DNA ($P = < 0.001$). Acid-insoluble radioactivity in the SAF, expressed per milligram of crude homogenate DNA is 4459±389 and 3140±251, respectively ($P = < 0.025$), in O_2 and air rats. These differences are not due to differences in free leucine in the tissue. Ultrastructural lineal analysis of granular pneumocytes reveal they are larger in O_2-exposed rats and contain more rough endoplasmic reticulum. The lamellar bodies are equal in size in O_2 and air rats after 96 h, whereas at 48 h, when protein synthesis was decreased, the lamellar bodies in O_2 rats were smaller. We conclude that during adaption to hyperoxia the lung regains its ability to synthesize protein including protein in a SAF; this is reflected in the ultrastructure of the granular pneumocyte.

198. Secondary Hyperparathyroidism and Hypocalcemia in Acute Renal Failure in Man: Evidence for Skeletal Resistance to Parathyroid Hormone. SHAUL G. MASSRY, ALLEN I. ARIEFF,* JACK W. COBURN, GENARO M. PALMIERI,* AND CHARLES R. KLEEMAN,** Los Angeles, Calif.

Skeletal resistance to parathyroid hormone (PTH) may underlie the hypocalcemia of chronic renal failure. In acute renal failure, hypocalcemia was thought to be due to elevated serum phosphorus. It is unknown whether resistance to PTH action develops in acute renal failure and causes hypocalcemia. Also data on blood levels of PTH in acute renal failure are limited. In 10 patients with acute renal failure, serum calcium, phosphorus, and PTH were measured frequently. Infusions of parathyroid extract (PTE) were given during the oliguric and diuretic phases of acute renal failure and 2–3 months after recovery. Hypocalcemia of 5.3 to 8.4 mg/100 ml occurred as early as 3 days after onset of acute renal failure and persisted during diuretic phase when serum phosphorus was normal or low. Blood levels of PTH were elevated 2–5 times above normal in nine of ten patients. Infusion of PTE caused a 15- to 40-fold rise in PTH levels but without calcemic response in nine patients during oliguric phase and in eight restudied during diuretic period. The change in serum calcium was 0–0.3 mg/100 ml (normal, 1.0–2.2 mg/100 ml). The failure to respond to PTE was unrelated to serum phosphorus. Five patients restudied with PTE when renal function was normal had a normal rise in serum calcium of 1.2–2.2 mg/100 ml. The data show that in acute renal failure in man (*a*) blood levels of PTH are elevated, (*b*) skeletal response to endogenous or exogenous PTH is impaired, and this abnormality is a major factor underlying

the hypocalcemia in these patients, and (*c*) normal skeletal responsiveness to PTH returns after recovery of renal function. (Supported by USPHS contract 43-68-1040.)

199. Jejunal Sodium and Water Transport in Uremic Man. EUGENE S. MAY,* H. JOHN REINECK,* HAGOP S. MEHKJIAN,* JAY H. STEIN,* AND THOMAS F. FERRIS,* Columbus, Ohio (introduced by James V. Warren).

It has been suggested that the increased fractional sodium excretion by the kidney in uremia is due to the presence of a humoral inhibitor of sodium transport. To investigate whether other epithelial membranes are also altered, we have evaluated sodium (Na^+) and water (H_2O) transport in the jejunum of uremic man before and after hemodialysis. Five patients with severe renal insufficiency maintained on chronic hemodialysis and ten normal controls were studied before and immediately after dialysis. In uremic patients net secretions of Na^+ (J_N^{Na+}) -400 ± 114 SEM μEq/min per 30 cm and H_2O (J_N^{H2O}) -2.72 ± 1.01 ml/min per 30 cm were consistently found, while absorption of Na and H_2O occurred in all controls, $+174.4\pm92$ and $+1.35\pm0.93$ ($P < 0.001$). This defect in Na absorption in uremics disappeared after dialysis: $J_N^{Na+} = +487\pm107$ μEq/min per 30 cm and $J_N^{H2O} = +3.79\pm0.91$. Studies of unidirectional Na^+ flux revealed inhibition of mucosa to serosa transport before, 386 ± 55, compared to 1029 ± 151 ($P < 0.001$) after dialysis, whereas serosa to mucosa transport before and after dialysis did not significantly differ, 621 ± 85 and 531 ± 49. The correction of the sodium transport defect occurred even when body weight was not altered by dialysis. In summary, (*a*) net secretion of water and electrolytes into the jejunum occurs in the uremic state in man; (*b*) the major alteration in transport is a decrease in mucosa to serosa flux of sodium; and (*c*) the altered transport of sodium in uremia is corrected by hemodialysis. (Research supported by grants from NIH.)

200. Serum Viscosity and Protein Changes in Early Diabetes. DONALD E. MCMILLAN,* Santa Barbara, Calif. (introduced by John W. Farquhar).

Serum viscosity is elevated in diabetes mellitus, especially when microangiopathy is detectable. The major cause of the elevated viscosity has been found to be an increase in the resistance to flow produced by the serum proteins, a property measured as the serum limiting viscosity number (LVN). Changes in serum viscosity produced by fluctuations in serum protein and glucose levels do not affect the LVN of dialyzed serum, making it a more stable measure of viscosity change in diabetes. The LVN of dialyzed serum was observed to be less elevated in latent and early diabetes than in established diabetes but elevation did not increase with duration of diabetes. This apparent paradox was resolved by studies of serum after removal of lipoproteins by ultracentrifugation. Lipid-free serum LVN values increased progressively from normal to diabetic levels as glucose intolerance advanced. Subjects meeting USPHS criteria for diabetic glucose tolerance had LVN values as high as those in well established overt diabetes, and subjects with lesser degrees of glucose intolerance had intermediate values. Electrophoresis of lipid-free serum demonstrated a reciprocal correlation between LVN and the percentage of albumin in serum proteins in both diabetic and nondiabetic subjects. Albumin levels fell and α_1- and α_2-globulin levels rose as diabetes became evident, indicating that change in serum protein pattern is important in producing elevated LVN values in diabetes. Removal of glucose and lipoproteins from serum has produced clear evidence that serum protein composition is disturbed early in diabetes; the disturbance may be detected before overt diabetes is present, and it produces an increase in serum viscosity which is more profound in diabetics with clinically recognizable microangiopathy. (Supported by Doris Palmer Fund, Kroc Foundation, and NIH grant AM13092.)

201. Preferential Association of β^S-Globin with Stroma in Sickle-Cell Disease. GREGORY MEARS,* CLAYTON NATTA,* JOYCE V. O'DONNELL,* AND ARTHUR BANK, New York.

Membrane-bound sickle hemoglobin (HbSS) has been associated with abnormalities of the red cell membrane and decreased cell deformability in patients with sickle-cell (SS) disease. The present study was undertaken to assess the role of individual globin chains in the pathogenesis of the abnormalities of the membrane of SS cells. The results indicate that newly synthesized β^S-chains are preferentially bound to the stroma of reticulocytes of patients with SS disease. Peripheral blood cells from patients with SS disease and with other hemolytic anemias (AA) were incubated with [^{3}H] leucine for 1 h at 37°C. The cells were then lysed hypotonically with 20 vol 1 mM EDTA at pH 7.2, and the stroma washed 5–10 times until the supernatant was clear. The AA stroma prepared using this method contained less Hb than that from SS cells. Relative α- and β-globin chain synthesis was determined using column chromatography after preparing globin by dropwise addition of either total stroma or stroma-free hemolysate directly into acid-acetone. Equal amounts of newly synthesized α- and β^A-chains were associated with both stroma and present in stroma-free hemolysates from AA cells. By contrast, twice as much radioactive β^S- as α-chain was associated with stroma in patients with SS disease, while equal amounts were present in stroma-free hemolysates. The selective association of newly synthesized β^S-chains with stroma in reticulocytes in SS disease could result from either precipitation of β^S-subunits in the stroma, precipitation of HbSS with rapid proteolysis of α-chains, or specific membrane binding properties of β^S-subunits. The association of β^S-chains with stroma may lead to preferential destruction of these chains as well as to membrane abnormalities which contribute to the shortened half-life of SS cells. (Supported by grants from NIH, NSF, and ACS.)

202. Crystalglobulinemia: Clinical, Morphological, and Physicochemical Studies. IRA MELNICOFF,* HENRICK BOGAARS,* ALBERT KALDERON,* CHAN PARK,* FRANCIS CUMMINGS,* STEPHEN R. KAPLAN,* ISRAEL DIAMOND,* AND PAUL CALABRESI, Providence, R. I.

A diagnosis of multiple myeloma was established in a 52-yr-old male who presented with generalized weakness, weight loss, and large, lamellar eschars involving the extensor surfaces of the upper and lower extremities. Biopsy of the ulcers revealed nonspecific acute and subacute vasculitis. Serum protein electrophoresis revealed an M-component comprising 65% of the total protein (12.4 g/100 ml). By immunoelectrophoresis, the myeloma protein was found to be IgG_1, with kappa light chains. The serum as well as the purified IgG_1 showed cryoprecipitation with spontaneous crystallization beginning at 31°C and cryogel formation at 4°C. This process was completely and repeatedly reversed at 37°C and found to be concentration dependent; 1:7 dilution of the serum abolished crystallization. No evidence of participation of other immunoglobulins in the crystal formation was detected by immunofluorescence. Light microscopic examination revealed the cryoprecipitate to be entirely crystalline, the crystals being fusiform, birefringent, and dichroic, 0.5–0.8 mm in length. Electron microscopy of the IgG_1 crystals showed a unique organized structure which was readily

recognizable in bone marrow plasma cell organelles when processed at low temperature. Immunofluorescent studies of clinically uninvolved skin and muscle showed no evidence of immunoglobulin deposition in vessel walls although there was specific interstitial staining for IgG in association with an active myositis. The patient underwent plasmapheresis using continuous cell flow centrifugation. Serial cryocrit determinations showed an initial sharp decline from 31% to 24% followed by a rise and subsequently reached a plateau at 29%. Combination chemotherapy was then instituted using vincristine, Alkeran, prednisone, and procarbazine. Evidence indicates that the spontaneous crystallization of this protein occurs on the basis of serum saturation. Spontaneously crystallizing immunoglobulins are rare although not unique. The readily recognizable structure of this protein provides an opportunity to study its intracellular synthesis and transport as well as its ultrastructural subunit composition.

203. Enhanced Effects of Prostaglandin E_1 (PGE_1) upon Human Lymphocytes in the Presence of Cortisol. JOHN MENDELSOHN,* MICHAEL MULTER,* AND ROBERT BOONE,* San Diego, Calif. (introduced by Mehran Goulian).

The inhibitory effects of cortisol and agents acting through cyclic AMP have been studied in cultures of purified human peripheral lymphocytes. In both phytohemagglutinin (PHA)-stimulated and unstimulated cultures, [^{3}H] thymidine incorporation is reduced when cells are incubated with cortisol, PGE_1, or dibutyryl cyclic AMP, and PHA-induced morphologic transformation is prevented. When cortisol and PGE_1 (or dibutyryl cyclic AMP) are added simultaneously to cultures, additive inhibitory effects are observed: [^{3}H] thymidine incorporation is reduced $>90\%$ in unstimulated cultures and $>99\%$ in PHA-stimulated cells. Peripheral lymphocytes contain 2.2 ± 0.2 pmoles cyclic AMP per 10^6 cells (mean±SEM). Within 20 min of adding 0.1 mM PGE_1 to cultures, the cyclic AMP levels rise to 7.2 ± 1.1. During the ensuing 1–6 h, the concentration falls to base line levels. Cortisol in concentrations as high as 0.1 mM does not affect cyclic AMP levels. However, when cells are incubated with PGE_1 in the presence of cortisol there is a significantly greater elevation of cyclic AMP levels, to 13.4 ± 2.2 pmoles/10^6 lymphocytes after 20 min, with a decline paralleling that observed with PGE_1 alone. Stimulation of lymphocytes with PHA does not result in significant changes in cyclic AMP levels, but the response to PGE_1 parallels unstimulated cultures. The PHA-stimulated cells also show a further elevation of cyclic AMP levels when cortisol is added to cultures with PGE_1. The data provide evidence that cortisol enhances the response to PGE_1 by human peripheral lymphocytes in both unstimulated and PHA-stimulated cultures, and that this enhancement may be mediated by a synergistic interaction with cyclic AMP. (Supported by NIH and Damon Runyon grants.)

204. Human Interferon Applied Locally Inhibits Respiratory Virus Infection in Man. THOMAS C. MERIGAN, SYLVIA E. REED,* THOMAS S. HALL,* AND DAVID A. J. TYRRELL,* Salisbury and Harrow, England.

Our in vitro studies have demonstrated that several strains of influenza and rhinoviruses are as sensitive to the antiviral action of human interferon as a known interferon-sensitive virus, provided that a yield-reduction type of assay is employed. In addition, influenza B/Hannover/1/70 was shown to be equally highly susceptible to interferon in the ciliated epithelial cells present in human fetal tracheal organ culture. Human respiratory tract infections would be an ideal target for the clinical application of interferon, since the antigenic diversity of viral pathogens in this area precludes immunological control. Randomized controlled double-blind studies were conducted on normal volunteers to evaluate the activity of human leukocyte interferon against two respiratory viruses. 16 applications of interferon or placebo were given by nasal spray during the 24 h before influenza B/Hannover/1/70 challenge of 22 volunteers who were selected on the basis of low serum antibodies and hence susceptibility to that virus. However, a single day of local interferon prophylaxis with a total dosage of 800,000 U appeared only to delay slightly the onset of clinical evidence of infection but failed to prevent or diminish the severity of disease by standard clinical scoring, virus shedding or sero-conversion. Next, 39 applications of interferon or placebo were given by nasal spray to influence rhinovirus 4 challenge in each of 32 volunteers who had low serum antibodies to that virus. By employing a greater daily interferon dosage and combining a day of prophylaxis with 3 days of treatment for a total dosage of 14,000,000 U, a statistically significant prevention of clinical symptoms and virus shedding after rhinovirus infection was achieved. The frequency and extent of sero-conversions in the treated volunteers were also lower than in the controls.

205. A Successful Approach to the Inhibition of Growth Hormone Secretion in Man. THOMAS J. MERIMEE AND S. EDWIN FINEBERG,* Boton, Mass.

This is to report the use of diet for suppressing growth hormone (HGH) secretion in man. This suppression is significantly greater than that achieved by pharmacologic means. HGH secretion was assessed in eight normal, nonobese subjects before and after each of four separate dietary regimens. With a high carbohydrate (525 g), normal protein (75 g) diet of 3600 calories (diet 1), the mean maximal HGH concentration in plasma after arginine decreased from 21.5 ± 3.5 mμg/ml to 4.2 ± 0.9 mμg/ml ($P < 0.01$). The mean sum of responses to arginine (30, 60, and 90 min) was reduced from 54.0 ± 10.0 mμg/ml to 10.5 ± 3.1 mμg/ml ($P < 0.01$). Total integrated 24 h secretion of HGH, assessed by hourly blood samples, was reduced to 19% of that noted in the control period. Virtually identical suppression of HGH secretion occurred when carbohydrate and protein were proportional to diet 1, but caloric intake was reduced to 2300 calories (diet 2). In diets 3 and 4, caloric intake was 3600 calories with protein reduced to 30 g. Diet 3 contained all essential amino acids; diet 4 lacked lysine and tryptophan. Mean maximal HGH responses to arginine before and after each diet were, respectively, 26.0 ± 5.1 mμg/ml vs. 10.0 ± 2.1 mμg/ml and 25.3 ± 6.4 mμg/ml vs. 9.1 ± 3.4 mμg/ml. HGH secretion was also examined after (*a*) β-adrenergic stimulation, (*b*) acute elevation of plasma free fatty acid concentrations, and (*c*) induction of hyperglycemia. Suppression achieved by these and other means was sporadic and did not exceed 50% of the control state. This data establishes for the first time an effect of prior diet on secretion of HGH and supports a new approach to altering its secretory pattern. (Supported by NIH RR-533.)

206. Serial Studies of T- and B-Lymphocytes in Patients with Systemic Lupus Erythematosus (SLE). RONALD P. MESSNER,* FOLKE D. LINDSTRÖM,* AND RALPH C. WILLIAMS, JR., Albuquerque, N. M.

Peripheral blood lymphocytes from 24 patients with active SLE were serially studied with respect to shifts in relative proportions of T- and B-lymphocytes in association with clinical disease activity. Peripheral blood B-cells were identified by direct immunofluorescence using enumeration of cells

showing surface Ig. T-cells were enumerated using indirect immunofluorescence with rabbit antisera prepared against human fetal thymocytes or thoracic duct lymphocytes. T-cell antisera were absorbed with B-cells from patients with chronic lymphatic leukemia to render them T-cell specific. T-cell specificity was indicated by 99% staining of human thoracic duct cells, excellent correlation between lymphocyte staining and cold rosette formation (E-binding) of sheep erythrocytes, and only 18% staining of normal human marrow lymphocytes. Lymphocyte studies also included determinations of E-binding by sheep erythrocyte rosettes, enumeration of percentages of B-lymphocytes showing the C3 receptor, and screening of plasma samples for lymphocytotoxic antibody. Patients with relatively stable SLE showed no prominent serial shifts in peripheral blood B- or T-cell populations. However, dramatic changes in peripheral T- and B-cells were recorded in many patients with active SLE. Most consistent was a rise in proportions of peripheral blood B-cells concomitant with exacerbations of SLE activity. Sudden or gradual falls in proportions of peripheral blood T-cells were often noted in association with a rise in B-cells from normal percentages of 22.9±7.1 to 50–70%. No correlations were evident between these changes and circulating lymphocytotoxins. In active SLE, a marked discrepancy was recorded between a relatively normal value for percent T-cells by immunofluorescence (75.3±14.0%) and extremely low values for T-cells using E-binding (10–20%). Percentages of B-cells recorded by sums of peripheral blood lymphocytes showing surface Ig was often 5 or 6 times higher than that recorded for B-cells showing identifiable C3 receptors.

207. Phytohemagglutinin Mitogenic Proteins: Structural Evidence for a Family of Isomitogenic Proteins. BRUCE MILLER,* CLAUDIA NOYES,* ROBERT HEINRIKSON,* HENRY S. KINGDOM,* AND STANLEY YACHNIN, Chicago, Ill.

Phytohemagglutinin (PHAP) mitogenic proteins consist of four subunits with mol wt of 34,000 as shown by SDS-acrylamide-gel electrophoresis. We have postulated (Yachnin and Svenson. 1972. *Immunology.* 22: 871) that L-PHAP (low-hemagglutinating PHAP) is composed of four L subunits, while H-PHAP (hemagglutinating PHAP) consists of a series of molecules composed of 3L + 1R, 2L + 2R, 1L + 3R, and 4R subunits. The amino acid sequence of both L- and H-PHAP were determined by the Edman degradation procedure. L-PHAP revealed the following sequence: NH_2-Ser-Asn-Asp-Ile-Tyr-Phe-Asn-Phe-Gln-Arg-Phe-?-Glu-Thr-Asn-Leu-Ile-Leu-Gln-Arg-Asp-Ala-Ser-Val- - -. Assuming a four subunit structure, recoveries ranged from 50 to 80% of the theoretical yield. Similar analysis of H-PHAP revealed a major sequence, as well as a minor sequence (the latter persisting only for the first seven amino acids, major: minor molar ratio = 3.7: 1, total recovery 76% of theoretical yield assuming a four subunit structure). The sequences are: (major) NH_2 - Ala - Ser - Gln - Thr - Ser - Phe - Ser.- Phe - Gln - - etc. (minor) NH_2 - Ser - Asn - Asp - Ile - Tyr - Phe - Asn -. Dissociation of H- and L-PHAP by guanidine HCl, followed by isoelectric focusing in 8 M urea, allowed isolation of the L (pI 5.3) and R (pI 6.0) subunits. Analysis of these materials confirmed that the L-PHAP sequence (and the minor sequence of H-PHAP) is that of the L subunit, while the major H-PHAP sequence is that of the R subunit. The latter, from the 8th to the 24th positions, is virtually identical with the L subunit. These data verify our isomitogen hypothesis and provide a structural basis for explaining the different biological properties of L- and H-PHAP.

208. Response of Plasma Dopamine Beta Hydroxylase (DBH) and Plasma Renin Activity (PRA) to Changes in Intravascular Volume (IVV). WALTER L. MILLER,* FRIEDHELM LAMPRECHT,* PHILIPPE V. CARDON,* AND FREDERIC C. BARTTER,** Bethesda, Md.

Plasma DBH is principally derived from sympathetic nerves, where by hydroxylation of dopamine it produces norepinephrine. Plasma renin is principally derived from juxtaglomerular cells. By its action on renin substrate it produces angiotensin. Norepinephrine and angiotensin are throught to be important in rapid adjustment to acute changes in IVV. DBH and PRA were determined at 4-h intervals in healthy volunteers. DBH showed no circadian variation, but PRA showed a significant rhythm with high values at 0400–0800 and low values at 2000–2400. Infusions of 1000 cc 7.5% human serum albumin over 2 h lowered DBH by 30% ($P < 0.01$), while PRA decreased by 50% ($P < 0.01$). After a nadir at 3.5 h PRA returned to a rhythmic pattern with a 12 h phase shift. Phlebotomy (5% IVV) increased PRA by 35% but did not alter DBH. In cats infused with 10% dextran to increase IVV up to 30%, DBH decreased by 80% ($P < 0.001$). Phlebotomy of up to 30% IVV increased DBH by 120% ($P < 0.01$). Whereas IVV expansion reduces the activity of the peripheral sympathetic nervous system and the renin-angiotensin system, volume depletion increases the activity of both systems. Thus both systems operate to reduce the impact of acute changes in IVV.

209. Prostaglandin Binding to Beef Thyroid Membranes. WAYNE V. MOORE,* AND J. WOLFF,** Bethesda, Md.

The binding of tritiated prostaglandin E_1 ([^{3}H]PGE_1) to beef thyroid membranes was investigated by a micro-discontinuous gradient centrifugation method. By studying the effects of selected cations, pH, and temperature on the binding of [^{3}H] PGE_1 to the membranes, two populations of binding sites were found. Binding of [^{3}H] PGE_1, occurring at pH 7.0, requires calcium, is heat labile, is inhibited by prostaglandin analogues and antagonists, and is inhibited by ITP, GTP, and TSH but not by fatty acids. Half-maximal binding occurs with 1 mM calcium. The binding at pH 7.0 with 5 mM calcium has an affinity constant of 2.6×10^8 liters/mole. The other population of sites has a pH optimum of 4.5, is heat stable, and has a partial calcium requirement. The pK for binding at this pH is 5.1± 0.2, which is similar to that of long-chain fatty acids. Scatchard plot analysis of the binding at pH 5.2 with 5 mM calcium revealed two components of binding with affinity constants of 1.5×10^{10} liters/mole and 1.6×10^8 liters/mole. Prostaglandin analogues and antagonists are relatively less effective in promoting debinding of [^{3}H] PGE_1 at pH 5.2. ITP, GTP, and TSH are ineffective in inhibiting binding at pH 5.2. The binding of [^{3}H] PGE_1 at pH 5.2 is inhibited by C_{18-24} fatty acids with the degree of inhibition increasing with chain length and degree of unsaturation. The results indicate that the binding of [^{3}H] PGE_1 is related to the ionic and lipid properties of the prostaglandin at pH 5.2 but has more specific structural requirements at pH 7.0.

210. On the Metabolic Pathogenesis of the Renal Disorder of Hereditary Fructose Intolerance (HFI). R. CURTIS MORRIS, JR., ELISABETH MCSHERRY,* AND ANTHONY SEBASTIAN,* San Francisco, Calif.

In patients with HFI, fructose induces a dose-dependent dysfunction of the proximal renal tubule like that of Fanconi syndrome, hypophosphatemia, and hyperuricemia. The metabolic abnormality induced depends on accumulation of fruc-

tose-1-phosphate (F-1-P) in tissues genetically deficient in F-1-P aldolase activity, including renal cortex. For each mole of fructose → F-1-P, one mole of ATP → ADP. We propose that in HFI, accumulation of F-1-P in the proximal tubule leads to impaired tubular function by acting both to deplete preformed ATP and to restrict ATP generation by diminishing cellular concentrations of inorganic phosphate ([P_i]) and total adenine nucleotides. Substantial reductions in [P_i] and [ATP] are known to remove the normal inhibition of enzymes that catalyze the essentially irreversible degradation of adenosine monophosphate to inosine monophosphate and adenosine, precursors of uric acid via inosine. Accordingly, our proposal predicts that prevention of cellular depletion of [P_i] would attenuate the experimental renal dysfunction and the hyperuricemia. When fructose was administered to rats, (20, 40 mM/kg), simultaneously administered sodium phosphate in amounts sustaining hyperphosphatemia, significantly attenuated an otherwise striking reduction in renal cortical [P_i], [ATP], and total adenine nucleotides, despite a significantly higher concentration of F-1-P. When the experimental renal dysfunction of HFI was induced during water diuresis, experimental hyperphosphatemia prevented an otherwise invariable increase in urine flow (an index of proximal tubular solute rejection) and significantly attenuated the acidification defect, aminoaciduria and the hyperuricemia, despite significantly lessened uricosuria. These results provide strong evidence that in HFI, the major pathogenetic consequence of F-1-P accumulation is not inhibition of glycolytic enzymes but sequestration of phosphate and greatly diminished tissue [P_i] that leads to restricted ATP generation.

211. Location of Lipid-Binding Sites in Human Plasma Lipoproteins: Synthesis and Properties of Peptide Fragments of Apolipoprotein-Alanine (apoLP-Ala). J. D. MORRISETT,* J. T. SPARROW,* AND A. M. GOTTO, JR., Houston, Tex.

ApoLP-Ala is an apolipoprotein which binds phosphatidyl choline (PC) and contains 79 amino acids of known sequence. In order to locate the lipid-binding site(s) of this protein, we have undertaken its chemical synthesis by standard solid-phase methods. We now report the synthesis of four fragments of this molecular corresponding to sequence positions 41–79 (I), 48–79 (II), 55–79 (III), and 61–79 (IV). The resulting peptides were cleaved from the resin with liquid HF and purified by chromatography on G-50 and DEAE-cellulose. Each purified peptide eluted as a single symmetrical peak, exhibited a single band on polyacrylamide electrophoresis, and gave an amino acid analysis in excellent agreement with the theoretical value. Circular dichroism studies indicated that fragments I and II became more helical in the presence of PC, and also inhibited the reactivation of delipidated β-hydroxybutyrate dehydrogenase which requires PC for activity. Electron microscopic studies showed that fragments I and II caused PC vesicles to become aligned into linear arrays of stacked discs. PC complexes of I and II could be isolated by density gradient centrifugation. Fragments III and IV exhibited none of these properties. These results indicate that residues 55–79 do not contain the minimum determinants required for the binding of PC. However, extension of this peptide's N-terminus by seven residues produces a molecule which binds PC. (Supported in part by HEW grant HL 14194 and by a grant from The John A. Hartford Foundation, Inc.)

212. Immunoregulatory Alpha-Globulin (IRA) Selectively Affects T-Lymphocytes. J. H. MORSE,* A. M. MORRIS,* AND R. MASCITELLI,* New York (introduced by V. P. Butler, Jr.).

Previous investigators demonstrated an immunosuppressive factor in plasma which inhibits antibody synthesis and graft rejection. This immunosuppressive factor (IRA), without inherent cytotoxicity, inhibited lymphocyte transformation induced by specific antigens, phytohemagglutinin, allogeneic lymphocytes, and antilymphocyte serum. IRA, a crude preparation of α-2-globulin, was prepared by DEAE chromatography from pooled plasma (27 individuals) and assayed for its ability to inhibit blood lymphocyte proliferation induced in vitro by the mitogens: phytohemagglutinin (PHA), concanavalin A (ConA), pokeweed (PWM), and *E. coli* lipopolysaccharide (LPS). All mitogens were used at concentrations producing maximum uptake of [^{3}H] thymidine. Control cultures included cells without mitogen and the substitution of serum albumin for IRA. Stimulation of cultures (1×10^6 cells/ml) by PHA or ConA was inhibited by concentrations of IRA varying between 0.25 and 4 mg/ml of culture medium. PHA and ConA are predominantly T-cell mitogens; therefore the effect of IRA on the action of B-cell mitogens was also studied. IRA failed to inhibit blastogenesis by PWM and LPS but did suppress stimulation by PHA or ConA in simultaneous cultures taken from normal individuals. Thus, IRA had no effect on lymphocytes undergoing proliferation by mitogens classified as B-cell stimulators, whereas T-cell mitogen proliferation was inhibited. Cultures stimulated by PWM and LPS incorporated about half the label seen in PHA- and ConA-stimulated cultures, consistent with estimates of 60% T and 30% B-lymphocytes in peripheral blood. The specificity of inhibition offers a new tool for evaluating T- and B-cell function in normal and disease states and offers a possible mechanism for the inhibition of PHA-stimulated cultures by plasma from patients with cancer and chronic renal disease. (Supported by NIH and New York Arthritis and LE Foundations.)

213. Association of Adenovirus and *E.Coli* Infections with Acute Hemorrhagic Cystitis (AHC) in Children. MAURICE A. MUFSON,* ROBERT B. BELSHE,* TERRENCE J. HORRIGAN,* AND LOWELL M. ZOLLAR,* Chicago, Ill. (introduced by Mark H. Lepper **).

AHC, characterized by gross hematuria and symptoms of bladder inflammation, occurs in infants and children apparently as a self-limiting disease which must be differentiated from serious renal disorders. Until recently no etiologic agent was associated with AHC. Studies from Japan and from our laboratory have implicated adenovirus 11 in the etiology of this disease. From October 1968 through September 1972 at the Cook County Hospital, 69 infants and children with AHC and 42 children without urinary tract disease (control) were tested for viruria and bacteriuria. Adenovirus 11 was recovered from the urine of 10 patients with AHC and adenovirus 21 from the urine of 2 patients with AHC. Children shed virus 5–7 days after the onset of illness. Two control children had adenovirus 11 viruria and may represent asymptomatic infection. Adenovirus 11 neutralizing antibody rises were detected in 4 of 5 AHC patients with adenovirus 11 viruria, 1 of 4 children with bacteriuria, and 1 of 14 children from whom no agent was recovered. None of 11 control children tested had such rises. 12 patients with AHC had *E. coli* bacteriuria. Bacteria were not isolated from the urine of 18 control patients tested. In the adenoviruric group, males predominated, but the bacteriuric group consisted of nearly all females. Hematuria was present in all and frequency and dysuria in nearly all cases. The average dura-

tion of AHC illness was 4.9 days. Overall, adenovirus or *E. coli* infections were detected in 39.1% of cases. These data provide evidence that AHC has a multiple etiology and that adenovirus and *E. coli* infections are the etiologic agents of significant segments of AHC illnesses.

214. Link of Porphyrias to Metabolism of Hemopexin (Hx), the Most Avid Serum Binder of Porphyrins. URSULA MÜLLER-EBERHARD, La Jolla, Calif.

Two facts are established: heme is taken up by hepatocytes as the heme-Hx complex (1970. *J. Lab. Clin. Med.* **76**: 426, and Hershko et al. 1972. *J. Lab. Clin. Med.* **80**: 624) and the K_d of Hx for heme and non metal-containing porphyrins are ca. 10^{-8} and 10^{-6} M, respectively (1972. *J. Biol. Chem.* **247**: 7181). Whether interaction of nonmetal porphyrins with Hx has pathophysiological significance has been investigated. Sera (samples) of patients analyzed were: 26 (33) with acute intermittent porphyria (AIP), 23 (34) with erythropoietic protoporphyria (EPP), and 64 (67) with porphyria cutanea tarda (PCT). Hx concentrations in AIP remained normal in 19 of 23 patients, in EPP and PCT approximately half were decreased. Therefore, porphyrin contents were related to Hx levels in EPP and PCT. 14 patients (65 samples) with active EPP showed an inverse correlation between levels of Hx and protoporphyrin (supplied by Dr. M. M. Mathews-Roth) in erythrocytes (correlation coefficient, $r = 0.915$), plasma ($r = 0.490$), and daily stool excretions ($r = 0.518$); significance level, $P < 0.001$. 20 patients (20 samples) with PCT showed $r = 0.625$, $P < 0.01$, for Hx levels and urinary uroporphyrin (supplied by Dr. J. H. Epstein), but for coproporphyrin $r = 0.232$, $P > 0.05$. Moreover, for 30 healthy individuals, concentrations of Hx and albumin were found to be 69.3±2.0 mg/100 ml and 4.27±0.14 g/100 ml, respectively. Levels of Hx, 52.9±1.8 mg/100 ml, were lowered simultaneously with those of albumin, 3.49±0.085 g/100 ml, in PCT ($P < 0.001$). In EPP, however, albumin levels, 4.20±0.13 g/100 ml, were unchanged, whereas those of Hx, 53.3±3.1 mg/100 ml, were lowered, $P < 0.001$, which was not due to hemolysis, as haptoglobin levels were normal. These data suggest an interrelationship between the metabolism of Hx and that of nonmetal porphyrins in PCT, very probably in EPP, but not in AIP. (Research supported by grant HL-08660 from NIH.)

215. Immunoglobulin Light Chains in Diabetes Mellitus. FRANKLIN MULLINAX,* CHARLES O. WATLINGTON,* AND BETTY HIMROD,* Richmond, Va. (introduced by David W. Richardson**).

Pancreatic amyloid is found frequently in autopsied diabetics and is apparently responsible for pancreatic hyalinization. Because of this relationship, the light chain content of certain amyloids, and the cryptic nature of diabetic vascular deposits, we have studied the light chains in serum and urine of diabetes free of clinical renal disease and in control subjects. Serum light chain levels, measured by radioimmunoassay, were higher ($P < 0.05$) in 32 diabetics (3.33±0.51 SEM μg/ml) than in matched controls (2.08±0.27 μg/ml). The 12 pairs over age 39 were largely responsible for this difference (5.48±0.87 and 2.69±0.46 μg/ml, $P < 0.01$). Urinary excretion of light chains, estimated by radial immunodiffusion, was also higher ($P < 0.05$) in 15 diabetics (9.20±177 mg/g creatinine) than in controls (4.84±0.72 mg/g creatinine). The paired diabetics and controls over age 39 were responsible for this difference (13.34±2.49 and 4.73±1.09 mg/g creatinine, $P < 0.01$). Serum concentration and urinary excretion of another low molecular weight protein, lysozyme, estimated by radial diffusion, were similar in the diabetics and controls. Diabectics with clinically normal renal function over the age of 40 have elevated levels of serum and urine light chains apparently due to over-production of free light chains. The finding of elevated levels of serum light chain provides a new approach to the etiology and complications of diabetes mellitus. (Supported by a grant from the Arthritis Foundation and NIH-AM 16564.)

216. Autonomy of Cortisol Secretion in the Human Fetus. BEVERLEY E. PEARSON MURPHY, SARAH JANE CLARK,* ROBERT DIEZ D'AUX,* IAN ROSS,* MICHAEL PINSKY,* AND DOREEN VEDAHY,* Montreal, Canada.

In sheep, where the placenta appears to present a significant barrier to the maternal to fetal movement of cortisol, the fetal cortisol level rises a few days before parturition and has been implicated as a factor in the initiation of labor. In human pregnancy it has been generally accepted that cortisol as such crosses the placenta freely and that the mechanism triggering parturition must therefore be different. We have studied the levels of bound and unbound cortisol in the fetal circulation and tissues, and also the fate of tritiated cortisol injected into the mother and found the following. High concentrations of cortisol [1540±1300 ng/g (SD)] were present in the fetal adrenal from 8 to at least 18 wk of gestation. In early pregnancy (8–18 wk), the cortisol level of blood obtained from the fetal end of the umbilical cord (essentially arterial) exceeded that from the placental end ($P < 0.01$). Mixed cord serum cortisol levels rose from 7.0±1.2 ng/ml (SE) in the first trimester to 23.3±3.4 ng/ml in the last trimester (elective Caesarean section) and were unchanged by induced labor (24.0±4.5 ng/ml). Spontaneous labor was associated with a further rise to 72.6±17.3 ng/ml at normal vaginal delivery and 87.1±20.0 ng/ml when Caesarean section was done after the onset of labor. The maternal-fetal gradient of unbound cortisol (10 a.m. to 3 p.m.) was estimated to be about 8:1 in early pregnancy and about 3:1 in late pregnancy. Tritiated cortisol injected into the mother was largely (about 85%) converted to cortisone in the placenta. These findings suggest (*a*) that the human fetal adrenal functions autonomously from early gestation, (*b*) that conversion of maternal cortisol to cortisone serves as a mechanism to prevent suppression of the fetal adrenal, and (*c*) that, although the nature of the placental "barrier" differs between the two species, the maternal-fetal cortisol relationships of man and sheep may yet be fundamentally analogous. (Supported by the Medical Research Council of Canada.)

217. Thymic Function in Murine Lymphoid Leukemia. HIROSHI NAGAYA,* Durham, N. C. (introduced by Herbert O. Sieker).

Although 90% of AKR mice die of spontaneous lymphoid leukemia before the age of 12 months, the mice do not develop leukemia until the age of 6 months, suggesting that a latent period is necessary for leukemia development. Moreover, the incidence of leukemia can be reduced to nil if thymectomized before the age of 6 months. In order to study thymic influence on leukemogenesis, 1- or 6-month-old AKR mice were thymectomized and received syngeneic 6- or 1-month-old thymus grafts. When 1-month-old thymectomized mice were grafted with thymus glands from 6-month-old mice, 13 of 21 mice died of leukemia before the recipient mice reached the age of 6 months, while only 3 of 17 mice thymectomized at the age of 1 month and grafted with 6-month-old spleens died of leukemia. In contrast, none of 19 mice thymectomized at the age of 6 months and grafted with thymus glands from 1-month-old mice developed leukemia for at least 5 months

until the age of grafted thymus reached 6 months. Normal thymus cells can be stimulated by concanavalin A (ConA) to increase [^{3}H] thymidine uptake 455-fold (median), while leukemic thymus cells can be stimulated only 3-fold, the latter response resembling the ConA response of normal bone marrow cells (2-fold). Preleukemic thymus cells obtained from 5-month-old mice were stimulated 269-fold. These results suggest that 1-month-old bone marrow precursor cells can become leukemic prematurely under the influence of 6-month-old thymus grafts because of failure in the thymic function to convert ConA-unresponsive bone marrow precursor cells to ConA-responsive normal thymus cells. (Supported by grant from ACS.)

218. Monozygotic Twin Kinships: a New Model for the Analysis of Quantitative Inheritance in Man. WALTER E. NANCE, PAO-LO YU,* PATRICIA I. BADER,* GLENN J. BINGLE,* AND PHYLLIS WINTER,* Indianapolis, Ind.

The genetic relationships within the families of monozygotic twins permit a more precise analysis of quantitative inheritance than has hitherto been possible in man. The offspring of identical twins are genetically related to each other in the same way as half-siblings, and comparison of monozygotic twin, full-sib, and half-sib correlations in these kinships permits simultaneous estimation of the effects of several degrees of genetic relatedness in the same body of data. In contrast to conventional half-sibs, monozygotic twin half-sibships have the same expected size and average age, and all the parents are usually available for study. Since the half-sibs live in different homes, the effects of genetic correlation can be largely separated from those of a common environment. The model permits estimation of the additive and dominance components of the total genetic variance as well as certain epistatic interactions, from observations on members of the same generation. Maternal effects can be detected by comparing the correlations of maternal half-sibships with those of paternal half-sibships, and the influence of assortative mating on husband-wife correlations can even be distinguished from those of common environmental factors by partitioning the covariance into within and between pair components. The model has been applied to the analysis of multiple anthropometric, dermatoglyphic, and biochemical variables including weight, height, blood pressure, total ridge count, cholesterol, and uric acid in 18 monozygotic twin kinships, which include a total of 95 offspring ranging in age from 6 wk to 44 yr. (This work was supported by PHS RR-00750 and a grant from The John A. Hartford Foundation.)

219. Decreased β-Messenger RNA Activity in Erythroid Cells in Heterozygous β-Thalassemia. CLAYTON NATTA,* JUDY BANKS,* GULZAR NIAZI,* PAUL A. MARKS,** AND ARTHUR BANK, New York.

The decreased β-chain synthesis characteristic of erythroid cells of patients with homozygous β-thalassemia is also observed when mRNA isolated from these cells is added to a cell-free system. The present studies demonstrate that mRNA isolated from erythroid cells in peripheral blood and bone marrow of heterozygotes, as well as homozygotes for β-thalassemia, directs decreased amounts of β-chain synthesis compared to that of α-chains. RNA's were extracted from total cells using phenol and SDS, and isolated by sucrose density gradient centrifugation. The 6-15S (mRNA) fractions were assayed in a Krebs ascites tumor lysate cell-free system. The ratio of α-chain synthesis to that of β-chains (α/β ratio) with mRNA from bone marrow cells of four nonthalassemic patients was 0.96, 0.95, 1.18, and 1.2, similar to that of peripheral blood cells. mRNA isolated from peripheral blood and bone marrow of a patient with homozygous β-thalassemia who had no demonstrable β-chain synthesis in whole cells directed no synthesis of β-chains when added to a cell-free system. A patient with clinical thalassemia intermedia had an α/β ratio of 1.9 in whole bone marrow cells, and 1.45 using isolated mRNA. A patient with high A_2 β-thalassemia trait had an α/β ratio of 1.0 in whole bone marrow cells, while mRNA isolated from peripheral blood and bone marrow led to α/β ratios of 1.6 and 1.75, respectively. These studies indicate that there is decreased β-mRNA activity in heterozygous as well as in homozygous β-thalassemia. Balanced globin chain synthesis in whole bone marrow cells of β-thalassemia heterozygotes may reflect translational control mechanisms which lead to limited α-chain synthesis or increased β-chain synthesis in these cells. (Supported by grants from NIH, NSF, and ACS.)

220. Lack of Insulin Effect on Human Lymphocytes in Culture and During In Vitro Transformation. W. MARCUS NEWBERRY* AND MARIA G. BUSE,* Charleston, S. C. (introduced by Joseph C. Ross).

Specific insulin binding sites have been reported to appear on the lymphocyte surface during transformation, suggesting a possible physiological role of the hormone. Human blood was layered on a mixture of Hypaque-Ficoll and the lymphocytes isolated by centrifugation. Lymphocytes were cultured for 24, 48, or 72 h in Eagle's minimum essential medium containing fetal calf serum with or without added phytohemagglutinin (PHA). The cells were transferred into Gey and Gey's balanced salt solution containing 5.5 mM glucose, with or without [^{14}C] leucine or [^{3}H] thymidine and incubated for 1–4 h. Insulin (10 mU/ml) was added to alternate vials. Resting lymphocytes (2×10^6) cultured for 24 h incorporated 0.3 nmoles [^{14}C] leucine into protein in 2 h PHA-stimulated cells 1.3 nmoles. Simultaneously, stimulated cells consumed 6 times more glucose than controls. DNA synthesis increased only after 48 h exposure to PHA, when stimulated lymphocytes incorporated approximately 50 times more [^{3}H] thymidine into trichloroacetic acid-precipitable material than controls. The addition of insulin during the 1–4 h incubation to resting or to PHA-stimulated lymphocytes did not affect either of the parameters measured; neither did the inclusion of insulin with PHA during 48 h transformation stimulate the utilization of glucose, [^{14}C] leucine incorporation into proteins, or DNA synthesis. By these criteria, insulin does not seem to regulate the metabolism of the unstimulated or maximally transformed peripheral blood lymphocyte. (Research supported by grants from NIH [AM-02001] and SCTRDA.)

221. Lamellar Body of the Large Alveolar Cell—a Misnomer? ALBERT H. NIDEN,* Philadelphia, Pa. (introduced by Arthur B. DuBois **).

The terminal airways and alveoli of the lung are lined with pulmonary surfactant (PS), predominantly saturated phospholipids, capable of markedly reducing surface-active forces on compression. Convincing indirect evidence indicates that the large alveolar cell (LAC) is probably the primary source of PS and that its inclusion bodies (lamellar bodies, LB) are secretory granules containing PS. Although there is some variation in the ultrastructural appearance of LB contents, a characteristic finding is concentric osmiophilic lamellae separated by clear spaces. This appearance indicates a partial extraction from and/or condensation of material within the LB. In an attempt to reduce this known extrac-

tion of saturated phospholipids during preparation of tissue for electron microscopy (EM), heavy metals known to reduce loss of lipids were combined with osmium (Os) and formaldehyde-glutaraldehyde (F-G) in varying proportions. A mixture of calcium, lead, Os, and F-G as a single primary fixative followed by rapid acetone dehydration and embedding in epon, resulted in good EM mouse lung preservation with a striking change in appearance of LB. Dense osmiophilic lamellae were no longer present. Most LB contained a uniform fine granular material throughout with a dense osmiophilic border. The ultrastructural appearance was of an organelle better preserved than that following standard fixation. Lipids were extracted (Folch) and phosphorous phospholipid determined (Fiske) from the fixative, dehydrant, and fixed tissue for both the standard and Ca-Pb-Os-F-G lung preparation. 57% lung phospholipid had been extracted by the former and 43% by the latter procedure. It is concluded that the lamellae of the LAC are an artifact of tissue preparation for EM due in part to extraction of phospholipid and in part to poor fixation of remaining tissue phospholipid. (This work supported by USPHS and Council for Tobacco Research.)

222. Plasma Fibrinopeptide A Concentration As an Index of Intravascular Coagulation. H. L. Nossel, R. E. Canfield, and V. P. Butler, Jr., New York.

Since thrombin cleaves fibrinopeptides A and B from the NH_2-terminal region of the fibrinogen molecule, measurement of fibrinopepetide levels in plasma provides a direct index of thrombin action. A sensitive radioimmunoassay (1971. *Proc. Nat. Acad. Sci.* **68**: 2350) capable of detecting 0.2 ng/ml fibrinopeptide A in plasma has been employed in clinical studies. Since fibrinogen cross-reacts with antifibrinopeptide antibodies, an extraction method was used which was capable of detecting more than 80% of peptide added to plasma. Thrombin treatment of plasma cleaves two molecules of fibrinopeptide A per fibrinogen molecule. Streptokinase treatment of plasma results in cleavage of a larger segment of the NH_2-terminal portion of the Aα-chain of fibrinogen including the A peptide. The A peptide and the plasmin-produced fragment can be distinguished by gel filtration and immunochemically. Extracts of plasma samples from 20 healthy men showed immunoreactivity equivalent to 0.1–0.8 ng/ml, some of which is thought to represent plasmin cleaved NH_2-terminal portion of the Aα-chain. Plasma from patients with intravascular coagulation contains peptide immunoreactivity equivalent to 4–80 ng/ml. The immunoreactive material had the gel filtration and immunochemical characteristics of the A peptide and not of the plasmin cleaved fragment. Infusion studies indicated that the plasma $t_{\frac{1}{2}}$ for the A peptide is approximately 3 min. On the basis of this estimate, it is possible to calculate that thrombin proteolyses fibrinogen at the rate of 500 mg to 10 g/24 h in patients with gross intravascular coagulation and at the rate of less than 50 mg fibrinogen per 24 h in normal subjects. After heparin infusion in patients with intravascular coagulation, the plasma fibrinopeptide A concentration fell to undetectable levels, implying that heparin therapy is associated with decreased proteolysis of fibrinogen and that A peptide levels may provide an index of the therapeutic effectiveness of anticoagulant therapy. (Supported by grants from the NIH and NYHA.)

223. Intestinal Fatty Acid-Binding Protein (FABP): Immunochemical Quantitation and Effect of Diet. Robert K. Ockner * and Joan A. Manning,* San Francisco, Calif. (introduced by Rudi Schmid **).

Recently we identified a protein (FABP, mol wt 12,000) which binds long-chain fatty acids in cytosol of intestine and other tissues, and suggested that it might participate in cellular fatty acid transport. We now provide evidence supporting this concept, based on studies using quantitative radial immunodiffusion with specific antiserum. FABP (~90% pure by disc-gel electrophoresis) was isolated by gel filtration and isoelectric focusing. FABP shared immunochemical identity with a protein in fractions (~12,000 mol wt) of liver and myocardium cytosol which bind fatty acids. No precipitin reaction was observed with serum, intestinal lymph, or partially delipidated lymph lipoproteins. Consistent with its proposed role in fat absorption, FABP concentrations in mucosa from proximal and middle thirds of small intestine (14.9±1.7 and 15.9±0.8 μg/mg soluble protein; 794±81 and 828±49 μg/g tissue) significantly exceeded that in distal third (7.3±0.9 μg/mg protein; 348±12 μg/g tissue). In liver, FABP concentration in cytosol was $\lesssim$ 1.0 μg/mg protein, $\lesssim$ 70 μg/g tissue, and was barely detectable in particulate fractions solubilized with deoxycholate. The effect of diet was studied in rats maintained for 3 wk on chow diets supplemented isocalorically with sucrose (low fat), corn oil (high fat), or medium-chain triglyceride (MCT). Weight gains were virtually identical. Concentrations of FABP in all three intestinal segments were significantly greater after high fat diet (14.4±1.6, 21.0±2.0, 10.5± 0.9 μg/mg protein) than after low fat (9.6±0.6, 14.7±1.1, 6.8±0.8) ($P < 0.025$ for all segments). Results expressed as micrograms per gram tissue were similar. MCT values were intermediate. The preponderance of FABP in proximal intestine and its response to dietary fat intake support the concept that FABP participates in intestinal fatty acid transport. Moreover, its immunochemical identity with FABP in other tissues is consistent with a more general role for this protein in fatty acid metabolism. (Supported by NIH grants AM-13328 and AM-14795.)

224. The Effect of Sexual Maturation on Testicular Sensitivity to Luteinizing Hormone (LH) Stimulation of Testosterone (T) Secretion in the Intact Rat. W. D. Odell, R. S. Swerdloff,* F. Wollesen,* and P. K. Grover,* Torrance, Calif.

We have previously shown that 5 days posthypophysectomy the sexually immature male rat (but not the sexually mature) becomes markedly insensitive to LH stimulation of testosterone secretion. Sensitivity may be restored by prolonged pretreatment with FSH; constant doses of LH produce a greater response dependent upon the time of exposure to FSH. In essence, a time-response relation exists. We have postulated thus that FSH induction of LH sensitivity is a major factor in sexual maturation in the male rat. We have now studied sensitivity of the intact rat to exogenous LH during sexual maturation. Varying doses (0.3–40 μg/100 g body weight) of NIH-LH B7 were administered intraperitoneally to male rats at 10, 22, 42, and 62 days of age. Serum testosterone was quantified by radioimmunoassay 1 h later, the time when the LH-induced testosterone rise was maximal. For all doses of LH which increased serum testosterone, increases were least in the 10-day-old animals, greater in the 22-day-old animals, and greatest at 42 and 62 days; the latter were indistinguishable. Given as per cent increase over base line in response to 30 μg LH/100 g body weight, the values were 10 days, 248±13%; 21 days 630±42%; 42 days 2295±225%; 62 days 1873±125. These studies demonstrate for the first time that the sensitivity to LH stimulation of testosterone increases with age in the intact male rat, and offers additional support for the hypothesis that changing

testicular responsiveness to LH is a major factor in sexual maturation. (Supported by NICHD grant ROI HDO6701.)

225. Zinc and Sickle-Cell Disease. F. J. OELSHLEGEL, JR.,* A. S. PRASAD, G. J. BREWER, D. OBERLEAS,* C. KNUTSEN,* AND E. B. SCHOOMAKER,* Ann Arbor and Detroit, Mich.

We hypothesized that the hemolytic process in sickle-cell disease (SCD) could lead to a state of zinc deficiency because of hyperzincuria. In 14 patients with SCD, red blood cell (RBC) zinc levels were normal in three (13.3–16.5 μg/ml RBC), low normal in two (10.5 and 10.4), and abnormally low in nine (5.8–9.8). Thus it appears that a fair proportion of SCD patients may be zinc deficient, which could partially explain clinical problems such as leg ulcers, growth problems, and hypogonadism. Since zinc is present in a number of enzymes and binds to various amino acids including histidine, we thought it important to investigate effects of zinc on RBC's before suggesting zinc supplementation in SCD. Surprisingly, zinc chloride added to either normal or SCD blood in a final concentration of 1.5×10^{-3} M caused a left shift of the erythrocyte oxygen affinity curve varying from 1.5 to 3.5 mm in eight SCD samples studied. Higher zinc concentrations led to precipitation of plasma protein(s) and aggregation of RBC's. When added to normal blood, zinc levels equilibrated between plasma and RBC's within 3 h. When comparable amounts of 2,3-diphosphoglycerate were added to normal and zinc-treated hemolysates, the P_{50} difference between the two samples did not change, suggesting the two are not competing for the same site. These studies suggest that zinc supplementation in SCD, in addition to its potential role in correcting wound healing and growth problems, may be beneficial in the basic pathological processes. Current evidence suggests that even small leftward curve shifts may be quite helpful. It remains to be seen if physiologically obtainable zinc levels are of therapeutic value in SCD. In vivo studies are planned. (Supported by a contract from NIH.)

226. Nuclear Receptor Sites for Triiodothyronine (T_3) in Rat Liver: Kinetics of Binding and Evidence for the Induction of New Building Sites in the Hyperthyroid State. JACK H. OPPENHEIMER, HAROLD L. SCHWARTZ,* DIONA H. KOERNER,* AND MARTIN I. SURKS, Bronx, N. Y.

In vivo displacement techniques with tracer and carrier T_3 have previously been used to demonstrate specific T_3-binding sites in the nuclei of rat liver and kidney. Additional isotopic experiments were undertaken to characterize the kinetic properties of these sites. Serial measurements of the subcellular distribution of T_3 in rat liver over a 4 h period allowed the calculation of extremely rapid rates of T_3 interchange between nucleus and cytoplasm. Displacement experiments with T_3 and T_4 showed that the nuclear sites bind T_3 at least 13 times more avidly than T_4. The estimated k_a of T_3 was 4.6×10^{11} M^{-1}. Chromatographic studies indicated that the small degree of cross-reactivity between T_3 and T_4 for the nuclear sites could not be attributed to T_4 to T_3 conversion in vivo. Saturation experiments with tracer and carrier T_3 were also used to determine a nuclear binding capacity. Sharp plateau values were achieved in each of six experiments over a 10- to 20-fold range of injected T_3: mean (ng/g liver), 0.99; range, 0.91–1.41. Moreover, the nuclear sites appear close to saturation at endogenous tissue levels of T_3 in euthyroid animals. When animals were rendered hyperthyroid by the daily injection of 25 μg T_3/100 g body weight for 6 days, a 5.7-fold increase in the capacity of nuclear binding sites was observed. Even 2 days after the cessation of T_3 injections the binding capacity was still 3.7 times normal. These findings suggest the possibility that induction of new nuclear sites is necessary before the effects of excess hormone can be manifested at the tissue level. The high degree of specificity of the nuclear sites for T_3 in liver and other tissues also supports previous indications that the hormonal activity of T_4 derives largely, if not exclusively, from its conversion to T_3. (Supported by NIH grant AM 15421-13.)

227. DNA of Epstein-Barr Virus Detected in Tissue of Burkitt's Lymphoma and Nasopharyngeal Carcinoma. JOSEPH S. PAGANO, M. NONOYAMA,* AND GEORGE KLEIN,* Chapel Hill, N. C., and Stockholm, Sweden.

The Epstein-Barr virus (EBV) is the sole virus consistently associated with malignant tumors in man. EBV is found in cultures of explanted Burkitt's lymphomas (BL) but never in the tumor itself. Having developed methods to detect the viral DNA in nonvirus-producing cells, we decided to search directly in BL tissue and also in nasopharyngeal carcinoma (NPC) and other tumors from the same region of Kenya for EBV DNA. The tumor DNA was extracted, denatured, fixed on nitrocellulose filters, and hybridized with EBV-specific radioactive complementary RNA (cRNA) to give the average number of EBV genome equivalents per cell. Of the 23 specimens of BL, 22 clearly contained EBV DNA in amounts ranging between 4 and 113 equivalents with a mean of 38. EBV DNA was less regularly found in NPC: 18 of 23 specimens contained detectable viral DNA ranging from 5 to 85 equivalents with a mean of 19. In contrast, only 4 of 23 other African tumors contained EBV DNA (5–12 genome equiv.). The titers of antibodies to viral capsid antigen (VCA) and to an early virus-specific antigen (EA-R) correlated with the number of EBV genomes in BL. These results strengthen the association of EBV with BL, although the single negative BL retested with the more sensitive DNA-DNA reassociation kinetics again revealed no EBV DNA (< 0.5 genome). Since EBV infects only lymphoid cells, at least in vitro, the EBV DNA in NPC and the other tumors may be due to infiltration of EBV-carrying lymphoid cells from a local site such as the tonsil rather than to EBV in the carcinomatous cells. (Supported by the NCI [SVCP].)

228. Treatment of Acromegaly by Radiofrequency Hypophysectomy. JOHANNA A. PALLOTTA,* NICHOLAS T. ZERVAS,* AND LOUIS M. SHERWOOD, Boston, Mass. (introduced by Louis M. Sherwood).

20 patients with acromegaly were operated on using stereotaxic thermal hypophysectomy for definitive treatment. Human growth hormone (HGH) was lowered into the normal range (10 mμg/ml) in all but three patients. The tumors were large and invasive, extending deeply and laterally into the sphenoid sinus. Studies included pre- and posthypophysectomy glucose tolerance tests with glucose, insulin, and HGH. Patients were restudied from 3 months to 6 yr after operation. Headache, hyperhydrosis, heat intolerance, soft tissue swelling, and joint stiffness began to subside within 1 wk. Preoperatively minor impairment of vision was detected in seven patients, varying from quandranopsia to minor defects on red field testing. Postoperatively visual fields were normal in all. All but one patient developed adrenal insufficiency. Two did not require thyroid replacement. Permanent complications failed to occur. The average hospital stay was 7–10 days. The mean preoperative level of HGH 2 h after glucose load was 76 mμg/ml (SEM was 23.1). The mean postoperative HGH in 18 patients tested for at least 6 months after the procedure was 8.6 mμg/ml (SEM was 2.6). HGH was 10 mμg/ml or less in 15 patients and ranged between 0 mμg and 6 mμg/ml in 11 patients. The average percentage drop in HGH was 88%

($P < 0.01$). Six patients had definite impairment of carbohydrate tolerance improving postoperatively with decreased insulin secretion. The main features of this procedure were a rapid drop in HGH, improvement of clinical symptoms, and halting of acral growth. Remission was distinctly related to the absolute drop of growth hormone to the normal range. This method of treatment is useful in patients with enlarged sella turcicas, with high HGH concentrations, and with serious medical complications which demand definitive and early intervention. The therapy of acromegaly has been multifaceted and controversial. Radiofrequency hypophysectomy offers a low morbidity procedure in reducing the oversecretion of HGH to normal and diminishing the clinical features of the disease.

229. Effect of the Kidney on Exogenous Parathyroid Hormone. Localization of PTH in Proximal Tubule Cells. G. M. A. PALMIERI* AND R. NORDQUIST,* Oklahoma City, Okla. (introduced by Jack Metcoff).

Parathyroid hormone (PTH) has a direct effect on renal tubular function. The kidney is also probably the main site of destruction of PTH. The effect of the kidney on exogenous PTH was studied in dogs receiving a constant infusion of partially purified bovine PTH (TCA-PTH) through a catheter in a jugular vein. Determinations of PTH by radioimmunoassay, using serum antibovine PTH that did not cross-react with canine PTH, were performed in plasma samples obtained with a catheter placed in the inferior vena cava (IVC) above the level of the renal veins. PTH became detectable in IVC within 30 min of the initiation of the TCA-PTH infusion. A plateau was reached at 60 min and remained constant for several hours. No detectable PTH was observed in urine obtained from the renal pelvis. Acute ligation of both renal pedicles provoked a 3- to 4-fold increase in IVC PTH, but only a slight increase was observed when the ureters alone were ligated. After 5 h of an intravenous infusion of [^{125}I]PTH + TCA-PTH or TCA-PTH alone, the kidneys were removed and snap frozen or fixed in formalin. Immunofluorescent studies of tissue sections using a 1:10 dilution of an anti-PTH guinea pig serum showed a selective localization of PTH in the cytoplasm of renal tubular cells. Autoradiographic studies demonstrated that PTH was only localized in the cells of the proximal tubules. Sections of other tissues of the same animals gave negative results. We conclude that in the dog, bovine PTH is selectively trapped by cells of the proximal tubules. (Supported by VA and Oklahoma Medical Research Foundation.)

230. Role of RBC Membrane Lipid Peroxidation in Paroxysmal Nocturnal Hemoglobinuria (PNH). N. V. PANIKER,* A. B. ARNOLD,* AND R. C. HARTMANN, Nashville, Tenn.

Increased susceptibility of PNH RBC membrane unsaturated fatty acids to peroxidation has been cited as a basic defect in PNH. Since iron catalyzes lipid peroxidation, support for this thesis has derived in part from the frequent occurrence of gross hemoglobinuria after iron therapy. An equally tenable hypothesis is that iron therapy stimulates the production of a greater number of "defective" cells in iron-deficient PNH patients. Supporting observations are: (*a*) frequent latent period with improvement in anemia long before the onset of gross hemoglobinuria; (*b*) lack of correlation of serum iron levels with degree of gross hemoglobinuria; (*c*) rise in reticulocyte count before and fall after onset of gross hemoglobinuria; and (*d*) failure of added iron to augment PNH in vitro hemolytic tests. Additionally, two of our PNH patients treated for more than 5 months with the biologic antioxidant, vitamin E, failed to show any response with respect to hemoglobinuria and transfusion requirements. Exposure of normal and PNH RBC to steady, low levels of H_2O_2 was carried out by diffusion technique and by the action of liver microsomes in the presence of TPNH and ATP. Formation of large amounts of malonaldehyde indicated adequate peroxidation, but there was no decrease from base line RBC acetylcholinesterase activtiy. Despite extensive lipid peroxidation of normal RBC, they showed no hemolysis in a variety of in vitro PNH hemolytic tests. Likewise, peroxidation after the inhibition of intracellular and membrane sulfhydryl groups in normal cells gave similar negative results. Moreover, addition of iron was without influence in these experiments. These data provide strong evidence against the thesis that PNH RBC membrane lipid peroxidation plays any significant role in this disorder. (Supported by grant HL-03509 from the NIH.)

231. The Origin of Glycoproteins in Human Alveolar Proteinosis. M. A. PASSERO,* S. N. BHATTACHARYYA,* R. W. TYE,* AND W. S. LYNN, Durham, N. C.

Two glycoproteins of mol wt 62 and 36×10^3 are found in lavage material from patients with pulmonary alveolar proteinosis. Each glycopeptide contains hydroxyproline (1%), hydroxylysine (0.5%), glycine (13%), and cysteine (5%). Each also contains about 8% carbohydrate, of which 1% is sialic acid and 2% is glucose. Antibodies to the glycopeptides do not precipitate human serum proteins. These glycopeptides were not found in isotonic lavage material from a normal human volunteer. Material prepared from human or rabbit whole lung plasma membranes, endocytoplasmic reticulum, mitochondria or soluble proteins, plasma membranes, and alveolar macrophages also did not contain detectable amounts of these glycopeptides. The observations of Nidin suggesting that Clara cells are apocrine cells which may secrete "surfactant" have led us to attempt to isolate these secretory products. Hypotonic washes of the rabbit airways with EDTA solutions contain two proteins similar to those obtained from the proteinosis patients. Characterization of these normal glycopeptides will be reported. Attempts to determine whether basement membrane or Clara cell secretions are the source of these glycopeptides using fluoroscein-labeled antisera to the purified alveolar proteinosis glycopeptides will be reported. (Supported by OH00302-N10SH and ES00124-N1EHS, both from USPHS.)

232. Mechanism of Parathyroid Hormone-Mediated Stimulation of Uridine Incorporation in Isolated Bone Cells. WILLIAM A. PECK* AND KIRK MESSINGER,* Rochester, N. Y. (introduced by John R. Jaenike).

Treatment of isolated bone cells in primary culture with parathyroid hormone (PTH) is known to increase bone cell cyclic 3′5′-AMP (cAMP) and subsequently to enhance the incorporation of [2-^{14}C] uridine into the RNA precursor fraction and into RNA, an effect which is mimicked by exogenous dibutyryl cyclic 3′5′-AMP (Bt_2-cAMP). To determine the mechanism of this effect, we examined the influence of these agents on [2-^{14}C] uridine incorporation into individual free ribonucleotide pools, and on activities of enzymes of uridine metabolism in isolated bone cells. Exposure to PTH (100 ng/ml) or to Bt_2-cAMP (800 μM) for 30 min significantly ($P < 0.01$) enhanced [2-^{14}C] uridine incorporation into cellular free uridine diphosphate (UDP) and uridine triphosphate (UTP) pools (PTH; +29%, Bt_2-cAMP; +25%) but did not increase free uridine monophosphate (UMP) radioactivity. UMP phosphorylation was significantly ($P < 0.01$) augmented in 100,000 *g* supernatant fractions prepared from sonicates of cells treated with PTH (1 μg/ml) or Bt_2-cAMP (800 μM) for 30 min (PTH; +37%, Bt_2-cAMP; +38%). UTP degradation was not suppressed, nor was uridine phosphorylation enhanced in the same supernatant fractions, although increased conversion of UMP to UDP and UTP was apparent

when uridine was the substrate. The effects of PTH and Bt_2-cAMP could not be explained by alterations in bone cell ATP, as assays were performed in the presence of excess (6 mM) ATP, and neither agent increased bone cell ATP levels. Hence, PTH appears to enhance [2-^{14}C] uridine incorporation by cAMP-mediated stimulation of UMP kinase. Since UMP phosphorylation is the first common step in UTP formation via salvage and *de novo* pathways, stimulation of this step could increase the availability of UTP for many metabolic processes, including RNA synthesis. (Supported by grants from NIH.)

233. Studies with Oral Cyanate in Sickle-Cell Disease. CHARLES M. PETERSON,* YANG S. LU,* JAMES M. MANNING,* PETER N. GILLETTE,* AND ANTHONY CERAMI,* New York (introduced by Attallah Kappas).

Cyanate diminishes sickling by specific carbamylation of amino terminal valines of hemoglobin S (1971. *Proc. Nat. Acad. Sci. U. S. A.* **68:** 1180). Aliquots of erythrocytes from patients with sickle-cell disease have an enhanced survival after treatment in vitro with cyanate (1971. *Proc. Nat. Acad. Sci. U. S. A.* **68:** 2791). Short-term administration of cyanate is well tolerated and decreases the hemolytic anemia (1972. *Adv. Exp. Med. Biol.* **28:** 261). No adverse effects have been observed to date in cyanate-treated animals maintained at 1.0–1.8 moles cyanate per mole hemoglobin tetramer (NCO/Hb) for 18 months (*J. Pharmacol. Exp. Ther.* In press). 26 patients with sickle-cell disease (S/S) received sodium cyanate orally in doses of 5–30 mg/kg per day for 8–15 months to determine toxicity and clinical efficacy. Maximal carbamylation was attained after 20–40 days, and the hematologic responses were dose related. Circulating hemoglobin increased 5–20% in patients maintained over 0.3 NCO/Hb, red cell mass increased 13% in six patients at 0.44 NCO/Hb, and oxygen affinity increased ($\Delta P_{50} \cong -3$ mm at 0.5 NCO/Hb). Subjective toxicity was minimal, and no objective adverse effects of cyanate were observed. 43 crises occurred; the mean incidence was 2.3/yr for 13 patients with carbamylation ranging from 0.05 to 0.26 NCO/Hb and 1.1/yr for 13 patients at 0.28–0.54 NCO/Hb. This evidence suggests that cyanate has promise as a possible therapeutic agent for sickle-cell disease and establishes the rationale for a randomized clinical trial. (Research supported by grants from NIH and NF.)

234. Differential Inhibition of Cholera and *E. Coli* Enterotoxins by Cholera Toxoids and Ganglioside. N. F. PIERCE,* Baltimore, Md. (introduced by C. C. J. Carpenter).

Cholera enterotoxin (CT) and the heat-labile component of *E. coli* enterotoxin (ECT) each induce gut secretion by activating mucosal adenyl cyclase. Yet the response to CT is slow in onset and of many hours duration, while that of ECT is rapid in onset and of brief duration, suggesting differences in mucosal binding of the enterotoxins. We investigated CT- and ECT-binding characteristics by comparing the inhibitory effects of natural cholera toxoid (NCT), formalinized cholera toxoid (FCT), and ganglioside on the secretory response of rabbit small bowel to CT and ECT. NCT acted as a competitive inhibitor of CT, being maximally effective when injected 4 h before CT. Mucosal binding of CT was more rapid than mucosal binding of NCT, but once bound the binding of each appeared to be irreversible. By contrast, FCT did not inhibit the secretory response to CT. FCT and NCT are each antigenic, but neither stimulates mucosal secretion. These data suggest that enterotoxic activity, mucosal binding capacity, and antigenicity are independent properties of CT. NCT had no inhibitory effect upon the secretory response to ECT, indicating different mucosal binding sites for ECT and CT. Prior studies suggest ganglioside is the mucosal receptor of CT. Premixing of CT with nanogram amounts of ganglioside completely deactivated CT. By contrast ganglioside was relatively inefficient in deactivating ECT, 1000-fold greater amounts of ganglioside being required. These findings suggest that ganglioside is not likely to be the mucosal receptor for ECT. These differences in mucosal binding of ECT and CT may explain, at least partly, the marked differences in time of onset and duration of their secretory effects. (Supported by NIH grant AI-07625 and a Career Development Award.)

235. Mass Testing for Hematological Disorders. SERGIO PIOMELLI,* BERNARD DAVIDOW,* PATRICIA YOUNG,* AND GISELLE GAY,* New York (introduced by Peter Elsbach**).

Microsamples of blood can be collected by untrained personnel from finger puncture on S & S 903 filter paper for shipment to a centralized laboratory. With a $\frac{1}{4}$" punch, aliquots equivalent to 11 μl of blood are obtained from the spots. (Quantification and reproducibility was confirmed with ^{51}Cr-labeled samples of varying Hct.) Thus Hgb can be quantitated by elution into a modified cyanmethemoglobin reagent. (1000 paired samples demonstrated an excellent correlation with existing techniques.) Hgb electrophoresis can be performed easily by direct implantation of the filter papers in agar gel. Free erythrocyte porphyrins (FEP) can be measured fluorometrically in 1.5 N HCl, after a rapid extraction in ethyl acetate/acetic acid in the presence of Celite. (Paired measurements in 25 samples with widely differing FEP content agreed closely with the standard technique of Wranne.) The ratio FEP/Hgb was an excellent indicator of Pb intoxication. In 5000 samples analyzed for Pb levels by the New York City Board of Health, FEP/Hgb increased exponentially with the blood Pb level. The FEP exceeded 6.2 μg/g Hgb in all 290 samples with Pb > 60 μg/100 ml, but only in 5% of those with normal Pb. FEP/Hgb was increased in 55 cases of Fe deficiency, but to a lesser degree than in Pb poisoning. A preliminary field screening of 1100 children with these techniques uncovered 42 cases of anemia (29 due to Fe deficiency) and 17 cases of Pb poisoning. These simple microtechniques offer a powerful tool for the mass detection of anemia, Fe deficiency, Pb poisoning, and hemoglobinopathies. (Supported by NIH grant AM 09274-08 and NYCHRC grant U 2282. Dr. Piomelli is a Career Scientist of the NYCHRC, contract I-383.)

236. The Effect of Histamine on the Cell-Mediated Immune Response. MARSHALL PLAUT,* CHRISTOPHER S. HENNEY,* AND LAWRENCE M. LICHTENSTEIN, Baltimore, Md.

Histamine inhibits lymphocyte-mediated cytolysis, as shown by in vitro experiments involving the killing (^{51}Cr release) of murine DBA/2 mastocytoma cells by splenic thymus-dependent (T) lymphocytes from sensitized C_{57}Bl mice. This inhibition is related to and presumably caused by histamine's ability to increase lymphocyte cAMP levels. Standard antihistamines (e.g., diphenhydramine) do not block this effect, but burimamide, a new type of antihistamine which affects histamine 2 receptors, reverses both the inhibition of cytolysis and the rise in cAMP. The inhibition of cytolysis by a standard (10^{-5} M) amount of histamine increased from $11 \pm 2\%$ at day 10 to $40 \pm 4\%$ on day 14, while the ability of histamine to increase the cAMP level of the entire pool of splenic lymphocytes remained constant throughout the immune response. The increasing inhibition observed was not an artifact of altered lytic activity or of a change in the threshold sensitivity of the lymphocyte to histamine, suggesting that the proportion of cytolytically active T-lymphocytes bearing histamine receptors increases with time after immunization. Histamine given intraperitoneally on day 4 markedly decreased the subsequent cytolytic activity of spleen cells measured in vitro on days 11 or 13, although the "killer" T-cells which remained still bore

histamine 2 receptors, as they were inhibited by histamine in vitro. Histamine also inhibited antibody production: animals treated with histamine on day 4 showed a decrease in mastocytoma agglutination titers assayed on day 11. These data suggest that histamine may affect antigen-driven cellular differentiation, as well as having marked suppressive effects on effector T-cells. (Supported by grants AI-10280 and AI-00423 from NIH and a grant from the NSF.)

237. Removal of Bilirubin from Human Plasma by Affinity Chromatography on Sepharose-Conjugated Albumin. PAUL PLOTZ,* PAUL BERK,* JOYCE GORDON,* AND JOHN VERGALLA,* Bethesda, Md. (introduced by Jacob Robbins**).

Substances in human plasma that bind tightly to plasma proteins cannot readily be removed. Unconjugated bilirubin (BR) is so firmly bound to albumin that it cannot be removed even by dialysis. In a study of possible methods for removing BR or other potentially toxic albumin-bound substances from plasma, we have coupled human albumin to Sepharose 6B with cyanogen bromide, attaining concentrations of 25–50 mg albumin per g wet weight of gel. Plasma from a patient with Crigler-Najjar syndrome, containing 19.5 mg/100 ml (195 μg/ml) BR, trace-labeled with [^{14}C]BR, was passed over such gels. 1 g of gel bound in excess of 90 μg of BR, determined by chemical assay or radioactivity. The radioactive material was eluted quantitatively with 50% ethanol in water and the column reused repeatedly without apparent loss of efficiency. An anion-exchange resin, Dowex 1-X2 (200-400 mesh), bound BR somewhat more efficiently per gram wet weight, and an uncharged resin, XAD-2, less efficiently than the Sepharose adsorbent (cf. Willson et al. 1972 *Gastroenterology.* **62**: 1191–1199). Two other substances which are partially bound to albumin were studied. [^{125}I] thyroxine, equilibrated with normal serum, bound to the Sepharose-albumin and was eluted by 50% ethanol; [^{14}C] salicylic acid, equilibrated with normal serum, also bound to the gel and was eluted with buffered saline. Protein adsorbents such as Sepharose-albumin may prove useful in the removal of BR or other protein-bound toxic substances from human plasma in patients with generalized hepatic failure, specific metabolic deficits, or intoxication with protein-bound drugs.

238. Paradoxical Effects of L-DOPA Administration in Huntington's Chorea. STEPHEN PODOLSKY* AND NORMAN A. LEOPOLD,* Boston, Mass. (introduced by Belton A. Burrows**).

It has been reported that L-DOPA administration increases the adventitious movements of patients with Huntington's chorea (HC), possibly due to an increased sensitivity to intracerebral dopamine in these patients. We therefore studied its effects on other parameters (glucose, insulin, and growth hormone). 10 patients with documented HC (mean age 48.5 yr and mean duration of disease 6.8 yr) and 10 control subjects (mean age 51.5 yr) had glucose and arginine tolerance tests before and after being given L-DOPA (1.5 g/per day) for 3 days. No clinical change was noted in controls or patients with HC. L-DOPA was associated with a rise in mean glucose level in controls 2 h after oral glucose (before = 95.6±6.8 mg/100 ml; after = 115.3±9.1 mg/100 ml), but a fall in the patients with HC (before = 125.9±11.0 mg/100 ml; after = 116.6±8.9 mg/100 ml). In controls the mean insulin level at 2 h was slightly lower with L-DOPA (before = 71.0±10.9 μU/ml; after = 61.2±8.8 μU/ml). In six HC patients who had abnormal glucose tolerance, the insulin levels 2 h after oral glucose were lower with L-DOPA (before = 197.5±41.8 μU/ml; after = 64.5±9.7 μU/ml). However, in the four patients with normal glucose tolerance, the insulin levels 2 h after glucose were higher with L-DOPA (before = 40.4±8.4 μU/ml; after = 61.5±12.3 μU/ml). In all patients basal growth hormone was increased by L-DOPA (before = 4.2±0.8 ng/ml; after = 17.0±4.9 ng/ml), but unlike normal controls was completely suppressed by oral glucose. In 5/6 patients with HC given L-DOPA, the peak growth hormone rise 60 min after arginine infusion (0.5 g/kg) was attenuated (before = 34.4±7.5 ng/ml; after = 15.9±5.2 ng/ml). It is clear that L-DOPA has significant effects on glucose, insulin, and growth hormone metabolism different in Huntington's chorea than in normal subjects. (Supported by a VA Clinical Investigator grant.)

239. Scanning Electron Microscopy of Normal and Leukemic Human Blood Cells. AARON POLLIACK,* BAYARD D. CLARKSON, NINA LAMPEN,* AND ETIENNE DEHARVEN,* New York.

Until now, blood cells have been prepared for scanning electronmicroscopy (SEM) mainly by air drying. This procedure causes distortion and loss of surface detail and consequently precludes distinction between different leukocytes under SEM. The present report describes the surface features of normal and leukemic blood cells dried either in the air or by CO_2 critical point. Cell suspensions obtained from buffy coats were collected by aspiration and filtration on to silver filters, fixed with gluteraldehyde and osmium, and dehydrated through a graded series of alcohols. One half of the filter was air dried, while the other was critical point dried after further dehydration in a graded series of amyl acetate. Comparison of these procedures showed that critical point drying was superior and enabled one to distinguish different leukocytes. After critical point drying finger-like surface projections were consistently present in most leukemic lymphocytes. Air drying caused shrinkage of cells and considerable loss of surface detail and did not allow ready characterization of the different cells. These findings indicate that SEM is useful in the recognition of different leukemic cells prepared by critical point drying. (Research supported in part by grant CA-08748 from the NCI. Dr. Polliack is from the Department of Hematology, Hadassah University Hospital and Medical School, Jerusalem, Israel, on an International Fellowship from the NIH and USPHS.)

240. Feedback Control of Cholesterol Synthesis in Circulating Granulocytes and Deletion of Feedback Control in a Granulocytic Leukemia. FRED I. POLSKY,* MICHAEL S. BROWN,* AND MARIVIN D. SIPERSTEIN,** Dallas, Tex.

To demonstrate feedback control of cholesterol biosynthesis by dietary cholesterol in blood cells, a method was developed to isolate rat granulocytes, lymphocytes, and platelets and to measure their sterol synthesis from [^{14}C] acetate. The rates of acetate incorporation into cholesterol in granulocytes, lymphocytes, and platelets were 0.441, 0.263, and 0.001 mμmoles/h per 10^6 cells. However, the cholesterol synthetic rates per milligram of protein were nearly equivalent: 0.549, 0.817, and 0.225 mμmoles/h. Feedback control was found in circulating granulocytes, but not in lymphocytes or platelets; a 5% cholesterol diet suppressed cholesterol synthesis in granulocytes by 91%. The activity of HMG CoA reductase, the rate-limiting enzyme in hepatic cholesterol synthesis, was similar in microsomes from granulocytes and liver (0.357 vs. 0.280 mμmoles/min per mg microsomal protein). After 21 days of cholesterol feeding, granulocyte HMG CoA reductase activity decreased by 87%, a degree of depression sufficient to account for the observed 91% inhibition of cholesterol synthesis. Since we have observed previously that feedback control of cholesterol synthesis in liver cells is deleted after their malignant transformation, the effect of cholesterol feeding on malignant granulocytic cells was examined. A spontaneous rat granulocytic leukemia maintained in ascitic fluid synthesized

cholesterol at about the same rate as normal granulocytes isolated from peritoneal fluid (0.579 vs. 0.495 mμmoles/h per 10^6 cells). However, although the normal granulocytes showed a 90% inhibition of cholesterol synthesis after 21 days of cholesterol feeding, no reduction occurred in the leukemic cells. This finding suggests that loss of the cholesterol feedback system may be a more general characteristic of malignant change than was generally believed.

241. Clinical and Genetic Heterogeneity of Pseudoxanthoma Elasticum. F. MICHAEL POPE,* Baltimore, Md. (introduced by Victor A. McKusick**).

Whereas pseudoxanthoma elasticum (PXE) was previously considered a single autosomal recessive disorder, we have found clinical and genetic evidence of heterogeneity. An attempted total ascertainment of affected persons in Britain provided data on 182 patients. Pedigrees with two or more affected persons showed two patterns: one with multiple affected generations typical of autosomal dominant inheritance, and the other with a single affected generation, typical of autosomal recessive inheritance. When the pooled genetic data were examined, correcting for bias of ascertainment, the affected-unaffected ratios were close to the one-to-one ratio expected for heterozygous dominant with normal matings, and close to one-to-three for the unaffected heterozygous matings of the recessive group. Clinical separation of two dominant and two recessive groups was also possible. Dominant type I disease is characterized by classical, flexurally distributed rash, severe angina, hypertension, intermittent claudication, and very severe degenerative retinopathy. In contrast, type II dominant disease is much milder with hardly visible cutaneous infiltration, few vascular complications, and mild retinal disease. Type I recessive PXE is of intermediate severity, except that there is a particular liability to gastrointestinal bleeding. It accounted for 66% of such complications in this series. Type II recessive disease is very rare and is characterized by a generalized cutaneous infiltrate, but no evident ocular or vascular complications. Of the 122 index patients, 12 were dominant type I, 52 were dominant type II, 54 were recessive type I, and only 3 were recessive type II.

242. The Role of Cell Swelling in Myocardial Ischemia and the Protective Effect of Hypertonic Mannitol. WM. JOHN POWELL, JR.,* JORGE FLORES,* DONALD R. DIBONA,* AND ALEXANDER LEAF,** Boston, Mass.

In brain and kidney, elevation of extracellular osmolality, through reduction of ischemic cell swelling, improves reflow of blood after arterial occlusion. Recently it has been demonstrated that hyperosmolar mannitol increases collateral blood flow to ischemic myocardium with improved myocardial function and decreased ischemia. The present studies in 55 dogs anesthetized with chloralose and urethane document and study the mechanism of impaired reflow after coronary occlusion and examine the possible salutary effect of mannitol in improving reflow and in lessening the extent of myocardial damage. Infusions of silastic into the aortic roots of 10 dogs after 40–60 min of left circumflex artery occlusion and 15 min of reflow revealed impaired distribution of silastic to the region of the posterior papillary muscle. Parallel electron microscope studies demonstrated myocardial and endothelial cell swelling with partial closure of vessel lumina. This cell swelling was prevented by elevation of serum osmolality by 30–40 mOsm above control with hypertonic mannitol administered during and after occlusion. These morphologic findings were associated with a 42 ± 10 (SEM) % ($P < 0.02$) reduction of vascular resistance after occlusion if mannitol was administered. Microscopic examination of tissue obtained 12 h after the period of occlusion revealed less damage in hearts which received mannitol. Thus, this study documents impaired reflow of blood after coronary artery occlusion and points to cell swelling as at least a partial mechanism. Reflow is substantially increased, cell swelling reduced, and subsequent damage lessened by the administration of hyperosmotic mannitol. (Research supported by grants from NIH.)

243. DNA Synthesis and Thymidine Kinase in Zinc-Deficient Tissue. ANANDA S. PRASAD AND DONALD OBERLEAS,* Detroit, Mich.

Growth retardation is a consistent manifestation of zinc deficiency in various animal species and man. Zinc is known to be essential for many enzymes, but its precise role in cellular growth has not been established. In this study, effects of zinc deficiency on DNA and activity of thymidine kinase (TK) in rapidly regenerating tissue were investigated. After 1 wk on zinc-deficient diet, rats were implanted with polyvinyl sponges subcutaneously. 6 and 10 days later, sponges were removed and connective tissue capsules studied in vitro. Continuously pair-fed and ad lib.-fed rates were used as controls. DNA was measured by a modified method of Schmidt-Thannhauser. Activity of TK was assayed in vitro by the method of Lieberman. Enzyme activity was expressed as nanomoles of thymidine monophosphate generated per hour per milligram protein. Incorporation of [2-^{14}C] thymidine as a measure of DNA synthesis was determined in vitro in 6 day tissue in zinc-deficient and pair-fed control rats. TK activity in sixth day connective tissue was as follows (units mean $\pm$SE): deficient, 0.58 ± 0.02; pair fed, 2.4 ± 0.59; and ad lib., 1.65 ± 0.12. The difference between deficient and controls was statistically significant ($P < 0.025$). In tenth day tissue, deficient rats showed no measurable TK activity (pair fed, 2.7 ± 0.6; and ad lib., 2.68 ± 0.7). [2-^{14}C] thymidine incorporation into DNA was also significantly reduced in sixth day deficient tissue (DPM/mg DNA: deficient, $(18\pm5.7)\times10^3$ and pair fed, $(136\pm15.2)\times10^3$, $P < 0.001$). In conclusion, this is the first demonstration of an adverse effect of zinc deficiency on activity of thymidine kinase in animals. Inasmuch as this enzyme is essential for cell division, our data suggest that this early metabolic defect may account for growth retardation in zinc-deficient rats. (Research supported by VA grant and NIH contract.)

244. Tissue and Sera Factors Affecting Organic Anion and Cation Transport in Hypertensive Rats (SHR). H. PREUSS,* M. ZMUDKA,* K. GRANT,* R. PARRIS,* L. SLOTKOFF,* AND G. SCHREINER,** Washington, D. C.

Altered renal metabolism may be causally related to essential hypertension. The present study was performed in a strain of spontaneously hypertensive Wistar rats (SHR), thought by many to be the best animal model of this disease. Organic anion (PAH) and organic cation (TEA) transport in renal slices were compared in SHR and normotensive Wistars (C). In 14-wk-old SHR rats, blood pressure taken via tail plethysmography averaged 146 mm Hg compared to a control of 113 mm Hg. In nine experiments, the accumulation of PAH by slices from SHR exceeded control ($\uparrow$36%, $P < 0.01$), while TEA transport was significantly decreased ($\downarrow$7%, $P < 0.05$). In 7-wk-old SHR that had not yet developed hypertension (BP 110 mm Hg to control 107 mm Hg), the same pattern was noted: PAH accumulation $\uparrow$12% ($P < 0.05$) and TEA transport $\downarrow$10% ($P < 0.001$). Within a given experiment, the ΔBP between C and SHR correlated significantly with the ΔPAH ($r = 0.56$, $P < 0.02$). The presence of changes in tissue are present before the development of elevated BP and constitute a physiologic difference in renal function in SHR. Sera taken from SHR compared to sera from control depressed significantly PAH transport in normal slices. When the sera

was fractionated on Sephadex 25, the depressor in SHR sera to kidney slice PAH transport was found in the fourth fraction, the same fraction that suppressed PAH transport and Na excretion in azotemic sera (1971. *J. Clin. Invest.* **50:** 303). Fraction 4 from C had no effect on PAH transport. The observation of renal metabolic abnormalities and factors in sera affecting PAH transport early in the development of hypertension may have implications in the pathogenesis of hypertension. (Supported by NIH grant 15458.)

245. In Vitro Hybridization of C-Type Viral RNA to Human DNA. PETER M. PRICE,* NORMAN GABELMAN,* KURT HIRSCHHORN,** AND SAMUEL WAXMAN,* New York.

We have previously reported the probable chromosomal localization of human hemoglobin genes by *in situ* hybridization. With this technique, we subsequently detected sequence homology between a D-group chromosome of uninfected human cells and the RNA of a putative human oncogenic C-type virus (RD114). This data lends credence to the "oncogene" theory proposed by Huebner and Todaro which proposes that all mammalian genomes contain the information for transcription of oncogenic RNA tumor viruses. We have now investigated the product of in vitro hybridization of solubilized human placental DNA with the high and low molecular weight RNA components of Feline leukemia and RD114 viruses. Our observations have shown that there is a large degree of hybridization of the high molecular weight RNA species (35 to 70S) of both viruses with human DNA, and that the low molecular weight species (4S) extracted from these viruses demonstrates much lower saturation kinetics with this DNA. These results indicate that the low molecular weight RNA species contained within C-type RNA tumor viruses is not a degradation product of the high molecular weight component. The low saturation kinetics also make it unlikely that the 4S RNA is a contaminating human cellular RNA species. This is supported by the finding that low molecular weight human RNA hybridized with human DNA does not reach saturation at even much higher concentrations. Our results indicate that a portion of human (and perhaps most mammalian) DNA contains closely related nucleotide sequences with mammalian C-type viral RNA, and that there is a substantial number of oncogenic viral genome copies preexisting in the genome of uninfected human cells. (This study was supported by grants from NIH.)

245a. "Little" Luteinizing Hormone (LH): the Appearance in Serum of an hLH-Related Peptide, Possibly a Subunit, After LHRH Stimulation. D. RABINOWITZ,* R. BENVENISTE,* L. FROHMAN, J. BELL,* AND I. SPITZ,* Jerusalem, Israel (introduced by C. R. Kleeman).

This report describes the identification of a human luteinizing hormone [hLH]-related peptide which can be prepared from pituitary hLH powder and is found in serum after LH-releasing hormone [LHRH] stimulation. Two purified pituitary preparations of LH, LER 960, and Hartree IRC-2 were labeled with ^{125}I and submitted to gel filtration on a Sephadex G-100 column. The IRC-2 preparation showed two peaks, "standard LH" and "little LH" with $K_{av} = 0.24$ and 0.40, respectively. LER-960 appeared as a single peak ($K_{av} = 0.24$) which could be partially converted to little LH [$K_{av} = 0.40$] by 8 M urea treatment. Molecular weights for standard LH and little LH were estimated to be 32,000 and 18,000 respectively, and it is likely that the peaks represent "associated" and "dissociated" (i.e., individual subunits) forms of LH. Purified standard LH and little LH material were used as tracers in a double antibody radioimmunoassay (RIA) with rabbit anti-HCG antibody. By choosing appropriate antibody concentrations, standard and little LH could be selectively assayed by these RIA procedures. Selected human sera were chromatographed on Sephadex G-100, and the eluted fractions were assayed in the two systems. Results follow. (*a*) Low LH sera gave negative results in both RIA's. (*b*) High LH serum, obtained at the time of the midcycle LH surge in a normal menstruating woman, gave a single peak in the position of associated LH, which reacted only in the standard LH RIA. (*c*) High LH serum, obtained from a young patient with ovarian failure, gave two peaks, the majority being standard LH, with a small but definite little LH peak. (*d*) Between 5 and 20 min after LHRH stimulation in this patient, a large increase in little LH occurred, this little LH constituting 39% of total serum LH. Little LH had fallen to 18% of total LH by 90 min after LHRH. (*e*) Appearance of little LH has been observed also in two other patients with high LH levels after LHRH. We conclude that little LH[possibly dissociated LH subunits, has been demonstrated in serum, and its appearance can be triggered by LHRH.

246. Ultrastructural and Physiological Evidence for Corticosteroid-Induced Alterations in Heptaic Production of Very Low Density Lipoproteins. EVE P. REAVEN,* ORVILLE KOLTERMAN,* AND GERALD M. REAVEN, Stanford, Calif.

We recently suggested that the cause of hyperlipoproteinemia in corticosteroid-treated (CS) patients is overproduction of hepatic very low density lipoproteins (VLD). We now have additional evidence to support this observation. Methylprednisolone (0.3 mg/100 g) treatment of young adult rats for 8 days produced an increase in plasma levels of triglyceride of 148% ($P < 0.01$) and cholesterol of 72% ($P < 0.01$). In CS rats injected with Triton WR 1339 (600 mg/kg), the estimated rate of accumulation of triglyceride into the measured plasma compartment was 176 mg/2 h as compared to 117 mg/2 h for control rats ($P < 0.01$). Liver samples were prepared for electron microscopic morphometric analysis of VLD localization, size, and abundance. The results show that in both corticosteroid and control animals, VLD are primarily associated with hepatocyte Golgi complexes. The number of Golgi complexes per area of hepatocyte cytoplasm does not change with corticosteroid treatment. However, in 15 large Golgi analyzed from each of five control and five CS animals, the mean ($\pm$SE) number of VLD per Golgi complex increased ($P < 0.02$) in CS animals ($121 \pm 11 \rightarrow 187 \pm 19$) and the average diameter of VLD per Golgi increased ($P < 0.01$) in CS animals (535 A $\rightarrow$ 707 A), representing a 120% increase in VLD volume. Thus, CS treatment results in (*a*) an increase in plasma triglyceride, (*b*) an increase in rate of accumulation of triglyceride after the inhibition of VLD clearance by Triton, and (*c*) an increase in the number and size of VLD present in hepatocyte Golgi complexes. These combined results suggest that CS induces hyperlipoproteinemia through increased hepatic production of VLD. (Research supported by grant HL 08506 from NIH.)

247. Proteases from Skeletal and Heart Muscle. M. K. REDDY,* J. ETLINGER,* R. ZAK,* D. A. FISCHMAN,* AND M. RABINOWITZ, Chicago, Ill.

Although intracellular muscle proteases are likely to be involved in the turnover of proteins in both normal and diseased muscle, their localization and mode of action remains obscure. We have studied the distribution and specificity of neutral and acid proteases in subcellular fractions. Recently it has been reported that a Ca^{2+}-activated sarcoplasmic factor (CASF) causes the removal of the myofibrillar Z line. We find that CASF causes the removal from myofibrils of α-actinin without myosin degradation as displayed on SDS-acrylamide gels and without loss of the EGTA sensitivity of myofibrillar ATPase and contractility. Myofibrils digested with trypsin or papain

lose this EGTA sensitivity and degradation bands of myosin appear before complete Z line disappearance, indicating that CASF Z line removal differs from trypsin or papain digestion. The CASF preparation catalyzed a Ca^{2+}-activated proteolysis of casein and hemoglobin at neutral pH. Studies are in progress to ascertain whether Z line release is proteolytic in nature. A second neutral protease activity has been observed associated with the myofibrillar fraction. It is not Ca^{2+}-activated. Mitochondrial and membrane fractions are enriched in acid protease activities. Unlike the neutral proteases, these show tenfold activation in the presence of 0.2% Triton X-100. These studies indicate that muscle contains highly compartmentalized proteases which are activated by specific and different conditions. (Supported by grants from MIRU, USPHS, AEC, and Chicago and Illinois Heart Association.)

248. Chemical and Biological Properties of Purified Native and Desialylated Human Thyroxine-Binding Globulin. S. REFETOFF,* V. S. FANG,* AND J. S. MARSHALL,* Chicago, Ill., and Cleveland, Ohio (introduced by R. L. Landau**).

Thyroxine-binding globulin (TBG) and partially desialylated or slow TBG (sTBG) were prepared as previously described (1972. *J. Clin. Invest.* **51**: 3173). Purified TBG was electrophoretically identical with TBG in serum. sTBG moved more slowly. Both bound thyroxine (T_4) equimolarly. sTBG had lower T_4 affinity and was less heat labile. Iodinated TBG and sTBG with from approximately 1 to 6 atoms of ^{131}I or ^{125}I per molecule had increased anodal mobility. Nevertheless, T_4 binding, antiserum precipitation, and disappearance rates in rabbits of simultaneously injected [^{131}I] TBG (1 atom I per mole) and [^{125}I] TBG (6 atoms I per mole) remained unchanged. 15 min after intravenous injection of [^{131}I] sTBG and [^{125}I] TBG mixture in rats, 94% of sTBG was in liver with an $^{131}I/^{125}I$ ratio of 33.2-fold that of serum. With time, sTBG-derived ^{131}I declined in liver and increased in bile, intestinal content, and urine. In normal rabbits, serum [^{125}I] sTBG/[^{131}I] TBG ratios declined from 1.0 to < 0.2 in 10 min. The ratio remained unchanged for 1 h in acutely hepatectomized rabbits. The serum $t_{\frac{1}{2}}$ of sTBG was 4–5 min and 2–3 days for TBG. Electrophoretic, immunologic, and chromatographic analyses identified 50% of sTBG-derived ^{125}I in bile as iodinated fragments and 98% as iodide in urine. In man, liver [^{125}I] sTBG/[^{131}I] TBG ratio was 51.8 times that of serum 30 min after intravenous injection during diagnostic open liver biopsy. Since radioiodinated TBG and sTBG preserve their biologic and immunologic properties, they are useful for studies of TBG metabolism. In rat, rabbit, and human, sTBG is rapidly cleared by liver. Conversion of TBG to sTBG may regulate the metabolism of TBG. (Supported by grants AM-15,070 and 15,308 from NIH.)

249. Description of a Serological Reaction Characteristic of Dermatomyositis. MORRIS REICHLIN AND MARTHA MATTIOLI,* Buffalo, N. Y.

Dermatomyositis (DM) is an inflammatory disease of muscle and skin for which no specific laboratory diagnostic test is available. We report a specific complement (C) fixation reaction involving human sera with calf thymus extract (CTE) as antigen which has thus far been found only in the sera of patients with DM. To identify the specificity of the immunological reaction in which the "antigen" is a complex mixture, the following method was developed. Papain digestion of whole serum results in complete conversion of IgG to Fab and Fc fragments. Serum containing C-fixing antibody to a specific antigen becomes a potent, specific inhibitor of that reaction after papain digestion. Thus, papain-digested human sera can be assayed for their ability to block the serum reacting with a specific antigen in a crude extract. We have found that the sera of three cases of DM fix C with an antigen in CTE. Papain digests of any one of these sera completely blocks the reaction of any of the three sera in their C-fixation reactions with CTE. Papain digests of human sera from patients with systemic lupus erythematosus (SLE) (20), rheumatoid arthritis (20), multiple myeloma and macroglobulinemia (9), polymyositis syndromes associated with other diseases (scleroderma, Sjogren's syndrome) (6), and normal human sera (19) do not block this reaction characteristic of these DM patients. The SLE sera contained a diversity of antibodies to various tissue antigens. Despite a multiplicity of such antibodies, no inhibitory activity for the reaction characteristic of DM sera was found. Thus far, therefore, antibodies to this antigen appear specific for dermatomyositis. Further studies of the specificity of this reaction for dermatomyositis are underway. (Supported by research funds from VA, NIH, and MDAA.)

250. Positive Effect of Intravenous Triiodothyronine (T_3) on Thyrotropin (TSH) Secretion in Primary Hypothyroidism. E. CHESTER RIDGWAY,* BRUCE D. WEINTRAUB,* AND FARAHE MALOOF,** Boston, Mass., and Bethesda, Md.

That thyroid hormone exerts a negative feedback on pituitary TSH secretion has been well established; that it may also exert a positive feedback has been demonstrated in the following study. Eight hypothyroid patients were given intravenous T_3, 100 μg on day 1, then 50 μg intravenously daily for 9 days. Before and after T_3 therapy, TSH metabolic clearance (MCR, nl = 48±15 cc/min) and production rates (PR, nl = 66±42 mU/day) were correlated with peak serum TSH concentrations (nl = 11.9 μU/ml) and pituitary TSH reserve (PTSHR, nl = 1.1 mU·min/ml), determined by integrating the total area under the TSH curve after intravenous thyrotropin-releasing hormone (TRH, 200 μg). In six of eight patients the mean base line TSH was 107 μU/ml (nl = 1.6±0.6), TT_4 = 2.5 μg/100 ml, FT_4 = 0.5 ng/100 ml, TT_3 = 86 ng/100 ml (nl = 200±25), RAI uptake = 14%. After the initial dose of T_3, the serum TT_3 rose to a peak of 500 ng/100 ml at 15 min and leveled at 112 ng/100 ml by 10 days. After 10 days, the serum TSH had decreased to 67 μU/ml. In five of these six patients, TSH-PR decreased from 4254 to 2461 mU/day, and the PTSHR decreased from 45 to 32 (mU·min/ml) with little change in the MCR (from 33 to 34 cc/min). In two of eight patients, the mean base line TSH was 81 μU/ml, TT_4 = 0.5 μg/100 ml, FT_4 = 0.1 ng/100 ml, TT_3 = 43 ng/100 ml, RAI uptake = 5%. After T_3 therapy, the serum TT_3 rose to a similar peak at 15 min and leveled at 130 ng/100 ml by 10 days. At this time, the basal serum TSH in these two cases had increased to 106 and 110 μU/ml, respectively. In one patient, the peak serum TSH after TRH increased from 270 to 315 μU/ml, the PTSHR increased from 38 to 41 (mU·min/ml), and the PR from 3046 to 4277 mU/day. In the other patient, the peak serum TSH after TRH increased from 180 to 590 μU/ml, the PTSHR increased from 20 to 66 (mU·min/ml), and the estimated PR increased from 2220 to 3630 mU/day with minimal changes in the MCR. In summary, intravenous T_3 has been shown for the first time to exert a positive central effect on TSH secretion under appropriate metabolic conditions. (Research supported by USPH Research, Metabolic Research Grant, and NIH Training Grant.)

251. The Interaction of Hemoglobin E and β-Thalassemia. RONALD F. RIEDER AND ROBERT FELDMAN,* Brooklyn, N. Y.

Hemoglobin E is found with high frequency in Southeast Asia and often occurs in association with a β^0-thalassemia gene. The interaction of the two genes produces a moderately severe thalassemia syndrome with complete absence of HbA. The discovery of a 5-yr-old girl with HbE-β-thalassemia, who had an Iranian father (β-thalassemia trait) and a Burmese mother

(HbE trait), provided the opportunity of studying the effects on hemoglobin production of the interaction of HbE with β-thalassemia derived from a gene pool from Western Asia. Hemoglobin synthesis was studied in vitro, in blood and marrow of the proposita, and in blood of family members. In the subjects with HbE trait, the ratio in blood of the quantity of HbA to HbE was 3:1. The β^A/β^E synthetic ratio in reticulocytes was 1.5 and the specific activity of β^E was greater than β^A, suggesting instability of HbE with preferential destruction of abnormal hemoglobin. The blood of the proposita exhibited only HbF and HbE; reticulocytes and marrow showed no β^A synthesis. Therefore, this Iranian β-thalassemia gene is also of the β^0 type. The β^E/α synthetic ratio (0.6) in blood of the proposita was similar to the β^A/α ratio in mildly affected relatives with thalassemia trait. The marrow β^E/α synthetic ratio was estimated to be 0.94. These results suggest that the severity of hemoglobin E-β-thalassemia is attributable to instability and defective synthesis of HbE in association with absent β^A synthesis due to a β^0-thalassemia gene. In addition, the data provide further evidence that in some examples of β-thalassemia there may be greater balance of α- and β-globin synthesis in marrow than in blood. (Research supported by grants from NIH and Life Insurance Medical Research Fund.)

252. Scintiphotographic Evaluation of Left Ventricular Function in Patients with Acute Myocardial Infarction. PIERRE RIGO,* H. WILLIAM STRAUSS,* DEAN R. TAYLOR,* DAVID T. KELLY,* MYRON L. WEISFELDT,* AND BERTRAM PITT,* Baltimore, Md. (introduced by Richard S. Ross**).

24 patients with acute myocardial infarction (AMI) were studied noninvasively by the gated scintiphotographic technique within 48 h of the onset of symptoms. Biplane scintiphotographic images in the right and left anterior oblique projections allowed calculation of left ventricular end diastolic volume (EDV), end systolic volume, ejection fraction (EF), and extent of asynergy (expressed as a percentage of the end diastolic circumference of the left ventricular free wall). These studies were correlated with the patient's clinical class: class I—uncomplicated AMI; class II, mild to moderate congestive failure; class III, pulmonary edema; class IV, cardiogenic shock; and the patients' hemodynamic evaluation. 22 patients had akinesis ranging from 11 to 59% and two hypokinesis. Mean EDV was 132 ml/m² (range 60–214) and mean EF 37% (range 19–53). EF in class I (42.6%) was significantly greater than in classes II (33%), III (31%), and IV 19% ($P < 0.001$). There was an inverse correlation between extent of asynergy and EF ($r = 0.87$, $P < 0.005$). Correlation between EDV and pulmonary capillary wedge pressure (PCW) was, however, relatively poor ($r = 0.56$, $P < 0.05$), suggesting a change in left ventricular compliance. These noninvasive studies demonstrate depressed function and regional abnormalities in left ventricular contraction in patients with AMI. Caution should be used in the analysis of left ventricular function using PCW in patients with AMI because of possible changes in compliance. (Supported by PH 43NHLI 67-1444 from NIH.)

252a. Phospholipid Requirement of Jejunal Lipid-Reesterifying Enzymes. JOHN RODGERS* AND RICHARD O'BRIEN,* Albany, N. Y. (introduced by John Balint**).

Previously, we reported that lipid-reesterifying enzyme activities were decreased in jejuna of bile fistula (BF) rats. This was associated with reduced esterification of absorbed fatty acid in vitro. As bile is rich in phospholipid (PL), it was decided to determine whether enzyme abnormalities in BF rats correlated with changes of PL content of jejunal microsomes. Four groups were studied: controls (CD) and BF (BFD) infused intraduodenally with liquid diet for 48 h and controls (CS) and BF (BFS) infused for a similar period with saline. Specific activity of monoglyceride-acyltransferase (MAT), total protein (P), total PL, and total lipid (L) were then determined for jejunal microsomes. Data are presented as ratios of microsomal lipid to protein content and nmoles product formed per milligram P per min. L/P ratios were similar in all groups. PL/P ratios were significantly reduced to 0.139 and 0.095 for BFD and BFS, compared to 0.197 and 0.199 for CD and CS respectively, ($P < 0.01$) and were associated with significantly ($P < 0.005$) lower MAT activities: BFD = 87, BFS = 81, CD = 140, CS = 142. This association suggests that PL is necessary for normal activity of MAT perhaps by altering the binding properties of microsomal membranes. Bile diversion, by removing bile PL or by reducing absorption of dietary lipid, impairs synthesis of microsomal PL, thus producing impaired enzyme activity. CD controls had greater P in response to diet compared to CS. PL was increased proportionally so activity of MAT and PL/P ratios were nearly identical. This again emphasizes the importance of PL to P ratio for normal microsomal enzyme activity. (Research support from grants NIH AM 11979 and AM 15281.)

253. Deuterium-Labeled Folic Acid: Synthesis and Application to Studies in Man. I. H. ROSENBERG, D. HACHEY,* AND P. D. KLEIN,* Chicago, Ill.

Quantitative studies of folate absorption and metabolism have been limited in man and avoided in infants and pregnant women by the dose of radioactive folate, which can be safely administered. Stable isotopes offer an alternate tool for tracing intestinal absorption and metabolic pathways, and for determination of body pool sizes without the hazards of administration or radioactive compounds. We have prepared deuterium-labeled folate (D-PteGlu) as a nonradioactive tracer for study of folate absorption and metabolism. D-PteGlu labeled in the 3′–5′ position of the *para*-aminobenzoyl moiety (PABA) was prepared by reductive dehalogenation with deuterium gas of 3′–5′ dibromofolate prepared from commercial PteGlu. The product was purified by column chromatography and migrated chromatographically as tetrahydrofolate. D-PteGlu was administered orally to human subjects and the absorbed D-PteGlu was flushed into the urine with an intramuscular dose of unlabeled PteGlu. The folate was concentrated from the urine using charcoal. Since folate is difficult to derivatize for gas chromatography, the deuterium-labeled PABA was isolated for isotopic analysis. PABA and glutamic acid (Glu) were released by oxidative cleavage and alkaline hydrolysis. Both were recovered as *N*-trifluoracetyl methyl (*N*-TFA-CH_3) esters and quantified by gas chromatography-mass spectroscopy-accelerating voltage alternation. The *N*-TFA-CH_3-PABA gives a spectrum with a prominent peak at mass to charge ratio (m/e) 216, while the deuterated product was displaced to m/e 218. A dilution curve of deuterated to unlabeled PABA was constructed and a dilution of 1:1000 was readily detected. This sensitivity and discrimination was easily adequate for studies of folate absorption where isotope ratios were 1:100 or greater. Isolation of Glu allowed internal standardization. Deuterium-labeled folates are versatile new probes for studies of folate absorption, disposition, and metabolism. (Research supported by NIH grant AM 15351 and the U. S. Atomic Energy Commission.)

254. Transcobalamin II-Facilitated Uptake of Vitamin B_{12} by Cultured Fibroblasts: Studies in Methylmalonicaciduria. LEON E. ROSENBERG, ANNE LILLJEQVIST,* AND ROBERT H. ALLEN,* New Haven, Conn., and St. Louis, Mo.

We showed previously that intact, cultured fibroblasts from a child with inherited methylmalonicaciduria are unable to convert extracellular vitamin B_{12} to its methyl- and deoxya-

denosylcobalamin coenzymes, whereas broken fibroblast extracts synthesize coenzymes normally. We now report studies of uptake of [^{57}Co] vitamin B_{12} (B_{12}) by confluent fibroblast monolayers incubated in serum-free medium for intervals of 15 min to 8 h. In normal cells, the uptake of B_{12} bound to homogeneous transcobalamin II (TC II), purified 2 million-fold from human serum, was equal to that for B_{12} bound to TC II in unfractionated human serum. TC II-B_{12} uptake was most rapid during the first 30 min, increased throughout the 8 h interval, and proceeded at a rate 4–8 times greater than that observed for uptake of free B_{12} or B_{12} bound to a homogeneous granulocyte B_{12}-binding protein. Uptake of free and TC II-B_{12} was saturable. The capacity for TC II-B_{12} far exceeded that for free B_{12} (V_{max}: 100 pg/mg protein per h for TC II-B_{12}; 10 pg/mg per h for free B_{12}), but the apparent affinity for the free and bound forms was the same (K_m: 1000 pg/ml). Cells from the child with methylmalonicaciduria took up free and TC II-B_{12} normally during the first 2 h, but then uptake plateaued. After preloading with TC II-B_{12} for 15 min, these cells released B_{12} normally; when preloaded for 6 h, their rate of B_{12} release was 30% faster than normal. We conclude (*a*) that TC II facilitates B_{12} uptake by cultured human fibroblasts; (*b*) that no other serum protein is required for this facilitation; and (*c*) that the site of the primary defect in this child with methylmalonicaciduria lies between the initial binding of TC II-B_{12} to the cell membrane and the intracellular conversion of the vitamin to its coenzyme forms.

255. Altered In Vitro Granulopoiesis in the Presence of Leukemic and Normal Marrow Cells. ALAN L. ROSENBLUM,* JOAN M. BULL,* AND PAUL P. CARBONE, Bethesda, Md.

Patients with leukemia and preleukemia manifest deficient hematopoiesis in vivo and depressed granulopoiesis in vitro although the bone marrow may show only minimal infiltration with abnormal cells. Colony formation is not linearly related to the proportion of abnormal to normal cells in the marrow of these patients, and normal colony formation in vitro occurs regularly only when the proportion of blasts is under 10%. Using the in vitro assay for granulocyte colony formation (CFU-C), we examined the response of normal marrow to the addition of small numbers of leukemic cells and unrelated normal marrow cells. Human marrow was cultured in semi-solid methylcellulose, each plate containing $1.0–2.0 \times 10^5$ normal test (recipient) cells to which 1.0×10^3 to 1.0×10^4 unrelated (donor) cells were mixed before plating. Results were expressed as a percentage of control plates. Leukemic cells in concentrations of 0.5–5.0% uniformly inhibited growth of normal marrow cells to 48% of control levels (range 32–63%, 10 values from three patients). Addition of normal marrow cells to a normal recipient marrow produced marked stimulation to 255% of control (range 210–290%, 10 values from six normals) if the donor/recipient pair was HLA identical or resulted in inhibition to 48% of control (range 45–56%, 11 values from five normals) if the donor/recipient pair was mismatched for 3/3 or 4/4 antigens. Mismatches for 2/4 or 3/4 antigens resulted in intermediate responses. These patterns were reproduced using irradiated or freeze-thawed donor cells was well as supernates from the freeze-thawed cells. These results demonstrate that granulopoiesis in vitro can be significantly altered by the addition of small numbers of normal or leukemic marrow cells. The relationship to HLA compatibility suggests one mechanism of inhibition by leukemic cells in vivo may involve recognition and response to neo-antigen.

256. Diminished Membrane Protein Kinase Activity in Erythrocytes from Patients with Myotonic Dystrophy. ALLEN D. ROSES* AND STANLEY H. APPEL, Durham, N. C.

Myotonic muscular dystrophy is a systemic disorder possibly of membrane origin which is inherited as an autosomal dominant trait. Although muscle is one of the target organs, the delineation of the primary biochemical defect is made extremely difficult on minimal biopsy material by the presence of atrophy, fibrous tissue, and changes of denervation which may result in multiple secondary biochemical changes. If the erythrocyte membrane is found to express the same biochemical genetic traits, the difficulties with interpretation of muscle tissue are obviated. A significant difference was demonstrated in the phosphorylation of membrane proteins. RBC ghosts were frozen at − 20° for 1 wk and examined under initial rate conditions for transfer of P^{32} from [γ-^{32}P]ATP to membrane proteins by SDS-gel electrophoresis. There were 17.5 pmoles P^{32}/min per mg ghost protein incorporated in eight normal age- and sex-matched control patients, but only 8.73 pmoles incorporated in seven myotonic patients from three different families ($P < 0.001$). Each individual membrane component which was phosphorylated reflected this difference. However, there was no difference in the cyclic AMP stimulation of normal and myotonic erythrocyte protein phosphorylation. Furthermore, using purified F_2C histone as a substrate with membrane protein kinase, there was no difference in ^{32}P transfer between normal and myotonic membranes. No differences were observed in membrane ATPases, protein and lipid-bound carbohydrates assessed by GLC, phospholipids and gangliosides assessed by TLC, or membrane proteins assessed by SDS-gel electrophoresis. Studies are presently in progress to characterize this membrane-associated enzyme further and to determine its significance in myotonic dystrophy. (Research supported by grant NS07872 from NIH; and the National Multiple Sclerosis Society.)

257. The Effect of Prednisone in Paroxysmal Nocturnal Hemoglobinuria. WENDELL F. ROSSE AND J. R. MARION,* Durham, N. C.

Prednisone markedly reduced hemolysis in patients with paroxysmal nocturnal hemoglobinuria (PNH). When it was given in high doses (30–60 mg/day) to six patients, the plasma and urine hemoglobin and the endogenous carbon monoxide production fell abruptly. In one patient, hemoglobinuria was decreased from 22 g/day to > 0.05 g/day in 2 days. With amelioration of the hemolytic episode, the hematocrit and the proportion of complement-sensitive cells all rose abruptly. The effect is not due to adrenal suppression since the same effect can be obtained with ACTH. In patients with nightly hemolytic episodes, prednisone given in the evening abolished the nocturnal hemoglobinuria. Prednisone was administered chronically (6–30 months) in doses of 15–30 mg every other day to six patients. No response to 30 mg/day was seen in one patient. An excellent response was obtained in five patients, all of whom maintained a normal hematocrit without transfusion. The proportion of markedly abnormal complement-sensitive cells (population 3) rose in each case (45–74% before, 55–94% after). Lysis of moderately abnormal (population 2) cells was also decreased in two patients. No side effects of prednisone were observed. The mechanism of decreased lysis was investigated in vitro. No change in the complement sensitivity of the cells or in their lysis in acidified normal serum was seen when prednisone was administered in vivo or added in vitro. Serum from PNH patients or normal donors did not change in complement content or in ability to induce lysis of PNH cells when acidified, reduced in ionic strength, or treated with cobra venom or inulin. Prednisone may decrease in vivo lysis in PNH by altering the activation of the alternate pathway of complement activation in an unknown way. (Supported by a grant from NCI.)

258. The Ph¹ Chromosome: Evidence for a Specific Chromosomal Translocation. JANET D. ROWLEY,* Chicago, Ill. (introduced by Leif Sorensen).

Chromosomes from five consecutive Ph¹-positive patients with chronic myelogenous leukemia were examined with quinacrine fluorescence and the new Giemsa techniques. Except for the Ph¹ chromosome, chromosomes from three patients appeared to be normal when conventional stains were used. A change in the karyotype occurred in one of these patients (case 3) in blast crisis. Two other patients were first seen in blast crisis and had chromosomal abnormalities in addition to the Ph¹ chromosome. *Cells of all five patients* showed a consistent abnormality in addition to the Ph¹ chromosome. With quinacrine fluorescence, the abnormality appeared as additional dull band on the terminal portion of the long arm of chromosome 9 (9q+). Giemsa-stained preparations of the same cells showed an additional faint band at the end of the long arm of the same chromosome 9. The appearance of this material is similar to that of the long arm of 22, suggesting that it may represent a translocation of the portion of 22 missing from the Ph¹. Furthermore, the amount of material deleted from 22 is approximately equal to the additional material on 9q+. Other findings, in addition to the 9q+, noted in blast crisis were (*a*) the second Ph¹ observed in case 3 was *not* identical to the single Ph¹ seen before blast crisis; (*b*) additional C group chromosomes were present in three patients and, in two patients, appeared to resemble chromosome 8; and (*c*) a metacentric marker chromosome, noted in the same three patients, contained at least one arm that resembled the long arm of 17. These findings illustrate the value of analyzing human cytogenetic abnormalities with the newer techniques of chromosomal identification.

259. A Hemoglobin S-Specific Radioimmunoassay for the Quantitation of Sickle Hemoglobin in Fetal Blood. PETER T. ROWLEY,* RICHARD A. DOHERTY,* CHERYL ROSECRANS,* AND ELSA CERNICHIARI,* Rochester, N. Y. (introduced by Ralph F. Jacox**).

The quantitation of hemoglobin S in fetal blood requires an analytical method which is specific and highly sensitive. A radioimmunoassay has been developed to take advantage of the specificity characteristic of immunological reactions and the sensitivity possible with isotopic measurement. Hemoglobin S was purified by column chromatography and injected with complete Freund's adjuvant into goats. Each goat serum was tested for reactivity against hemoglobins A and S by immunodiffusion and by quantitative precipitation. Hemoglobin A reactivity was removed by cross-reaction. One serum so treated was specific for hemoglobin S and did not react with hemoglobins A or F. Hemoglobin S was labeled with ^{125}I by the chloramine-T method. In the radioimmunoassay, complete precipitation of the antigen-antibody complex was insured by the addition of rabbit anti-goat gamma globulin. This assay offers reliable and specific quantitation of as little as 1 ng of hemoglobin S. Assuming that 10% of the hemoglobin in fetal blood at 14 wk gestation is of adult type, this assay is capable of detecting the amount of hemoglobin S in 10^{-7} ml of homozygous hemoglobin S blood. The prenatal diagnosis of sickle-cell anemia will require in addition a method for demonstrating the absence of hemoglobin A and a method for obtaining fetal blood safely. (Research supported by contract from NIH-NIHLI-71-2402B.)

260. A Therapeutic Role for Dichloromethylene Diphosphonate (Cl_2MDP) and 25-Hydroxycholecalciferol (25HCC) in Renal Osteodystrophy. JEAN E. RUSSELL* AND LOUIS V. AVIOLI, St. Louis, Mo.

It has been well established that chronic renal disease is attended by alterations in 25HCC metabolism as well as an increased bone resorption and defects in the maturation of skeletal mineral and osteoid, each of which is resistant to vitamin D therapy. Diphosphonates (Cl_2-MDP) have also been shown to decrease bone resorption and stabilize its crystalline integrity. Rats with experimentally induced renal failure of 4 wk duration were treated with either Cl_2MDP alone in doses of 2 mg/kg per day (DP), 25HCC at 500 IU/day (25HCC), or both compounds (DP-25HCC) for a 2 wk period. When compared to pair-fed nontreated uremic controls, plasma and tissue calcium and phosphate in all treated groups decreased with the greatest fall noted in the DP-25HCC group. Analyses of bone mineral and collagen maturation, using density gradient bromoform toluene techniques, demonstrated a reversal of the immature crystalline and collagen moieties toward more mature forms. Changes were pronounced in the DP group and greatest in the DP-25HCC group. The results strongly suggest that Cl_2MDP and 25HCC either alone or in combination may prove to be therapeutic for the osteodystrophy and soft tissue calcification which attends chronic renal insufficiency. (Research supported by NIH contract 70-2219.)

261. Synergistic Cytotoxicity of Bacterial Proteins Produced by Different Species. L. D. SABATH, S. J. WALLACE,* V. LORIAN,* AND C. WILCOX,* Boston, Mass., and Bronx, N. Y.

The concept of two or more species of bacteria interacting to cause disease has been suggested, but details for a mechanism of such synergistic pathogenicity have been lacking. A diffusible product from *Staphylococcus aureus*, the "β-hemolysin" that by itself does not effect human red cells but does cause "incomplete lysis" of sheep erythrocytes, may cause synergistic hemolysis of human and sheep erythrocytes if it acts with diffusible substances from certain strains of *Staphylococcus epidermidis*, *Diplococcus pneumoniae*, *Streptococcus agalactica*, *Herellea* sp., and diptheroids. The clear hemolysis produced on erythrocytes in agar is a result of lysis if the membrane and diffusion away of unchanged hemoglobin. The material produced by *S. epidermidis* has mol wt 280,000, and it is neither the epsilon hemolysin nor a lipase (E.C. 3.1.1.3)—substances previously suggested; dialysis against tap water of pH 7 phosphate buffer causes loss of hemolytic activity that can be restored by adding cysteine. Sheep erythrocytes treated sequentially with the *S. aureus* protein and the *S. epidermidis* protein are more readily lysed than when exposed in the reverse sequence. These results demonstrate that identifiable proteins from different bacteria may act together to damage specific cells in a way not possible with either alone. The possibility should be investigated that apparent variations in host susceptibility or "changes" in pathogenicity of some organisms might be due to the simultaneous presence or absence of apparent "nonpathogens" which are the source of factors necessary for the cytotoxicity usually attributed to the "pathogens." (Research supported by grants from NIH.)

262. Immunological Cross-Reactions among Enterotoxins of Human *Escherichia coli*. R. BRADLEY SACK,* Baltimore, Md. (introduced by Douglas Carroll**).

Within the last few years enterotoxigenic *E. coli* have been recognized as distinct etiological agents in human diarrheal disease. Previous studies from this laboratory have indicated considerable immunological cross reactions between the enterotoxins of *E. coli* and *V. cholerae*. These studies now have been expanded to include additional serotypes of enterotoxigenic *E. coli* from India and one strain isolated from a patient with diarrhea in the United States. Heat-labile (100°C, 15

min) enterotoxins of six isolates of *E. coli* have been examined for immunological similarities. A single lot of antiserum was prepared in rabbits by hyperimmunization with an enterotoxin preparation of *E. coli* 078:H12 (strain 408-3). Enterotoxin preparations consisted of dialyzed, lyophilized culture filtrates of organisms grown in stationary shallow syncase cultures for 48 h. Twofold dilutions of antisera were incubated with 3 ED_{50} of enterotoxin for 1 h at 37°C, and the residual enterotoxin activity assayed in 18-h rabbit ileal loops. 1 U of antitoxin was defined as the reciprocal of the amount of serum required to neutralize 1 ED_{50} of enterotoxin. Preimmune rabbit sera had titers of < 10 U/ml for each of the enterotoxins studied. Enterotoxins from five different serotypes of *E. coli* from India (078:H11, 078:H12, 085:H7, 0126:H12, 015:H11) were neutralized by the single hyperimmune sera at titers of 450–2500 U/mil. Enterotoxin from the *E. coli* from Whiteriver, Ariz. (serotype not yet determined; not of "classical" enteropathogenic serotype), was neutralized at a titer of 1505 U/ml. These studies suggest that human *E. coli* of different serotypes and geographical locations produce enterotoxins which are immunologically similar, thus suggesting the feasibility of developing immunological techniques to identify both the organisms and specific antitoxin responses in patients. (Supported by NIH grant 7 R22 AI11358-01 and NIH contract 71-2260.)

263. Quantitative Health Measurements in Randomized Trial of Nurse-Practitioners. DAVID L. SACKETT,* WALTER O. SPITZER,* ROBIN S. ROBERTS,* MICHAEL GENT,* JOHN C. SIBLEY,* AND ANTHONY OLYNICH,* Hamilton, Ontario, Canada (introduced by Alvan R. Feinstein**).

We have now completed final quantitative analysis of end results for patients in a randomized clinical trial of the efficacy and safety of nurse-practitioners in providing primary medical care. In two nonuniversity family medical practices, 1500 families were randomly allocated to either a control group, where they continued to receive care from family physicians, or to an experimental group in which their primary medical care was provided by nurse-practitioners who had completed a special training program in clinical judgment and in the evaluation and treatment of common disorders. The experimental and control groups were initially identical with respect to three measures of physical health and satisfaction with previous medical care. During the 1 yr experimental period, the drop-out rates among patients randomized to the family physicians and to the nurse-practitioners were 1.1% and 1.7%, respectively, and the mortality rates in the two groups were 0.8% and 0.3%. In measurements performed at the end of the trial in the control vs. experimental groups, the following respective values were obtained: for unimpaired physical function, 88% vs. 86%; for ability to execute usual daily activities, 90% vs. 90%; and for freedom from bed disability in the prior 14 days, 87% vs. 86%. For 12 measures of positive social function, the average percentages of maximum possible function were 76% vs. 75%, and for nine measures of positive emotional function, 74% vs. 73%. These results, together with concomitant studies of the quality of clinical care delivered, indicate that a nurse-practitioner can be both efficacious and safe as a provider of primary medical care. (Supported by research grants from the Province of Ontario and the Government of Canada.)

264. Fletcher Trait: Defects in Coagulation, Fibrinolysis, Permeability Enhancement, and Kinin Generation. HIDEHIKO SAITO,* VIRGINIA H. DONALDSON,** AND OSCAR D. RATNOFF,** Cleveland and Cincinnati, Ohio.

Wuepper reported that the defective assays for coagulation, permeability enhancement (PF/Dil), and kinin generation in Fletcher trait plasma were corrected by a plasma prekallikrein. All of these functions are dependent upon activation of Hageman factor (HF), but Fletcher trait plasma has normal amounts of HF by immunologic and functional tests. Defective clotting, fibrinolysis, and PF/Dil generation in Fletcher trait were corrected by small amounts of normal, HF-deficient, or PTA-deficient plasmas, and were partially corrected by partially purified HF, activated by kaolin or ellagic acid. These agents were less effective in correcting defective esterolysis and did not correct defective kinin generation. HF fragments (mol wt approximately 30,000D), prepared by digesting partially purified HF with insoluble trypsin, induced kinin formation in normal, HF-deficient, or PTA-deficient plasmas, but not in Fletcher trait or 61°C-heated normal plasma. Partially purified plasma kallikrein repaired the clotting and fibrinolytic defects in Fletcher trait plasma. Normal esterolytic and kinin-generating activities were eluted from Celite with which Fletcher trait plasma had been adsorbed. All these experiments suggest that Fletcher trait plasma lacks the capacity to activate HF at a normal rate and there is the apparent deficiency of a plasma prekallikrein, but no single hypothesis can yet explain these data. Although Fletcher trait plasma appears deficient in a prekallikrein, a plasma prekallikrein may be present in a nonfunctional form, not activable by HF but activated by exposure to Celite. (This study was supported in part by grants HL 11933 and HL 01661 from the USPHS and by grants from the AHA.)

265. Thyroid Hormone Action: Demonstration of Nuclear Receptors and Transcriptional Control in Cell Culture. HERBERT H. SAMUELS* AND JIR TSAI,* New York (introduced by Saul J. Farber**).

We have previously demonstrated that triiodothyronine (T_3) and thyroxine (T_4) induce a 3-fold increase in the rate of growth of GH_1 cells in culture. Within 3 h of incubation of cells with T_3, nuclear template activity, as assayed with *E. coli.* RNA polymerase, increases 2-fold. To study further the action of T_3 and T_4, we examined the binding of purified [^{125}I] T_4 and [^{125}I] T_3 to cellular fractions after 2 h of incubation with intact cells in serum-free media. High-affinity, low-capacity, binding sites for T_3 and T_4 were demonstrated in nuclear but not in mitochondrial or cytosol fractions. Chromatographic analysis of the bound nuclear radioactivity from cells incubated with [^{125}I] T_4 demonstrated 97% T_4, 2% iodide, and 1% T_3. Dissociation constants determined by Scatchard analysis were 2.9×10^{-11} M for T_3 and 2.6×10^{-10} M for T_4. The maximal binding capacity was identical for T_3 and T_4 with approximately 5000 molecules bound per cell nucleus. [^{125}I] T_4 binding was competitively inhibited by T_3. These data suggest that T_3 and T_4 interact with identical nuclear receptors, and that conversion of T_4 to T_3 may not be a prerequisite for biologic activity. Similar high-affinity, low-capacity, nuclear binding sites were also demonstrated by incubation of [^{125}I] T_3 or [^{125}I] T_4 directly with isolated nuclei. Incubation of cells with increasing concentrations of nonradioactive T_3 resulted in a subsequent increase in binding when [^{125}I] T_3 was then incubated directly with isolated nuclei. This suggests that the number of nuclear receptors are not fixed, but increase after exposure of intact cells with hormone. This increase in nuclear receptor content may result from the transfer of an unstable cytosol receptor to the nucleus. (Research supported by grants 1 K04 AM 46546-01 from NIH and P-595 from ACS.)

266. Antibiotic Therapy in Experimental Staphylococcal Endocarditis. MERLE A. SANDE* AND MARK L. JOHNSON,* Charlottesville, Va. (introduced by Edward W. Hook**).

This study was undertaken to investigate factors influencing killing of staphylococci by antibiotics in experimental endocarditis. Rate of bactericidal activity was determined for each antibiotic by standard techniques in broth using a strain of *Staphylococcus aureus* (SA). Endocarditis was produced in 87 rabbits by intravenous injection of 10^6 SA after 5 days of aortic valve trauma (polyethylene catheter transarterially into left ventricle). Within 24–36 h after challenge, cardiac vegetations containing 10^{9-11} SA/g were present and animals had fever and constant bacteremia (10^{3-4} SA/ml). Antibiotics were started 24 h after infection in doses that achieved sustained serum bactericidal levels of $> 1:8$. Bacteria in vegetations were enumerated after therapy for 1–3 days (period I), 4–6 days (II), and 7–12 days (III). Penicillin eradicated SA from vegetations in 1 wk (period I, geometric mean [Log_{10}] 8.38 viable units SA/g vegetation; II, 2.86; III, 0). The combination of gentamicin and penicillin was synergistic in vitro and eradicated bacteria from vegetations more rapidly (I, 2.56; II, 0; III, 0) than penicillin alone. Vancomycin was also rapidly bactericidal in broth and sterilized vegetations more rapidly (I, 2.53; II, 0; III, 0) than penicillin. Rifampin was less effective than penicillin or vancomycin in vitro and in vivo (I, 7.61; II, 3.96; III, 4.32). SA highly resistant to rifampin emerged in 3 of 14 rabbits treated with rifampin. Rifampin antagonized the bactericidal action of penicillin in vitro and in vivo (I, 5.36; II, 3.15; III, 2.21). Clindamycin had a slow rate of bactericidal activity in broth and failed to consistently eliminate SA from vegetations within 12 days (I, 8.27; II, 6.17; III, 2.32). The capacity of an antibiotic or combination of antibiotics to kill SA in broth seems to correlate well with ability to eradicate SA from cardiac vegetations in experimental endocarditis.

267. Neutral Protease and Histonase Activity in Human Articular Cartilage. ASHER I. SAPOLSKY,* J. FREDERICK WOESSNER,* AND DAVID S. HOWELL,** Miami, Fla.

Recently we reported increased activity of cathepsin D in osteoarthritic (compared to control normal) cartilage with cathepsin D, the predominant proteoglycan digesting protease. The current report is, to the authors' knowledge, the first to show neutral protease activity in human cartilage not due to cathepsin D or B. In addition the activity of the cathepsin D on proteoglycan was limited to the acid pH range. Evidence for these statements is as follows. Human cartilage enzyme extracts prepared from postmortem patellae, as well as purified cathepsin D from bovine uterus (D_1), did not digest hemoglobin but degraded proteoglycan subunit (PGS) considerably at neutral pH. Pepstatin and 1-2-epoxy-3-phenoxypropane, new powerful inhibitors of cathepsin D, scarcely affected this degradation at neutral pH but inhibited almost all PGS digestion at pH 5 and all hemoglobin digestion at pH 3.1 Moreover, highly purified bovine cathepsin D (D_2) did not degrade PGS at pH 7. The human extracts and D_1 also degraded histone and casein at neutral pH, but D_2 did not. Pepstatin did not inhibit the neutral histonase and caseinase activity but completely inhibited their action at pH 5. This neutral activity was inhibited by human serum and chloroquine (20 mM). However, it was neither activated by cysteine (10 mM) nor inhibited by diisopropylfluorophosphate (10 mM), ϵ-aminocaproic acid (50 mM), or trypsin inhibitor (250 μg/ml); these findings differentiate it from cathepsin B and neutral proteases derived from leukocytes. Thus, the current human articular cartilage contained unique neutral protease(s) capable of degrading the cartilage matrix proteoglycans at neutral pH and also histonase activity which might play a role in the chondrocytic proliferation believed to occur in advancing osteoarthritis. (This work was supported by grants AM-08662, AM-05038, and HE-11035 from the NIH and by USVA, Part I Research Program.)

268. Isotonic Volume Reabsorption and Transepithelial Potentials in Proximal Straight Tubules. JAMES A. SCHAFER* AND THOMAS E. ANDREOLI, Birmingham, Ala.

These experiments were designed to evaluate factors affecting isotonic volume reabsorption (J_v, nl min^{-1} mm^{-1}) and transepithelial electrical potential difference (V_e, mV) in proximal straight tubules isolated from rabbit kidney. The perfusing solution contained: 105 mM NaCl, 25 mM $NaHCO_3$, 10 mM Na acetate, 5 mM KCl, 4 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 1.0 mM $MgSO_4$, 8.3 mM glucose, and 5 mM alanine; the bath contained rabbit serum bubbled with 5% CO_2–95% O_2. At 37°C, J_v was 0.54±0.17 (SD; 27 tubules) and V_e (lumen relative to bath) was − 1.5±0.8 (31). Both V_e and J_v were independent of perfusion rate (2.5–50 nl min^{-1}) and perfusion pressure (5–30 cm H_2O). At 22°C, J_v fell 0.46±0.17 (6) to 0.04, and V_m rose + 1.7±0.6 (5) to + 0.1. Similarly, ouabain (10^{-4} M) reduced J_v by 0.52±0.08 (4) to ~0.02, and V_m rose + 1.7±1.0 (5) to + 0.1. These results agree qualitatively with those of Burg and Orloff (1968. *J. Clin. Invest.* **47**: 2016; 1970. *Am. J. Physiol.* **219**: 1714) for proximal convoluted tubules. Significantly, when the perfusion solution was altered by replacing $NaHCO_3$ and Na acetate with 17 mM Na_2SO_4 and glucose and alanine with 15 mM urea, J_v was reduced 30% and V_e fell to − 2.6±1.0 (5). We assume that these relatively small changes in V_e and J_v were referable to the poorly reabsorbed sulfate counterion. Taken together, these data support the view that isotonic volume reabsorption in the proximal straight tubule depends primarily on active transport of Na^+ from lumen to peritubular medium. (Supported by AHA Established Investigator Award, NIH RCDA, and research grants from the NIH, NSF, and AHA.)

269. Hyporeninemic Hypoaldosteronism. MORRIS SCHAMBELAN,* NORMA BRUST,* AND EDWARD G. BIGLIERI,** San Francisco, Calif.

Previous reports from this laboratory suggested that isolated hypoaldosteronism in adults may be secondary to renin deficiency. Additional experience indicates that this syndrome is common (20 patients recognized) and lends further support to a primary hyporeninemic mechanism. Detailed studies have been completed in 13 patients (age 41–81 yr, mean 64). All had hyperkalemia (5.9–6.9 mEq/liter) that was corrected by mineralocorticoid replacement. Creatinine clearance ranged from 18 to 72 ml/min, (mean 42). Plasma renin activity (PRA) (angiotensin I generation) and plasma aldosterone (PA) were determined by radioimmunoassay and urinary steroids, by double-isotope dilution techniques. Generalized adrenal insufficiency was excluded by demonstration of normal excretion of tetrahydrodeoxycorticosterone, tetrahydrocorticosterone, and 17-hydroxycorticoids, which increased normally during 3 days of ACTH. Urinary aldosterone was subnormal (3.0±0.7 (SE) μg/24 h, N 4–17) and failed to increase normally during salt restriction (6.7±1.4, N 20–67) or ACTH (6.5±1.0). Supine PA was subnormal (4.7±0.9 ng/100 ml, N 7.8±1.6) and failed to increase normally with upright posture (4.4±1.0, N 22.0±3.4) or salt restriction (supine 7.2±1.5, N 18.8±5.8; upright 12.1±4.5, N 50.4±8.6) in the six patients so studied. Supine PRA was subnormal (1.7 ±0.3 ng/ml per 3 h, N 5.5±0.9) and failed to increase normally with upright posture (2.5±0.4, N 13.9±3.2), salt restriction (supine 3.9±0.7, N 12.1±1.2; upright 6.3±1.3, N 25.3±4.8) or intravenous furosemide, 20 mg (7.8±2.4). In general, PA correlated with PRA under these study conditions. Four patients with Addison's disease on glucocorticoid replacement had similar degrees of hyperkalemia and hypo-

aldosteronism but markedly elevated PRA (mean supine 82, right 124, salt restriction 118 ng/ml per 3 h). Thus isolated, hypoaldosteronism, in contrast to generalized adrenal insufficiency, is definitely associated with hyporeninemia and is a frequent cause of otherwise unexplained hyperkalemia. (Research supported by grants AM-06415 and HL-11046 from NIH).

270. Parallel Discharge of Secretary Proteins from the Exocrine Pancreas of the Guinea Pig. GEORGE A. SCHEELE* AND ALAN M. TARTAKOFF,* New York (introduced by James G. Hirsch**).

We used an in vitro system of pancreatic lobules to study the discharge of proteins from exocrine cells. Lobules remained morphologically intact for at least 5 h by light and electron microscopy, displayed linear incorporation of [^{3}H] leucine through the period tested (3 h), and showed low levels of resting discharge ($\sim$ 5%/h). Stimulation with optimal doses of carbachol (10^{-6} M), pancreozymin (7×10^{-9} M), and cerulein (10^{-9} M) caused discharge of 60 90% of secretory proteins in 2 h and 5 h, respectively. 75 mM KCl ($\pm$ atropine) caused a 45% discharge of secretory protein within 2 h. Enzyme activity was used to monitor the kinetics of discharge of amylase, lipase, RNase, and the zymogens chymotrypsinogen and procarboxypeptidase B (after activation with trypsin). In addition, SDS-gel electrophoresis allowed analysis of secretory protein independent of enzyme activity. We have recently reported the identification of the majority of gel bands with known secretory enzymes or zymogens. Maximal sensitivity was achieved by prior mass-labeling of the secretory proteins with mixed ^{14}C-labeled amino acids. Resting secretion and secretion induced by each stimulant (carbachol, pancreozymin, cerulein, and 75 mM KCl) collected over 2 h showed identical proportions of secretory protein by enzyme activity measurements and by measurement of radioactivity in gel bands. Furthermore, a detailed analysis of the time course of carbachol-induced secretion, collected sequentially in 15-min periods over 3 h, showed constant relative proportions of secretory protein by both methods studied. The evidence suggests that all secretory proteins in the guinea pig pancreas are discharged in parallel. (Supported in part by USPHS and NIH Special Fellowship AM 49448.)

271. Cooperative Multicenter Evaluation of Intra-Aortic Balloon Pumping in Cardiogenic Shock. S. SCHEIDT,* G. WILNER,* H. MUELLER,* D. SUMMERS,* M. LESCH,* G. WOLFF,* J. KRAKAUER,* M. RUBENFIRE,* P. FLEMING,* G. NOON,* T. KILLIP, AND A. KANTROWITZ,* Detroit, Mich., and New York.

87 patients with rigidly defined cardiogenic shock refractory to standard medical therapy were treated with intra-aortic balloon pumping (IABP) in 10 institutions under a common protocol. Favorable physiologic effects were observed in most patients. After 4 h of IABP, aortic systolic pressure ("afterload") fell from 76$\pm$22 to 57$\pm$17 mm Hg ($P < 0.001$), peak aortic diastolic pressure rose from 53$\pm$12 to 83$\pm$19 mm Hg ($P < 0.001$), heart rate fell from 110$\pm$24 to 103$\pm$21 (NS), cardiac output increased from 2.4 to 2.9 liters/min, urine output increased from 11$\pm$21 to 37$\pm$34 ml/h ($P<0.001$), and myocardial lactate extraction improved in all 19 patients studied. During IABP, oliguria was reversed in 70% of patients, acidemia in 69%, and need for pressor agents in 64%, but abnormal mental status was improved in 44% and pulmonary congestion in only 34%. In spite of favorable clinical and physiologic responses, 52 patients died during IABP. Of the 35 in whom IABP could be discontinued, 15 were discharged from the hospital and 8 survived 1 yr. Neither initial hemodynamic measurements nor responses to IABP were useful in predicting the final outcome. Several patients survived after unsuccessful attempts to terminate IABP, but none survived hospitalization if > 48 h of IABP was required. In a group of 22 patients with shock studied at one participating institution, myocardial damage averaged 50.2% of left ventricular mass and could not be related to the duration of shock or of IABP. It is concluded that refractory cardiogenic shock is associated with massive myocardial necrosis. Although IABP is physiologically effective, few patients (18% in this study) will survive shock, despite IABP. (Supported by The John A. Hartford Foundation.)

272. Cycling Characteristics of Human Peripheral Blood Lymphocytes in Culture. LEWIS SCHIFFER,* ALAN WINKELSTEIN,* ARNOLD MARKOE,* AND JANET NELSON,* Pittsburgh, Pa. (introduced by Jessica Lewis**).

By means of a new in vitro technique of demonstrating DNA polymerase in individual cells, it may now be possible to estimate the fraction of lymphocytes in phytohemagglutinin-stimulated peripheral blood cultures that are in cycle at any given time. The method entails coating unfixed lymphocyte smears with soft agar, with and without DNase-activated, sonicated DNA, and incubation of these slides with all four nucleotide triphosphates—one tritiated—at 37°C in the presence of Mg^{++}. Slides are processed further by fixation, autoradiography, and staining. Insoluble, tritiated DNA is represented by exposed silver grains over the cells containing DNA polymerase. Unlike human cells in vivo, cultured lymphocytes have a short G1 period once induced into proliferation and can thus be expected to maintain measurable amounts of DNA polymerase during 3 days of growth. Our results indicate that DNA polymerase-containing cells are present at least 10 h before there is significant DNA synthesis taking place. Thereafter, the percentage of cells containing DNA polymerase assayed with exogeneous DNA primer increases at 20%/day to reach 90% at 4 days. When the only primer is the cellular DNA, the labeled cells increase at 15%/day, starting from the same point, to reach 50% in 4 days. If one assumes that the latter assay is equivalent to the growth fraction, as we have demonstrated in three animal tumor systems with short G1 phases, the cell cycle time of these lymphocytes can be estimated at 33, 17, 14, and 23 h at 1, 2, 3, and 4 days, respectively. It may also be theorized that as few as 10–20% of the original, cultured lymphocytes may actually enter into proliferation. (Research supported by grants from the NIH.)

273. Mechanisms of Hypotension Induced by Quinidine and Procaine Amide. P. G. SCHMID,* F. M. ABBOUD, A. L. MARK,* AND D. D. HEISTAD,* Iowa City, Iowa.

The antiarrhythmic drugs, quinidine (Q) and procaine amide (PCA), may cause significant hypotension. Effects on neurogenic transmission, adrenergic receptors, and vascular smooth muscle might explain the hypotension, but the specific mechanisms involved are not known. In humans, norepinephrine (NE) (0.075 and 0.15 μg/kg per min intravenously) produced increases in mean arterial pressure averaging 14.5$\pm$2.2 (SE) and 21.8$\pm$2.1 mm Hg before Q (200 mg base intravenously) and 9.8$\pm$1.7 and 15.9$\pm$2.2 mm Hg after Q ($P < 0.05$). Corresponding increases in forearm vascular resistance (plethysmograph) were 5.9$\pm$2.2 and 9.2$\pm$2.9 U before Q and 2.4$\pm$1.4 and 2.5$\pm$2.4 units after Q ($P < 0.05$). In the perfused gracilis muscle and hindpaw of the dogs, Q produced marked, dose-related inhibition of vasoconstrictor responses to postganglionic nerve stimulation (NS) and to intra-arterial NE, but not angiotensin and serotonin. PCA (20 mg/kg intravenously) inhibited reflex vasoconstrictor

responses to bilateral carotid artery occlusion and stimulation of carotid chemoreceptors with nicotine by factors of 25 and 50, respectively; responses to preganglionic NS also were reduced ($P < 0.05$), but only by a factor of 2. Responses to postganglionic NS were not reduced. The results suggest that Q and PCA have negligible direct relaxant effects on vascular smooth muscle since intra-arterial infusions of the drugs did not cause vasodilatation in denervated muscle and paw. Q inhibits stimuli which activate α-adrenergic receptors. PCA inhibits responses to reflex stimuli more than preganglionic NS, suggesting a predominant effect on vasomotor centers or afferent reflex pathways in addition to a ganglionic blocking action.

274. The Mechanism of Renal Excretion of Potassium in Uremia. DONALD A. SCHON,* PATRICIO SILVA,* AND JOHN P. HAYSLETT,* New Haven, Conn. (introduced by L. R. Freedman).

An increase in the fractional excretion of K occurs in uremic subjects fed a normal diet rich in K. Recent studies in this laboratory demonstrated that the augmented excretion of K in normal rats on a high K diet (2.60 mEq/g) was associated with an increase in Na-K-ATPase in both cortex and outer medulla. In order to study the role of Na-K-ATPase in K excretion in uremia, normal and uremic animals (75% renal ablation) were pair fed a normal diet (0.13 mEq/g K) for 14–21 days, when fractional K excretion (FE_K) during control periods and after the acute infusion of 0.5 M KCl was examined. While serum K levels were similar in both groups, FE_K during control periods was 6.71±0.66% (mean±SE) in normal and 21.5±5.79 in uremic rats ($P < 0.05$). 60–90 min after beginning KCl infusion, FE_K rose to 27.94±5.22% in normal and 53.71±5.01 in uremic animals. In contrast, control FE_K in high K rats was 79.74±24.52% and did not increase further with KCl infusion. There was no difference in maximal FE_K in uremic rats compared to high K rats. This increased capacity to excrete K in uremic animals was associated with elevated levels of Na-K-ATPase (cortex/medulla: 12.51±1.2/33.33±0.89 μm P_i/mg protein per hr, normal; 16.81±0.89/40.23±2.32, uremic $P < 0.05$). Lowering control FE_K in uremic rats to values present in normal rats, by decreasing dietary K (0.07 mEq/g), abolished both the increased levels of Na-K-ATPase and the augmented response to KCl loading. These data indicate that Na-K-ATPase plays an important role in the renal mechanism of K excretion in uremia to maintain K balance despite reduced GFR. (Supported by NIH and PHA grants.)

275. Effects of Dietary Carbohydrate Exchange for Fat on Cholesterol Balance in Man. PAUL H. SCHREIBMAN* AND E. H. AHRENS, JR.,** New York.

Carbohydrate (CHO) induction of plasma triglycerides (TG) has been extensively studied; the effects on plasma cholesterol (CH) are less well defined. To determine effects of CHO feeding on sterol metabolism, 10 hyperlipidemic patients were studied for 4–5 months on the metabolic ward. Weights were held constant. High-fat formula feedings furnished 45–70% of calories as polyunsaturated fat (PUF); high-CHO formulas contained 0–20% PUF, with isocaloric substitution of dextrose for PUF. Sterol balance studies were carried out simultaneously with isotope kinetic analyses after intravenous administration of [4-^{14}C] CH. Since all feedings were CH-free, CH absorption was not measured. In 7/10 patients on the high-CHO diet, plasma TG rose (mean 147%) and also plasma CH (45%). Total fecal steroid excretion decreased in four (−39%), was not significantly changed in two, and increased in one. Plasma CH specific activity decay curves flattened markedly in all seven. In the other three patients plasma TG rose (163%), but plasma CH was unaffected in two and fell in one. Sterol excretion increased in all (+39%); plasma decay curves were unchanged. These two kinds of responses were unrelated to differences in type or severity of hyperlipidemia, body weight, age, or sex. Specific activity of plasma CH and fecal neutral steroids remained identical in both groups on both diets. Interpretation: In all patients, TG and CH synthesis increased on high-CHO diets with a concomitant increase in isotopic CH exchange between plasma and slowly-turning-over pools. In one group, the increment in newly synthesized CH was excreted into the intestinal lumen. In the other, CH retention in the plasma compartment was followed, in the new steady state, by feedback inhibition of synthesis. (Research was supported by grants from NIH—NHLI and GCRC.)

276. Interaction of Calcium, Magnesium, and Adenosine Triphosphate (ATP) in Human Erythrocyte Ghost Endocytosis. STANLEY L. SCHRIER, MURIEL SEEGER,* IRENE JUNGA,* AND KLAUS BENSCH,* Stanford, Calif.

Our purpose was to explore the nature of the interaction of calcium (Ca), magnesium (Mg), and adenosine triphosphate (ATP) at the interior of the ghost membrane as it undergoes endocytosis and vacuole formation. Endocytic vacuoles are induced in ghosts by Mg ATP and blocked by 2.5 mM Ca. The proposal that Mg ATP energizes the membrane to form vacuoles by displacing membrane-bound calcium was tested. Fresh ghosts were resealed with 2.5 mM ATP and 1.0 or 2.5 mM Ca labeled with ^{45}Ca. After incubation, ghost ATP was measured and ghost Ca was determined both by isotopic measurements and atomic absorption. In parallel tubes conditions were identical except that vacuoles were induced by addition of 2.5 mM Mg. The results were as follows. (*a*) Ca addition reduced ghost mean corpuscular volume perhaps by activating an actomyosin. Electron microscopy confirmed that the Ca induced ghost contraction. Mg antagonized this Ca effect and produced vacuoles. (*b*) Mg addition reduced the amount of ghost ATP; the ghost preparation containing most Ca had least ATP. (*c*) Mg sharply reduced the amount of calcium resealed with ghosts between 3- and 10-fold. Therefore, it appeared that vacuole formation involved displacement of Ca from the ghost interior by Mg ATP. AT^{32}P was resealed within ghosts and incubated for 10 min in order to discover why less ATP was recoverable in ghosts containing both Ca and Mg. AT^{32}P did not leak from ghosts. Ca alone produced no loss of AT^{32}P, but upon addition of Mg, as well as Ca, there was loss of AT^{32}P and production of $^{32}P_i$. The findings point to the activation of a Ca Mg ATPase which could displace Ca from membranes and which may have a mechanical role in endocytosis. (Work supported by grant R1 AM 13682 from the NIH.)

277. The Antiarrhythmic Effectiveness of Intramuscular Lidocaine; Influence of Different Injection Sites. MORTIMER L. SCHWARTZ,* VIRENDER SETHI,* AND RAVINDER M. NARANG,* New York (introduced by Edward E. Fischel**).

To determine the effectiveness of intramuscularly administered lidocaine on ventricular premature contractions (VPC's), 18 patients with at least 5% VPC's received injections randomly in either the deltoid (nine patients) or vastus externus muscle (nine patients). 10% lidocaine was administered as 4.5 mg/kg. Statistically significant reductions from 20 VPC's/min to 5 VPC's/min occurred within 15 min after the deltoid injections which were associated with concomitant rise in plasma lidocaine levels (3.5 μg/ml) which remained at 1.5 μg/ml or above for 90 min. At this time the frequency of VPC's was still significantly lower than control (8 VPC's/min). After injection into the vastus, no sustained reduction

in VPC's resulted. This correlated with generally lower lidocaine levels (less than 1.5 μg/ml). Lidocaine levels above 1.5 μg/ml were effective in causing a reduction of VPC's. Intramuscular injections of lidocaine into the deltoid caused a prompt increase in blood levels and prompt reduction of VPC's. Obvious differences in results occurred when two different muscle masses were used. If lidocaine cannot be administered by vein, our results suggest that the intramuscular injection of 4.5 mg/kg (deltoid) may be used to produce a significant antiarrhythmic effect. (Research supported by Astra Pharmaceutical Company grant.)

278. Reduction of Infarct Size in Hypertensive Patients with Acute Myocardial Infarction. WILLIAM E. SHELL,* ALI A. EHSANI,* AND BURTON E. SOBEL, La Jolla, Calif.

Prognosis after acute myocardial infarction (AMI) is affected significantly by the extent of myocardial damage. Accordingly, this study was designed to reduce infarct size by decreasing systemic arterial blood pressure (BP) and myocardial oxygen requirements by administration of trimethaphan. 11 patients with AMI, hypertensive on admission (BP > 150/90 mm Hg), were treated after infarct size had been predicted by analysis of early (7 h) serum creatine phosphokinase (CPK) changes with a new nonlinear curve fitting technique. In each patient, infarct size predicted before trimethaphan was compared to the extent of the completed infarct determined from serum CPK changes after reduction of BP. Intraarterial BP, pulmonary artery wedge pressure (PAW), cardiac output (CO) (determined by dye dilution), and ejection fraction (EF) (determined by isotope angiocardiography) were measured serially. In 30 consecutive control patients with AMI, completed and predicted infarct size correlated closely ($r = 0.93$, mean difference < 5%). In treated patients, completed infarct size was consistently less than that predicted (predicted = 84±20 [SE] CPK-g-eq; completed = 61±19, $p < 0.001$; mean difference = 31±5%). Trimethaphan also decreased mean BP (22% average), heart size (4%), and PAW (20%) and increased CO (12%), EF (47%), and circumferential fiber-shortening velocity (40%). Heart rate was not altered appreciably. Mortality within 30 days after infarction in treated patients was < 50% of that in controls with corresponding infarct size. Thus, reduction of blood pressure in patients with AMI and hypertension results not only in improved ventricular performance but also in considerable salvage of myocardium. (This research was supported by NIH MIRU contract PH-43-68NHLI-1332.)

279. The Role of Cellular Adenosine Triphosphate (ATP) in Regulating Erythrocyte Lysophosphatide Concentration, Erythrocyte Shape, and Erythrocyte Membrane Stability. STEPHEN B. SHOHET AND JAMES E. HALEY, San Francisco, Calif.

To evaluate the effect of adenosine triphosphate ATP depletion on cell membrane renewal, erythrocytes were incubated in autologous serum at low hematocrit without added glucose for 24 h. Cell and plasma levels of ATP, lysophosphatidylcholine (LPC), and cell LPC-acylation activity were followed. Red cell shape was recorded by scanning electron microscopy. Cell membrane stability was evaluated by an in vitro system in which the transfer of ^{14}C-labeled erythrocyte membrane lipids to human splenic macrophage monolayers was measured. When cell ATP levels fell below 0.25 μm/cc cells, acylation activity failed and membrane LPC levels rose (0.12 → 0.33 μm/cc cells). Proportional to rising membrane LPC levels, progressive echinocytic transformation of the erythrocytes occurred. These morphologic changes could be duplicated by adding sufficient LPC to fresh, ATP-replete cells suspended in heated serum in order to induce similar membrane LPC levels. However, if cell metabolism was maintained by the addition of glucose, these changes were reversed as membrane LPC levels fell. Echinocytic RBS's with elevated LPC levels showed a nearly threefold increase in membrane instability in comparison to normal cells, as measured by membrane lipid transfer to the macrophage monolayer. This was seen in both incubated and fresh, LPC-treated echinocytes. In the latter, it was independent of ATP content and cell cation content. Both membrane instability and membrane LPC content of echinocytes could be reduced by modification of the membrane ⇄ plasma LPC equilibrium by the addition of defatted albumin to the incubation media. Membrane instability can be induced by increased membrane LPC levels. Such deleterious levels, in turn, can be induced by the failure of cell LPC-acylation activity consequent to ATP depletion. (Supported by NIH grants).

280. Radioimmunoassay for Human β_2-Microglobulin. J. SHUSTER,* P. GOLD, S. O. FREEDMAN, AND M. D. POULIK,* Montreal, Quebec, Canada and Royal Oak, Mich.

β_2-Microglobulin (BMG) is a low molecular weight protein (11,600 daltons) which was first isolated from the urine of humans suffering from renal tubular malfunction. Amino acid sequence analysis has shown that BMG has extensive amino acid sequence homology with the heavy chain of human IgG_1 molecules. A preliminary, semiquantitative study, employing gel precipitin techniques, suggested that serum BMG levels were increased in the plasma cell dyscrazias. In order to fully evaluate this problem, a quantitative assay for BMG was necessary. Therefore, a radioimmunoassay to quantitate serum BMG was developed. BMG was radiolabeled with ^{125}I by the chloramine-T technique. A standard inhibition curve was established using a double antibody procedure. This assay utilizes 100 μl of patients serum, has a low limit of sensitivity of 0.2 ng, and can be completed within 18 h. BMG levels were measured in the serum of 100 individuals (controls and hospital patients) who had normal renal function as indicated by BUN and serum creatinine levels. In normal individuals the serum BMG values ranged fron 0.86 to 1.83 μg/ml. 17 of 21 patients with multiple myeloma or Waldenström's macroglobulinemia had evaluated μ_2M values varying from 2.6 to 12 μg/ml. The sera of two of seven patients with monoclonal gamma globulin peaks associated with disorders other than multiple myeloma had values of 3.75 and 2.76 μg/ml. Several patients with a variety of other tumors also showed elevations in BMG. The significance of BMG elevated in these disorders requires the study of a larger number of patients. (This work is supported by grants from the NCI of Canada and the NIH.)

281. Heterogeneity of 5′-Nucleotidase in Chronic Lymphocytic Leukemia (CLL). ROBERT SILBER, DOROTHEA ZUCKER-FRANKLIN, MARYROSE CONKLYN,* AND JOHN LOPES,* New York.

In view of the importance of the cell membrane in neoplastic processes, the membranes of lymphocytes isolated from blood of normal subjects and patients with chronic lymphocytic leukemia (CLL) were compared. Membrane purification was monitored with electron microscopy and by the assay of two membrane marker enzymes, NADH diaphorase and 5′-nucleotidase. The membrane preparation contained no detectable nuclear or mitochondrial contamination. While the ultrastructure of normal and CLL membrane preparations appeared identical, a marked difference was found in the 5′-nucleotidase level of these preparations. The average activity of this enzyme in lymphocyte plasma membrane from 12 normal subjects was 6 μmoles/h per mg (range 3–12 μmoles/per mg). In membranes from seven patients with CLL, the activity was either not detectable or less than 0.4 μmoles/h per mg, while

the level in the membranes of the other three patients was within normal limits. None of the patients had received therapy; the values were unchanged for any given patient tested on multiple occasions. In contrast, NADH diaphorase activity was not diminished in CLL membranes, indicating that the lack of 5′-nucleotidase was not associated with the loss of this other membrane function. 5′-nucleotidase could not be detected in crude lymphocyte homogenates from the same patients whose lymphocyte membrane activity was lacking, indicating that the absence of the membrane enzyme did not stem from a loss during purification. No evidence for an inhibitor in CLL cells was obtained. The absence of 5′-nucleotidase in 7 out of 10 patients with CLL is the first demonstration of the lack of a plasma membrane enzyme in this disorder. Since the activity is present in the cells of some patients, it appears that lymphocytes from CLL patients are heterogeneous with respect to this biochemical marker. (Research supported by NIH.)

282. A New Role for Na-K-ATPase: Enhancement of Potassium Excretion by the Kidney. PATRICIO SILVA,* DONALD SCHON,* JOHN HAYSLETT,* AND FRANKLIN H. EPSTEIN,** Boston, Mass., and New Haven, Conn.

Adaptation to potassium loads requires an increase in renal potassium excretion, thought to be mediated by distal tubular secretion and influenced by mineralocorticoids. Na-K-ATPase appears to play a major role in this process, since in the course of potassium adaptation, renal enzyme activity increases markedly. Tripling the usual intake of potassium for 7 days increased the specific activity of Na-K-ATPase by 30% in the outer medulla, but not in the cortex, of normal rats. Increasing potassium intake and excretion to 10 times normal induced a further increase in enzyme content of both medulla and cortex. The increase in renal Na-K-ATPase activity was specific in that it was not accompanied by changes in Mg-ATPase or 5′-nucleotidase and did not occur in brain, liver, or diaphragm. Although potassium loading accelerates aldosterone secretion, stimulation of renal Na-K-ATPase activity was not exclusively dependent on the adrenal, since it could be elicited by potassium loading in adrenalectomized animals. The rise in renal Na-K-ATPase seen in compensatory renal hypertrophy in rats is also related to an increased excretory load of potassium per nephron, and this adaptive change after partial nephrectomy was also unaffected by prior adrenalectomy. Finally, sodium restriction did not induce enzyme changes, though it too increases aldosterone secretion. Studies of QO_2 and PAH accumulation by kidney slices suggest that the increase in Na-K-ATPase induced by potassium loading is localized to distal tubules. Potassium is thought to enter distal tubular urine from renal cells down an electrochemical gradient. An increase in Na-K-ATPase in the peritubular plasma membrane of distal tubular cells would increase the intracellular pool of potassium and therefore accelerate its passive movement into the urine, thus playing a key role in the process of potassium secretion by the kidney.

283. Heterogeneity of Parathyroid Hormone: Clinical and Physiologic Implications. ROBERT SILVERMAN* AND ROSALYN S. YALOW,* Bronx, N. Y. (introduced by Stanley Ulick**).

Four fractions of immunoreactive human parathyroid hormone (PTH) were demonstrable in parathyroid glandular tissue extracted by three different solvents, 20% acetone in 1% acetic acid (AA), 8 M urea (U), or normal saline (NS), and subjected to Sephadex G-100 gel filtration and immunoassay using two antisera (273 and C-329). The first (I), a void volume peak, was detected by both antisera, as was a second (II), which eluted from Sephadex and sedimented in the ultracentrifuge with purified bovine PTH; a third (III) eluted between [^{125}I] growth hormone and [^{125}I] insulin, sedimented similarly to [^{125}I] insulin, and was detected primarily by 273; a final fraction (IV), detected primarily by C-329, eluted just before [^{125}I] insulin. The AA and U extracts were similar, containing fraction II as their major component, while in NS extracts fraction III predominated. Three fractions, having gel filtration and immunologic characteristics similar to fractions II, III, and IV of NS glandular extracts, were detected in the plasma of patients with primary and secondary hyperparathyroidism. In every plasma, the intermediate component was the most abundant, and the final component, the least abundant form. The first eluted plasma fraction disappeared from uremic plasma during calcium infusion with a half-time of about 20 min and probably represented biologically active hormone; the intermediate and final fractions had turnover times more than 100 times longer, remained elevated during postparathyroidectomy hypoparathyroidism, and were presumed biologically inactive. The evidence favored a glandular origin for the fragments. A striking advantage was observed using antiserum 273 in the preoperative diagnosis of primary hyperparathyroidism and was presumed due to the enhanced sensitivity occasioned by its detection of biologically inactive as well as active hormonal forms.

284. Glucose Transport by Synovial Microvasculature. PETER A. SIMKIN* AND JOSEPH E. PIZZORNO,* Seattle, Wash. (introduced by Mart Mannik).

The small molecules of synovial fluid are known to be in equilibrium with those of plasma, but the kinetics of the equilibria have remained unclear. In this study, rates of transynovial exchange were determined by a new experimental model. 30 ml of saline were injected into one knee of 25 normal subjects and sampled periodically over 35 min. Increasing concentrations of physiologic molecules (urea, creatinine, urate, and glucose) and decreasing concentrations of marker substances (tritiated water and ^{14}C-labeled urea, urate, glucose, or sucrose) were analyzed by the Fick diffusion equation for DA/x (D = diffusion coefficient, A = area, x = path length of diffusion). DA/x, here termed the synovial diffusion capacity (SDC), is the volume of fluid equilibrating per minute. SCD applies equally to plasma and intrasynovial fluid and is analogous to the physiological concept of clearance. Respective mean SDC values in milliliters per minute±SD were: tritiated water 0.94±0.29, [^{14}C] urea 0.70±0.11, urea 0.81±0.29, creatinine 0.48±0.13, [^{14}C] urate 0.65±0.31, urate 0.51±0.19, [^{14}C] glucose 0.36±0.08, glucose 0.83±0.36, and [^{14}C] sucrose 0.42±0.13. Except for glucose, SDC correlated well with molecular diffusion coefficients ($r = 0.897$, $P < 0.001$) suggesting that transynovial exchange of these molecules occurs primarily by free diffusion. Glucose, however, enters the knee more rapidly. Within individual studies, comparison of bidirectional rates showed glucose ingress to be 177±19% (SD) of its rate of egress, whereas respective values for urea and urate were remarkably symmetrical: 100±7% and 98±7%. These findings indicate that specific glucose transport, consistent with facilitated diffusion, occurs across the normal human synovium. Since the synovial lining is discontinuous, this system is presumably within the endothelial cells of the synovial microvasculature. (Supported by grants from NIH and Arthritis Foundation.)

285. Immunoreactive Arginine Vasopressin (AVP) and Arginine Vasotocin (AVT) in the Fetal Pituitary of Man and Sheep. RONALD SKOWSKY* AND DELBERT A. FISHER, Long Beach Calif., and Torrance, Calif.

AVT has been tentatively identified by bioassay in the fetal pituitary of the sheep and seal. To quantify the changes in pituitary AVP and AVT during gestation, we have developed

radioimmunoassay (RIA) systems for AVP using antisera with and without cross-reactivity to AVT. Both RIA systems are sensitive to 0.4 μU AVP or AVT. Measurement of samples in both RIA systems enables determination of the AVT content of the sample. Using this dual RIA we measured the AVP and AVT content of 17 human fetal pituitaries (11–19 wk gestation) and 9 fetal sheep pituitaries (109–137 days gestation). In the human fetus, AVP and AVT were first detectable at 12 wk (0.6 and 1.6 mU/mg gland weight) and tended to increase throughout 18 wk (AVP = 2.8 mU/mg and AVT = 2.9 mU/mg). In these specimens AVT exceeded AVP (AVP/AVT = 0.71). In the fetal sheep AVP and AVT were detected in six of the nine pituitaries, but AVP exceeded AVT (AVP/AVT = 8.78). Mean (and SEM) of AVP and AVT in the sheep pituitaries was 6.9±4.7 mU/mg and 0.79±0.37 mU/mg. The data indicate that immunoreactive AVT is present in the fetal pituitary of the human and the sheep and suggests that the pituitary content of this peptide decreases throughout gestation, while that of AVP increases. (Supported by USPHS grants HD-04270 and HD-06335 from the NICHHD of the NIH.)

286. Physical State of Lipids of Atherosclerotic Lesions. DONALD M. SMALL, G. GRAHAM SHIPLEY,* CARSON R. LOOMIS,* AND MARTIN J. JANIAK,* Boston, Mass.

Human atherosclerotic lesions are associated with the accumulation of lipids, specifically cholesterol and its esters, and although the histology and biochemistry of aortic intima are well documented, little is known of the physical state of the lipids. Using calorimetric, optical, and X-ray diffraction techniques, we have examined the physical state (crystalline, liquid-crystalline, liquid) of aqueous mixtures of the predominant lipid types found in both normal and atherosclerotic intima. Multicomponent phase diagrams corresponding to different temperatures were constructed and indicate the compositional limits, molecular structure, and structural variations of individual phases. Mixtures of the major intimal phospholipids (lecithin, sphingomyelin, cephalin) form lamellar liquid-crystals which can incorporate cholesterol to a molar ratio 1:1. The liquid-crystalline phase can incorporate traces of cholesterol esters (CE), the excess CE separating as liquid (oil), thermotropic liquid-crystal, or crystal, depending upon the CE and the temperature. The liquid and liquid-crystalline phases of CE solubilize small amounts of cholesterol but not phospholipid. Cholesterol, exceeding that solubilized by phospholipid and CE phases, separates as crystalline cholesterol. When the chemical composition and physical state of lipids in different stages of intimal disease are compared, we conclude that the development of atherosclerotic lipid lesions progresses as follows: first, cholesterol saturates the lamellar phospholipid phase (plasma membrane); second, CE is incorporated into this lamellar phase, exceeds its solubility, and separates as a liquid or liquid-crystalline phase (microscopic fat droplets); third, the amount of CE increases producing the "fatty streak" lesion; finally, cholesterol exceeds its solubility in phospholipid and CE phases and separates as crystals. Thus, advanced plaques consist of lipids partitioned into several phases; membranes saturated with cholesterol and CE, liquid and/or liquid-crystalline "oil droplets" of CE saturated with cholesterol, and crystalline cholesterol. (Supported by NIH-AM 11453.)

287. Assay of Gentamicin with Gentamicin Adenyl Transferase (GAT). ARNOLD L. SMITH* AND DAVID H. SMITH, Boston, Mass. (introduced by Fred Rosen).

Previous studies have emphasized the wide variations in serum concentrations of gentamicin after a given dose, and the potential toxicity of this antibiotic. Clinical management would be facilitated by an assay that is rapid, specific, and accurate and utilizes small samples. Certain R factors mediate the synthesis of gentamicin adenyl transferase (GAT). GAT has been purified from a mutant of *E. coli* W677/JR 66 selected for high gentamicin resistance. The enzyme was assayed by trapping antibiotic-adenylate produced from radioactive ATP on phosphocellulose paper. GAT has mol wt 35,000, a pH optimum of 8.6–9.1, an absolute requirement for sulfhydryl groups, and is stimulated by Mg^{++}. It utilizes certain ribonucleotides and 2′-dATP, and adenylates only gentamicin, kanamycin, and tobramycin. The K_m for ATP is 61 μM; that for tobramycin, gentamicin, and kanamycin is 0.85, 1.54, and 2.25 μM, respectively. GAT is inhibited by high concentrations of these antibiotics and pyrophosphate, the other product of the reaction. This assay is not affected by anticoagulants, fluoride, urea, bilirubin, albumin, or other antibiotics; it can be applied to all body fluids and requires specimens of 0.01 ml and 1 h to complete. With mock unknowns in 59 sera, half from patients with abnormal renal and/or hepatic function, the arithmetic correlation coefficient (r) between the observed and theoretical gentamicin concentrations was 0.988; $r = 0.945$ for an 18 hr microbiological assay. With unknowns in 19 urines, $r = 0.930$ for both the enzymatic and microbiological assays. For 63 clinical specimens, the r between enzymatic and microbiological methods was 0.956. These observations suggest that the GAT method meets the criteria for an ideal antibiotic assay.

288. Phenotypic Distinction of R Factors Associated with Epidemic Bacilli. DAVID H. SMITH AND TERRY MARSH,* Boston, Mass.

A strain of *Shigella dysenteriae* is causing a pandemic in Central America and a strain of *Salmonella typhosa* is epidemic in Mexico. Genetically similar, drug-sensitive isolates of each strain existed in the areas before the respective epidemics; both epidemic strains contain an R factor mediating resistance to identical antibiotics. These and other epidemiological findings led Gangarosa et al. to propose that these R factors may be genetically related and/or contain a locus-mediating "virulence." These questions and the possible use of phenotypic analysis to distinguish R factors were studied with a strain of *E. coli* infected with each of 12 coded R factors received from Gangarosa. The R factors could not be distinguished by patterns of drug resistance. None of the R factors restricted phages T7, λ, or P1, or mediated susceptibility to phage f2. On the basis of 11 other tests, the R factors were categorized into five groups. When the codes were broken, it was found, for example, that all *S. typhosa* R factors mediated low conjugal fertility and low specific activities of chloramphenicol acetyl transferase (CAT) and possessed a CAT locus that was stable in *S. typhimurium*; all those from *Sh. dysenteriae* had identical phenotypes which differed significantly in these properties. Both of these groups of R factors were fi^- and incompatible with R factors of existing compatibility groups. Certain R factors associated with isolates of other genera recovered in relation to the epidemic strains had an "epidemic R factor" phenotype. These results suggest different genetic origins for the "epidemic R factors" or dramatic evolutionary divergence and indicate the potential of phenotypic analysis of R factors in epidemiological studies. (These studies were supported by a grant from NIAID.)

289. Effects of Antibodies Specifically Directed Against Na^+, K^+-ATPase. THOMAS S. SMITH,* HENRY WAGNER, JR.,* MICHAEL YOUNG, AND JACK KYTE,* Boston, Mass.

Antibodies have been obtained which specifically interact with the transport enzyme Na^+, K^+-activated ATPase. The

antigen used was Na^+, K^+-ATPase from canine renal medulla purified to homogeneity as judged by polyacrylamide-gel electrophoresis. Pre- and postimmunization sera from rabbits challenged with this antigen in complete Freund's adjuvant were fractionated by ammonium sulfate precipitation, DEAE cellulose ion-exchange chromatography, and prolonged dialysis to obtain the γ-globulin fraction. Gamma globulin from immunized animals, but not from control animals or preimmune serum, inhibited Na^+, K^+-ATPase activity of canine renal medullary extracts up to 68 ± 4 (SD) % in a concentration-dependent manner with maximum inhibition occurring within 5 min at 37°C. The Mg^{++}-dependent, non-Na^+, K^+-activated, and non-ouabain-inhibited component of activity (Mg^{++}-ATPase) was unaffected. Fab fragments obtained by papain cleavage of the γ-globulin fraction had similar inhibitory activity and specificity. These antibodies also produced concentration-related inhibition of canine myocardial microsomal and human red cell ghost Na^+, K^+-ATPase activities by up to 50 ± 6 and 98 ± 5%, respectively. Control and preimmune γ-globulin fractions had no effect on canine myocardial or human red cell ghost Na^+, K^+-ATPase. Mg^{++}-ATPase was unaffected by all preparations including those with marked Na^+, K^+-ATPase inhibitory activity. Despite marked inhibition of Na^+, K^+-ATPase activity in these particulate enzyme preparations, experiments with canine renal slices and human red cells showed no specific effect of antibody on ouabain-inhibitable ^{86}Rb uptake, indicating a lack of inhibition of active monovalent cation transport in the intact cell. These experiments demonstrate immunologic cross-reactivity among Na^+, K^+-ATPases from different organs and different species. In addition, they indicate that the antibody response is directed against an antigenic determinant or determinants inaccessible to macromolecules at the outer cell surface. (Supported by grants from NIH and AHA.)

290. The Role of the Liver in Metabolism of Low Density Lipoproteins (LDL). A. D. SNIDERMAN,* T. E. CAREW,* J. G. CHANDLER,* S. HAYES,* AND D. STEINBERG,** La Jolla, Calif.

Elucidation of the normal mechanisms for lipoprotein secretion into and removal from plasma is prerequisite to an understanding of the metabolic errors in inherited hyperlipoproteinemias. Our initial studies in pigs showed that autologous labeled plasma LDL (d 1.019–1.063; > 95% ^{125}I in protein) was in rapid equilibrium with a pool of LDL in the liver, the only major extravascular pool identified. To assess the role of the liver in LDL removal, the disappearance of [^{125}I] LDL was studied before and after total hepatectomy, each animal serving as his own control. Intact pigs and dogs showed a biexponential ^{125}I disappearance: phase I $t_{\frac{1}{2}}$ (equilibration with extravascular pools), 0.9 h and 1.7 h, respectively; phase II $t_{\frac{1}{2}}$ (irreversible removal from plasma), 20 h and 27.4 h, respectively. Three pigs and three dogs were re. tudied immediately posthepatectomy (porto-caval shunt and en bloc removal). The disappearance was now monoexponential over the entire 13–20 h study period and more rapid: $t_{\frac{1}{2}}$ 8.8 h in pigs; 11.3 h in dogs. In posthepatectomy pigs, LDL (d 1.006–1.063) was reisolated for measurement of protein specific radioactivity. This did not change significantly, while absolute LDL protein levels decreased by 55–73% over the intervals studied. The results show that under the conditions of study there was no significant *de novo* LDL synthesis and secretion from extrahepatic tissues. Barring the possibility that hepatectomy is accompanied by substantial shifts in rates and patterns of LDL removal in the periphery, irreversible LDL removal by the liver seems to be quantitatively minor and the liver may, directly or indirectly, play a role in stabilizing LDL to prolong its lifetime in the plasma. (Research supported by NIH grant HL-14197.)

291. Pituitary and Thyroid Responses to Repetitive Administration of Thyrotropin-Releasing Hormone (TRH). PETER J. SNYDER* AND ROBERT D. UTIGER, Philadelphia, Pa.

Previous studies showed that the thyrotropin (TSH) response to thyrotropin-releasing hormone (TRH) is readily inhibited by exogenous triiodothyronine (T_3) and thyroxine (T_4). In this study, repetitive TRH administration was used to produce small increases in endogenous serum T_3 and T_4 levels to determine if endogenously produced T_3 and T_4 inhibited the TSH response to subsequent TRH doses. Each subject received 13 consecutive doses of 25 μg TRH at 4-h intervals. Serum TSH, T_3, and T_4 levels were measured before (basal levels) and for 4 h after the first, seventh, and thirteenth doses of TRH. In 10 normal subjects, the mean increase in serum TSH levels fell from 14.6 μU/ml after the first TRH dose to 6.9 and 3.0 μU/ml after the seventh and thirteenth doses. The reduced TSH responses were accompanied by rises in mean basal serum T_3 levels from 81 to 115 to 114 ng/100 ml (normal range, 70–150) and in mean basal serum T_4 levels from 6.7 to 8.6 to 9.6 μg/100 ml (normal range, 5–11) at the time of the first, seventh, and thirteenth TRH doses. In a patient with primary hypothyroidism, repetitive TRH administration resulted in no increase in serum T_3 and T_4 levels and the TSH response to TRH was not blunted. These results further demonstrate the marked sensitivity of TRH-induced TSH release to suppression by small increases in serum T_3 and T_4 levels and show the suppression is not due to depletion of TSH from the pituitary. Inhibition of TRH-induced TSH release was preserved in two patients with hypothyrotropic hypothyroidism. In one patient, with presumed TRH deficiency, the TSH response was blunted by repetitive TRH administration, but only after the serum T_3 and T_4 levels increased to normal. In the other patient, with presumed pituitary insensitivity to TRH, the initially subnormal TSH response was blunted further by repetitive TRH administration even though the serum T_3 and T_4 levels did not rise to normal. Preservation of sensitivity of TRH-induced TSH release to inhibition by small rises in serum T_3 and T_4 despite insensitivity to TRH in this patient supports the concept that inhibition and stimulation of TSH release are mediated by different mechanisms.

292. RNA-Dependent DNA Polymerase of Erythroid Cells: a Possible Mechanism for Control of Hemoglobin Synthesis. ANTERO G. SO,* JOHN J. BYRNES,* AND KATHLEEN M. DOWNEY,* Miami, Fla. (introduced by William J. Harrington).

There are numerous defined or suspected disorders of hemoglobin synthesis. Although the regulatory mechanism underlying these disorders is not clearly understood, control of hemoglobin synthesis is usually held to occur at the translational level. We wish to present an additional mechanism for control of hemoglobin synthesis. To account for the exceedingly rapid rate at which hemoglobin is synthesized in developing erythroid cells, amplification of the structural genes for the α- and β-chains has been postulated. Amplification of the genes for ribosomal RNA has been found to occur in amphibian cocytes and shown to involve RNA-dependent DNA synthesis. We have found a RNA-dependent DNA polymerase in the cytoplasm of rabbit erythroid cells. The cytoplasmic synthesis of DNA using the endogenous template in inhibited by ribonuclease A, suggesting that RNA acts as the template for this polymerase. The endogenous activity is also inhibited by high levels of actinomycin D, suggesting that a DNA template is also utilized. The purified enzyme prefers native DNA to synthetic DNA/RNA hybrid. The control of hemoglobin synthesis involving this enzyme will be discussed, with possible applications to human disease. (Research supported by grants from NIH and AHS.)

293. IgG Half-Molecules in a Patient with Plasma Cell Leukemia. HANS L. SPIEGELBERG* AND VICTOR HEATH,* La Jolla, Calif. (introduced by Frank J. Dixon**).

The paraproteins of a patient with plasma cell leukemia who excreted 300 mg/100 ml of a κ, IgG paraprotein into the urine were characterized. The serum contained two monoclonal proteins, one having a sedimentation rate of 7S (0.5 g/100 ml) and the other 4.3S (1 g/100 ml); the 4.3S protein was identical to the urinary protein. The paraproteins were detected when quantitation of IgG in the serum was attempted by commercially available immunodiffusion plates, which showed two concentric precipitin rings. Similarly, analysis of the serum by immunoelectrophoresis showed a double line with anti-IgG, but only a single line with anti-κ antisera. Only the 7S protein precipitated with anti-Fc fragment antisera; the 4.3S protein, however, inhibited this precipitin reaction. After reduction and alkylation, both the 7S and the 4.3S proteins dissociated into heavy and light chains, the mass ratios being 2:2 and 1:1 respectively. The molecular weights were similar to normal γ- and κ-chains, excluding a large deletion in the heavy chain. The heavy chains of both proteins contained the peptide which forms the inter-heavy-heavy-chain disulfide bonds, its amino acid composition being identical to that of γ_1-chains. The electrophoretic mobilities and the amino acid compositions of the polypeptide chains of both the 7S and 4.3S proteins were indistinguishable, suggesting that the same clone of cells produced both proteins. In contrast to normal IgG, the 7S protein dissociated into half-molecules (4.3S) after mild reduction and alkylation in aqueous solution at neutral pH. These data indicate that the paraproteins of this patient lacked the noncovalent bonds normally present in the Fc portion of γ-chains and suggest that the absence of these bonds resulted in impaired assembly of 7S IgG molecules. (Supported by NIH and AHA.)

294. Studies on the Characterization of Transfer Factor. LYNN E. SPITLER,* DAVID WEBB,* CHRISTINE VON MULLER,* AND H. HUGH FUDENBERG,** San Francisco, Calif.

The nature of transfer factor was studied by immunological and biochemical analysis and by systemic transfers of coccidioidin reactivity in normal subjects using conversion of skin reactivity (SR), lymphocyte stimulation (LS), and production of migration inhibitory factor (MIF) as measures of reactivity. The crude dialyzable transfer factor preparation contained deoxyribonucleotides, ribonucleotides, and peptides. Transfer factor which had been heated at 56°C for 2 h transferred all three parameters of reactivity, and after heating at 80°C for 1 h it transferred SR and LS in $\frac{3}{4}$ normal subjects but transferred MIF capacity in only $\frac{1}{4}$. All parameters of reactivity were transferred by a preparation which had been treated with pancreatic RNase and TI RNase at low molarity, and controls run in parallel indicated that only single stranded RNA was hydrolyzed. Treatment with pronase destroyed the transfer of SR and NIF, and LS was transferred in only $\frac{1}{4}$ subjects who received this preparation, suggesting that a peptide must be part of the active molecule. After passage through a UM IO Diaflo membrane, transfer factor did not transfer SR or MIF, but did transfer LS. We conclude that there may be different transfer factor for the transfer of SR, LS, and MIF and that these may be distinguished on the basis of physicochemical characteristics.

295. Azurophil and Specific Granules Resolved for Human Polymorphs (PMN): Immunochemistry, Biochemistry, Enzyme Histochemistry. J. K. SPITZNAGEL,* F. G. DALLDORF,* M. S. LEFFELL,* J. D. FOLDS,* Chapel Hill, N. C. (introduced by C. W. Gottschalk**).

Granules deliver antimicrobial substances to phagocytic vacuoles. Intraleukocytic bactericidal defects have been described in PMN lacking myeloperoxidase or lactoferrin, antimicrobial proteins normally associated with granules. We have isolated normal granules and characterized their contents in order to define antimicrobial capacities of granules and degranulation and more readily to interpret studies with potentially abnormal PMN. Crude granule suspensions (from 99% pure PMN) applied in 11% sucrose to linear (50–53%) sucrose density gradients and centrifuged at $\int_0^t \omega^2 dt = 2.3 \times 10^{10}$ rad^{2}sec^{-1} with $R_{max} = 15.3$ and $R_{min} = 6.4$ cm yielded three turbid bands (I, II, and III in order of increasing sedimentation rate). Band III contained 88% of the myeloperoxidase measured immunochemically (IC) and 77% of the neutral protease measured spectrophotometrically (SP). Band II had 65% of the lactoferrin (IC). Lysozyme (SP) was bimodally distributed between II (50%) and III (46%). Alkaline phosphatase (85%) was in I. Crude granules applied in 25% sucrose to gradients and centrifuged yielded in place of III two narrowly separated bands (IIIs and IIIf). I and II were unchanged. The MPO (95%) and the neutral protease (95%) peaked in IIIs with a shoulder in IIIf, while lysozyme peaked in IIIf with a shoulder in IIIs. Ultarstructurally bands IIIs and IIIf contained peroxidase-positive 0.3 μ granules. II contained peroxidase-negative 0.12 μ granules. In I were empty vesicles with alkaline phosphatase positive membranes. Evidently the MPO-positive (azurophil) granules comprise two subgroups, one relatively richer in MPO and protease (IIIs) and one (IIIf) which is richer in lysozyme. The MPO negative granules (specific) are rich in lysozyme and lactoferrin. Perhaps IIIf granules arise fom the Golgi apparatus as its secretory cycle shifts from secretion of azurophil to specific granules. (Supported by NIAID and AEC.)

296. Peripheral Nerve Myelin in Experimental Diabetes. NORTON SPRITZ, BARBARA GEYER,* AND HARBHAJAN SINGH,* New York.

Abnormalities in Schwann cell function related to the maintenance of myelinization may play an important role in peripheral neuropathy in diabetic patients. We have quantified the amount of myelin in sciatic nerves of rats and rabbits with experimental diabetes, and have measured in vitro their incorporation of isotopic precursers into myelin lipids. Sciatic nerves were incubated with either tritiated water or [1-C^{14}] acetate and myelin isolated from the homogenate. Incorporation into myelin phospholipid had first-order kinetics, and myelin isolation was quantitative and uncontaminated. In rats with diabetes of 1 wk incorporation was like that of controls. In four groups of 4–6 diabetic rats with diabetes of 3–12 wk duration, specific activity ranged from 55 to 71% of controls for phosphotidyl choline—the main constituent and phosphotidyl ethanolaine and phosphotidyl serine—the other phospholipids with significant incorporation. In rabbits, specific activities were about $\frac{1}{3}$ that of the rat and were not different between diabetics and controls. Composition of isolated myelin did not differ between diabetics and controls in either species. In the rabbit, however, myelin content decreased in controls with age (1.80 mg/cm of nerve at 6, 1.54 at 7, 0.9 at 8, and 0.85 at 10 months of age). In age-matched diabetic rabbits (3–8 months duration) myelin content ranged from 65 to 90% of controls. These studies indicate that defective myelin synthesis in the rat and decreased myelin content in older rabbits occur in experimental diabetes. This suggests that, at least in part, neuropathy in diabetic man may be a consequence of insulin deficiency per se and that experimental diabetes provides a model for its study. (Supported by VA research and NIH grant AM13525.)