297. Posthepatic Delivery Rates of Proinsulin and Insulin in Normal Subjects. J. I. STARR,* D. J. JUHN,* D. F. STEINER,* AND A. H. RUBENSTEIN, Chicago, Ill.

Proinsulin:insulin ratios in serum are higher than in pancreatic islets. This ratio is highest in the fasting state and at late times after stimuli to insulin secretion. Differences in peripheral metabolism of endogenous insulin ($t_{\frac{1}{2}}$ = 4.8 min) and proinsulin ($t_{\frac{1}{2}}$ = 17.2 min) are partly responsible for these findings. To investigate whether differential pancreatic secretion or variable hepatic clearance of the polypeptides may also contribute, we have calculated posthepatic deliver rates (PHDR) of insulin and proinsulin after oral glucose. Specimens were obtained at 15- to 30-min intervals during 5-h oral GTT's in four healthy subjects. Proinsulin and insulin were separated by gel filtration and measured by radioimmunoassay against human proinsulin and insulin standards. Using a disappearance constant derived for endogenous proinsulin and insulin, PHDR were calculated assuming constant delivery during each time interval. The highest insulin level occurred at 30 min (2.63±0.80 ng/ml), while proinsulin peaked at 105 min (1.04±0.45 ng/ml). Basal insulin PHDR was 28±4 pg/ml per min, and increased to 408±124 pg/ml per min at 15–30 min. Thereafter a gradual decline to 20±4 pg/ml per min occurred by 5 h. Proinsulin PHDR rose from 7±1 pg/ml per min in the basal state to plateau between 36 and 46 pg/ml per min at 15–105 min and returned to vasal values by 5 h. The fasting ratio of insulin PHDR:proinsulin PHDR was 4.5± 0.8, rose to 12.0±2.4 in the 0–15 min interval, and returned to basal levels by 3 hr. These observations can be explained either by preferential beta cell release of insulin in the early poststimulatory period or relatively decreased hepatic insulin extraction when insulin secretion is maximal. The significant correlation observed between the PHDR ratio of the two polypeptides and insulin PHDR supports the latter interpretation. (Research supported by grants from NIH.)

298. Circulating High Density Lipoprotein (HDL): Measurement of an Apoprotein Component by Radioimmunoassay. J. I. STARR,* D. J. JUHN,* M. E. MAKO,* M. GUERIN,* A. M. SCANU, AND A. H. RUBENSTEIN, Chicago, Ill.

Recent studies have established that human serum lipoproteins contain distinct polypeptide chains, each exhibiting immunological specificity. As part of a program aimed at the quantification of these polypeptides, we have developed a specific and sensitive double-antibody radioimmunoassay for fraction III (apo LP-Gln-I or A-I), the major polypeptide of high density lipoprotein (HDL). Pure polypeptide III was labeled with ^{125}I (15–30 mCi/mg) and shown to retain its homogeneity by both electrophoretic and chromatographic criteria. Gamma globulin was purified by chromatography from antiserum to III raised in rabbits. In this assay system, with $B/B_0 > 0.4$, apo-HDL, HDL, delipidated serum, and serum demonstrated identical immunological reactivity. However, with B/B_0 below 0.4, standards of III, apo-HDL, and HDL resulted in progressively shallower slopes indicating an effect of the other apoprotein peptides and lipid moieties on the immunological reactivity of III. Fasting sera from healthy subjects contained 1.02±0.05 mg/ml III (range 0.63–1.39). Mean levels in females were higher than males (1.14±0.09 vs. 0.98±0.05). Patients with heaptic failure had markedly reduced levels (0.58±0.07 mg/ml, $P < 0.001$), while concentrations in renal failure were normal (1.07±0.06). Hypothyroidism was assoicated with elevated III (1.26±0.06 mg/ml, $P < 0.001$), but values in hyperthyroidism were normal (0.97 ±0.09). Striking increases occurred in the third trimester of pregnancy (2.04±0.13 mg/ml, $P < 0.001$) and fell towards, but did not reach, control levels by 6 wk postpartum (1.63± 0.19). Correlation of these results with serum cholesterol and triglyceride levels and lipoprotein electrophoretic patterns was undertaken. This approach to the quantitative study of serum lipoproteins should prove valuable in work concerning the structure and metabolism of these complexes. (Research supported by NIH grants.)

299. Comparison of 1,25-Dihydroxycholecalciferol and 5,6-Transcholecalciferol in Uremic Man. THOMAS H. STEELE,* M. ARIEF MANUEL,* GOEFFERY BONER,* MARGARET NEWTON,* MICHAEL F. HOLICK,* AND HECTOR F. DELUCA,* Madison, Wis. (introduced by Richard E. Rieselbach).

Whereas 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) is a potent tissue-active metabolite of vitamin D synthesized by the kidney, 5,6-transcholecalciferol ($transD_3$) is a synthetic analogue active in anephric rats. In three out of four anephric patients, $transD_3$ increased both serum calcium and intestinal ^{47}Ca absorption, and induced a more positive calcium balance, but only at dosages of 50–200 μg/day. In contrast, $1,25-(OH)_2D_3$ produced similar or greater changes in these parameters at a dosage of 1 μg/day, both in anephric patients and three other hemodialysis patients with far advanced chronic renal disease. In two of the three, intravenous $1,25(OH)_2D_3$ was more effective than the same dose administered orally. In addition, urinary calcium in these patients increased from 3.0 mg/day before treatment to 7.1 mg/day after $1,25(OH)_2D_3$, while urinary phosphorus decreased. The increased urinary calcium excretion probably did not reflect a direct inhibitory action of $1,25(OH)_2D_3$ on renal calcium reabsorption, since clearance studies in five normal persons failed to show any acute effect of intravenous $1,25(OH)_2D_3$ on renal calcium reabsorption within 6 h. Furthermore, administration of 2 μg of $1,25(OH)_2D_3$ to a patient with a GFR of 4.4 ml/min failed to affect calcium or phosphorus reabsorption acutely, although base line fractional phosphorus reabsorption was only 0.13. These results indicate that, while $transD_3$ is effective in anephric patients, it is far less potent than $1,25(OH)_2D_3$. They also suggest that the route of administration of $1,25(OH)_2D_3$ may be an important factor governing its efficacy in dialysis patients. In addition, changes in urinary calcium and phosphorus excretion do not occur immediately after intravenous administration of $1,25(OH)_2D_3$. (Supported by grant from NIH.)

300. Collagen Turnover in Ascorbic Acid Deficiency—a Cell Culture Model. JAMES STEINBERG* AND GEORGE NICHOLS, JR.,** Boston, Mass.

Optimal hydroxylation of specific proline and lysine residues in growing collagen polypeptides requires ascorbic acid, and may be important in their subsequent extrusion, extracellular fibril formation, and stabilization through cross-linking. The influence of ascorbate (250 μM) on the turnover of newly synthesized collagen was examined in the mouse fibroblast line, 3T6, by radioactive labeling and compartmental analyses. Cell growth and general protein synthesis were unaffected. Chemically detectable collagen deposition, delayed until cell multiplication ceased but linear therafter, was enhanced by daily ascorbate treatment to 16 $\mu g/10^7$ cells per day. Using [^{14}C] proline as a percursor of collagen [^{14}C] hydroxyproline (hyp) in cell layer and growth medium, an early effect of ascorbate was noted both in pulse and steady-state labeling experiments: It abruptly stimulated proline hydroxylation 3- to 5-fold, with a shift in the medium [^{14}C] hyp to macromolecular species. The collagen fraction available for breakdown was simultaneously reduced, although degradation products (soluble, free [^{14}C] hyp) continued to accumulate at the same rate (4%/day) in continously labeled cultures. Chase experiments demonstrated poor stability of the collagen deposited in ascorbate-deficient cell layers, 70–80% of the pre-

formed [^{14}C] hyp being lost despite subsequent ascorbate feeding. Conversely, ascorbate-replete cultures showed excellent retention in the cell layer, a 70% reduction in the immediately available collagen breakdown pool, and a 50% reduction in the fractional breakdown rate. These changes were accompanied by altered extractability of the residual collagen, which became enriched to 95% in highly cross-linked, acid-insoluble species. It is concluded that the reduced collagen content in scorbutic tissue is the result of both deficient synthesis and accelerated degradation. (Supported by NIH AM14749 and The John A. Hartford Foundation, Inc.)

301. Thermal Inactivation of Thyroxine-Binding Globulin for Direct Radioimmunoassay of Triiodothyronine in Serum. KENNETH STERLING** AND PETER O. MILCH,* New York.

A new radioimmunoassay procedure for triiodothyronine (T_3) is described. The method uses unextracted serum at a 1:4 dilution. The novel feature of the process is that preincubation and incubation with isotope take place at 60°C. At this temperature the binding capacity of the thyroxine-binding globulin (TBG) is virtually eliminated and the antigen-antibody reaction is greatly accelerated. No artifactual T_3 formation is demonstrable. Separation of antibody-bound hormone from free hormone is effected by polyethylene glycol solution (Carbowax 6000). By plotting the standard curve on probability × logarithmic paper, the curve is a strainght line. Thus, all parts of the curve are equally sensitive from 15 to 500 ng/100 ml. The heating procedure excludes the effects of TBG sufficiently to eliminate, for the most part, the discrepancy between standard curves made up in buffer or in T_3-free serum. Results obtained from two such curves can differ as much as 100% and the differences cannot be fully corrected by the use of conventional blocking agents such as ANS or salicylate. Sera of 16 normal individuals showed a mean value (±SD) of 186±36 ng/100 ml. 14 clinically thyrotoxic individuals had T_3 values of 838±398 ng/100 ml and the same number of clinically myxedematous patients had T_3 values of 15±22 ng/100 ml. These values were confirmed by using several different antisera and by performing the radioimmunoassay on previously extracted samples.

302. Coronary Artery Disease (CAD) in Familial Type II Hyperlipoproteinemia (HLP): Study of 116 Kindreds. NEIL J. STONE,* ROBERT I. LEVY, DONALD S. FREDRICKSON,** AND JOEL VERTER,* Bethesda, Md.

Conflicting data regarding CAD risk in type II hyperlipoproteinemia (HLP) compel reassessment of risk in members of affected families, omitting propositi to avoid bias. The relatives over age 19 of 116 propositi with type II were chosen for analysis of CAD risk. In over 90% of the 754 living relatives, interview by one investigator and 12 lead electrocardiogram were obtained. In these, and in 295 deceased relatives, physician and hospital records were utilized as needed to determine CAD events. Type II HLP was diagnosed when low density lipoprotein cholesterol exceeded age-corrected upper 5% limits. CAD was diagnosed in 24.6% of relatives with type II (II's) compared to 9.0% of normal relatives (N's) ($P < 0.001$). The II's and N's did not differ significantly with regard to age distribution, sex, hypertension, smoking, or diabetes. Angina pectoris by Rose questionnaire was noted in 18.7% of II's and 5.4% of N's ($P < 0.001$). Documented myocardial infarction (MI) occurred in 5.0% of II's vs. 0.8% of N's ($P = 0.002$). CAD death or MI occurred in 10.1% of II's contrasted with 1.8% of N's ($P < 0.001$). Life table analysis in males showed the cumulative probability (CP) of initial nonfatal CAD before age 40 to be 10.2% in II's and 0.5% in N's; before age 50 CP was 29.0% in II's compared with 4.4% in N's. CP of fatal and nonfatal CAD in males before age 40 was 16.0% in II's and 0.5% in N's. In female II's and N's, differences in CP were less striking. In this, the largest study of type II HLP kindreds thus far, CAD events unequivocally occur more frequently and earlier in life in II's than in N's.

303. Marrow Transplantation in Aplastic Anemia. R. STORB,* C. D. BUCKNER,* A. FEFER,* R. CLIFT,* P. E. NEIMAN,* H. GLUCKSBERG,* L. FASS,* AND E. D. THOMAS,** Seattle, Wash.

16 patients with complete marrow failure, ten due to unknown cause, four associated with hepatitis, one associated with paroxysmal nocturnal hemoglobinuria, and one drug related, did not show spontaneous recovery after 2–15 months of conventional therapy. Six were severely infected and four were refractory to random platelet transfusions. They were grafted with marrow from HL-A compatible siblings. Ten were conditioned for grafting by Cyclophosphamide (CY), 50 mg/kg on each of 4 successive days, and six by 1000 rads whole body irradiation. All were given intermittent methotrexate within the first 100 days of grafting to modify graft-vs.-host disease (GVHD). One patient died on the day of grafting of congestive heart failure possibly related to CY cardiac toxicity. One died on day 6 with septicemia. One died on day 24 without engraftment. Thirteen patients showed prompt hemopoietic engraftment indicated by rising peripheral blood counts, a return of marrow cellularity and confirmed by blood genetic markers. Of these, two rejected the graft and died on days 41 and 67. Four died between days 45 and 85 with GVHD. Seven are alive without GVHD, 46, 135, 293, 310, 432, 537, and 614 days after grafting and, with one exception, have returned to normal activity. These results show that normal stem cells will repopulate the marrow in aplastic anemia and demonstrate that long-term stable chimerism is possible in man. They suggest that marrow grafting in patients with complete marrow failure and HL-A matched siblings should be undertaken before major infections and refractoriness to blood transfusions complicate the course of their disease.

304. Isolation and Properties of Actin and Myosin from Polymorphonuclear Leukocytes. T. P. STOSSEL,* T. D. POLLARD,* AND E. JANTZEN,* Boston, Mass. (introduced by D. G. Nathan).

Proteins resembling skeletal muscle actin and myosin in structure and function were isolated from guinea pig peritoneal exudate granulocytes. Actin was extracted from acetone powders of granulocytes and purified by polymerization and washing with KCl, depolymerization by dialysis, and chromatography on Sephadex G200. Its identity was established by the following properties. It comigrated with rabbit skeletal muscle actin on SDS-acrylamide-gel electrophoresis (mol wt 46,000), appeared as 6-nm diameter filaments which assumed arrowhead configurations after addition of granulocyte myosin, and stimulated granulocyte myosin Mg^{2+}-ATPase activity. Myosin was purified from granulocyte extracts by precipitation at low ionic strength, ammonium sulfate fractionation, and chromatography on Bio Gel A15-M. It comigrated with rabbit skeletal muscle myosin on SDS-acrylamide-gel electrophoresis (mol wt 200,000), had EDTA-activated and Ca^{2+}-activated but not Mg^{2+}-activated ATPase activity in 0.6 M KCl, and was bound by rabbit skeletal muscle F-actin in the absence but not the presence of Mg^{2+}-ATP. It formed thin, short bipolar filaments in 0.1 M KCl only after incubation with divalent cations. Actin constituted 10% of the total protein in granulocyte homogenates prepared with sucrose. Actin and myosin were found primarily in 100,000 *g* supernatant fractions. Actin was identified in association with paraffin oil-laden phagocytic vesicles which are, in part, derived from plasma membranes. Phagocytosis is energy dependent, is

influenced by divalent cations, involves cellular movement, and therefore has properties resembling muscle contraction. The existence in granulocytes of actin, possibly attached to plasma membranes, and of myosin, which assembles into filaments only in the presence of divalent cations, suggests that these proteins may generate force for phagocytosis.

305. Modulation of Lymphocyte Blast Transformation by Cyclic Mononucleotides. T. B. STROM,* M. S. HIRSCH,* P. H. BLACK, C. B. CARPENTER, AND J. P. MERRILL,** Boston, Mass.

Graft-vs.-host-induced lymphocyte blast transformation has been studied by quantitating thymidine uptake ([^{3}H] TdR) into graft-vs.-host splenocytes (GVH cells) explanted from F_1 hybrid (CAF_1) mice which had been given 4-weekly injections of parental (Balb/c) splenocytes. GVH cells incorporated more [^{3}H] TdR than normal CAF_1 splenocytes, averaging 180% of the F_1 cpm after 24 h of culture. The relationship between cyclic nucleotides (cAMP and cGMP) and the proliferative rate of GVH cells was studied in six experiments. GVH cells were placed into media with and without pharmacologic agents for 20 h before [^{3}H] TdR pulse labeling. 10^{-3} M prostaglandin E_1, an adenylate cyclase activator, and theophylline (10^{-3} M), a phosphodiesterase inhibitor, inhibited [^{3}H] TdR uptake (64% and 27%, respectively). Dibutyryl cAMP also markedly inhibited proliferation. These data are in concert with observations that mitogen-induced T-lymphocyte proliferation is inhibited by exogenous or endogenous elevations of intracellular cAMP. Addition of carbamylcholine, a cholinergic agonist, in optimal dosages (10^{-10}–10^{-13} M) resulted in as much as 240% enhancement of [^{3}H] TdR uptake. 8-bromo-cGMP (10^{-6}–10^{-7} M) caused enhancement of proliferation (maximum 190%). Phytomitogens have recently been reported to elevate lymphocyte cGMP. The possibility that the effects of cholinergic stimulation are mediated by elevating intracellular cGMP has been hypothesized. The dual modulation of cytotoxic T-lymphocyte function by cAMP and cGMP has been reported (Strom et al. 1972. *Proc. Nat. Acad. Sci. U. S. A.* **69**: 2995; 1973. *Fed. Proc.* In press). These data demonstrate functional cholinergic receptors on proliferating cells from GVH spleens and provide direct evidence that cGMP augments their proliferative rate. (Supported by NIH grants AM-15579, AM-05700, AI-18516, CA-12464-2, and NCI SVCP 72-2012.)

306. Selective Deyciency of Tissue Triiodothyronine: a Proposed Mechanism of Elevated Free Thyroxine in the Euthyroid Sick. P. R. C. SULLIVAN, J. A. BOLLINGER, AND SEYMOUR REICHLIN, Boston, Mass.

Elevated free thyroxine (FT_4) has been found in the plasma of euthyroid patients with severe prolonged illness (euthyroid sick, ES). To test the hypothesis that this abnormality might arise from a relative deficiency of T_3, plasma T_3 (radioimmunoassay, RIA) and plasma T_4 in eight ES patients were compared with eight normals. Plasma T_3 was markedly depressed (< 25 ng/100 ml vs. 130 ± 20 ng/100 ml), FT_4 elevated (2.6 ± 0.6 ng/100 ml vs. 1.5 ± 0.3 ng/100 ml), and T_4I low normal (3.4 ± 0.9 μg/100 ml vs. 5.0 ± 0.3 μg/100 ml). The possibility that plasma T_3 deficiency was a reflection of tissue T_3 deficiency was evaluated by assaying T_3 and T_4 levels by RIA in acid butanol extracts of necropsy specimens of liver and kidney from six ES patients and of four patients dying after brief illness (IB). T_3 concentrations (ng/g) in liver were 1.6 ± 0.5 vs. 10.6 ± 4.8 and in kidney 0.9 ± 0.6 vs. 6.8 ± 3.2. T_4 levels (ng/g) in liver were 55 ± 15 vs. 48 ± 18, and in kidney 41 ± 8 vs. 14 ± 6. The relative changes in tissue hormone concentration led to striking changes in tissue T_3/T_4 ratio in ES compared with BI (liver 0.03 vs. 0.20 and kidney 0.03 vs. 0.49). It is concluded that ES patients have a marked deficiency of intracellular T_3 arising from impaired deiodination and/or reduced T_3-binding protein. The high tissue T_4 levels reflect the high plasma TF_4, together with a probable loss of T_4 deiodinating capacity. The high plasma FT_4 concentration can be looked upon as a compensatory response to deficiency of T_3 at pituitary sites regulating TSH secretion.

307. Successful Tissue and Organ Allotransplantation without Immunosuppression. W. T. SUMMERLIN,* G. E. MILLER,* AND R. A. GOOD, Minneapolis, Minn., and New York.

Allotransplants with best matched of donor and recipient require dangerous "immunosuppressive" treatment. New methods promise to overcome major transplantation barriers for certain allografts without immunosuppressive or anti-inflammatory treatment. Human or mouse skin in organ culture for a critical period can be transplanted across major histocompatibility barriers. Such tissues are not rejected by gross or histologic criteria. Human allografts lasting 4 yr and mouse allografts surviving 1 yr have been achieved. Chromosome markers in epidermal cells, pigmentation markers on mouse and human skin, histocompatible antigens on epithelial cells, and fibroblasts cultured from grafts identify the successful allotransplants as being of donor origin. Xenografts of human, guinea pig, and porcine skin have remained in excellent condition on mice for long periods. Similarly, organ culture permits long-term maintenance of corneal grafts in excellent condition for at least 4 wk. This method excels other techniques for storage of cornea. Allotransplants and xenotransplants of cornea near limbus are accepted and have exhibited good function for 6 months. Control allogeneic and xenogeneic grafts placed near the limbus are regularly rejected within 2 wk. These techniques have been applied to adrenal gland allografts. Functional reconstitution has been achieved in adrenalectomized C_3H mice by adrenal allografts grown in organ culture, whereas fresh adrenal allografts are always rejected. Organ cultured adrenal allografts under the renal capsule become beautiful, histologically intact organs and survive longer than 6 months. ATCH stimulation yields responsive adrenocorticoid secretion. These successful allografts initiate minimal or no immunological response as judged by quantifiable methods to evaluate T-cell-dependent killer function of lymphocytes, cytotoxic antibodies, and blocking antibodies. (Aided by ACS, The John A. Hartford Foundation, and the National Foundation.)

308. Apparent Nonspecificity of an Intermolecular Contact Region in Sickle Fibers. PAUL H. SWERDLOW,* BEATRICE MAGDOFF-FAIRCHILD,* AND JOHN F. BERTLES,** New York.

Intracellular aggregation of deoxygenated molecules of sickle hemoglobin (Hb S) into fibers constitutes the basis of the sickling phenomenon. Molecular organization of these fibers has recently been established (Magdoff-Fairchild. 1972. *Nature (Lond.).* **239**: 217; Finch. *Proc. Nat. Acad. Sci. U. S. A.* In press). Each fiber consits of six apposed right-handed helical filaments of hemoglobin molecules, in effect a stack of six-membered planar discs. Rotation from disc to disc of 7° about the fiber axis generates the six helices. The helical repeat is about 3000 A (approximately 45 molecules). Although the presence of hemoglobin molecules in which the $\beta6$ amino acid is valine (for example, Hb S) appears mandatory for fiber formation, clinical and direct analytical evidence exists that hemoglobins without valine at $\beta6$ (for example, glutamate in Hb A, lysine in Hb C) can participate with Hb S in fiber formation. The molecular orientation of these non-S hemoglobins in sickle fibers is the subject of this report. Fibers from deoxygenated mixtures of Hb S with Hb A, and Hb S with Hb C, were concentrated by ultracentrifugation and introduced into thin-

walled capillaries. X-ray diffraction patterns from these preparations were characteristic of those from fibers composed of Hb S alone. Layer-line spacings (a measure of the center to center distance between discs) and distribution of intensities (indicative of molecular orientation within fibers) were the same in all preparations. The significance of this finding lies in the strong similarity of intermolecular contact regions in sickle fibers, independent of whether partial occupancy of the $\beta 6$ positions is valine, lysine, or glutamate. This information, in view of the differing ease of copolymerization of Hb C and Hb A with Hb S, can accelerate the search both for areas on neighboring hemoglobin molecules complementary to the $\beta 6$ region and for agents capable of interrupting fiber formation. (Research supported by grants from NIH and National Foundation.)

309. Inhibition of Action of Vasopressin by Cytochalasin B. ANN TAYLOR,* HELEN GOLBETZ,* EVE REVEN,* AND ROY MAFFLY, Stanford, Calif.

Vasopressin stimulates smooth muscle contraction and promotes transcellular water movement in responsive tissues. Microtubules and microfilaments are associated with many types of intracellular movement and may participate in processes of mechanico-chemical transduction analogous to those occurring in muscle. We have recently found that agents which disrupt microtubules inhibit vasopressin-induced water movement in the toad bladder. Cytochalasin B (CB) has been reported to disrupt microfilaments in some cells; its effect on the response to vasopressin in the isolated toad bladder has therefore been investigated. While CB 1–20 μg/ml slightly enhances the basal rate of osmotic water movement, it inhibits (up to 64%) the increase in water movement induced by vasopressin 20 mU/ml; this inhibitory effect is concentration dependent and reversible. Water movement in response to cyclic AMP 5 mM is inhibited to an equivalent degree by CB, indicating that the inhibitory effect is exerted distal to stimulation of cyclic AMP synthesis by vasopressin. Exposure to CB results in leakage of ions across the bladder along the concentration gradient from serosal to mucosal bathing medium, apparently via intercellular channels; however, the decrease in osmotic gradient across the tissue does not account for the reduced response to vasopressin. CB has no effect on basal or vasopressin-stimulated active sodium transport by the bladder. The observed inhibitory effect of CB is consistent with the concept that microfilaments play a role in vasopressin-induced water movement in the toad bladder; we have indeed found conspicuous numbers of microfilaments within the bladder epithelial cells. However, the inhibitory effect of CB is also consistent with a direct action of this agent on the plasma membrane of these cells. (Supported by USPHS grant AM16327.)

310. Prostaglandin E (PGE) Control of Cell Growth In Vitro. DAVID R. THOMAS,* GORDON W. PHILPOTT,* AND BERNARD M. JAFFE,* St. Louis, Mo. (introduced by David H. Alpers).

Prostaglandins may be important modulators of cell growth. The purpose of this study was to correlate PGE levels with cell proliferation in established cell cultures. L, HEp-2, and Hela cells were cultured for 4–8 days in medium alone or with (*a*) 1 mM dibutyryl cyclic AMP, (*b*) 10^{-8} M indomethacin, or (*c*) 3×10^{-6} M PGE_1. Samples were taken at 1- to 2-day intervals for viable cell coounting by vital dye exclusion, and for radioimmunoassay measurement of PGE in cells and medium. Total PGE production (ng/10^6 cells per day) and cell counts were compared with controls. Addition of db-cAmp resulted in 220%, 305%, and 34% increase in PGE with L-cells, Hep-2, and Hela cells, respectively, and a 44%, 74%, and 22% decrease in their respective viable cell counts. Comparative results with addition of indomethacin were a 80%, 63%, and 23% decrease in PGE, with a concurrent rise of 23%, 21%, and 18% in cell counts. Adding 1 mM of PGE_1 resulted in a 28%, 48%, and 44% decrease in viable cell count in these cell lines. Under these conditions, PGE concentrations varied inversely with cell growth rates. Increasing PGE concentrations by adding PGE_1 or db-cAmp slowed growth, and more importantly, lowering endogenous PGE concentrations with a synthesis inhibitor (indomethacin) accentuated growth. When L-cells were cultured in the presence of both 10^{-8} M indomethacin and 10 ng/ml PGE, the growth curve approached normal, which further implicates prostaglandin E as a modulator of cell growth.

311. Lanthanum Permeability of the Tight Junction in the Rat Nephron. C. C. TISHER,* W. E. YARGER,* AND R. R. ROBINSON, Durham, N. C. (introduced by J. R. Clapp).

Measurements of transepithelial electrical resistance have permitted separation of transporting epithelia into those whose resistances are relatively low, such as rabbit gall bladder, or high, such as toad urinary bladder. Where examined, the tight junction of low resistance epithelia is permeable to the extracellular tracer, lanthanum, thus supporting the existence of a shunt pathway for ion and water movement. In this study the permeability of tight junctions (zonulae occludentes) in proximal and distal convoluted tubules and cortical collecting ducts of the rat were evaluated with lanthanum. Eight nonexpanded and nine volume-expanded rats were studied. Nonexpanded animals received 0.02 ml/min of isotonic saline. Volume-expanded animals received isotonic bicarbonate-Ringer's solution (0.375 ml/min) until 10% of the body weight had been administered. Individual nephrons from the two groups of animals were preserved for ultrastructural examination by intraluminal microperfusion with a glutaraldehyde-formaldehyde or an osmium tetroxide solution followed by microperfusion with lanthanum hydroxide. Other tubules were microperfused with 1 mM $LaCl_3$ before microperfusion fixation, or preserved via drip-fixation *in situ* before perfusion with lanthanum hydroxide. GFR, $U_{Na}V$, and EF_{Na} were all significantly higher in volume-expanded animals: 1.71 ± 0.17 vs. 0.84 ± 0.15 ml/min; 13.211 ± 1.372 vs. 0.203 ± 0.400 μEq/min; and 6.06 ± 1.16 vs. 0.24 ± 0.08%, respectively. In both groups of animals, tight junctions of proximal and distal convoluted tubules were permeable to lanthanum, while those of the cortical collecting tubules were impermeable. The results were independent of the type and osmolality of the fixative and the method of fixative application. They correlate well with previously measured transepithelial electrical resistances and provide morphological evidence for the existence of a paracellular shunt pathway for ion and water movement in proximal and distal convoluted tubules of the rat.

312. Phytohemagglutinin Induction of the Platelet Release Reaction. DOUGLAS M. TOLLEFSEN* AND PHILIP W. MAJERUS, St. Louis, Mo. (introduced by Stuart Kornfeld).

We previously postulated that thrombin initiates the platelet release reaction by inhibiting adenylate cyclase, a component of the platelet plasma membrane. We also demonstrated that several phytohemagglutinins bind to the surface of intact human platelets and that two of these (the erythroagglutinating [E-PHA] and leukoagglutinating [L-PHA] PHAs of *Ps. vulgaris*) inhibit adenylate cyclase activity. We now find that E-PHA causes platelet aggregation and induces release of [^{14}C] serotonin from platelets. Release follows binding of E-PHA, and haptenic inhibitors of E-PHA binding prevent induction of release. E-PHA does not produce platelet lysis and has little effect on [^{14}C] serotonin uptake. Platelets possess approximately 300,000 receptor sites for E-PHA per cell, and

we estimate that about 15% of these sites must be occupied by E-PHA to initiate the release reaction. Prior incubation of platelets with prostaglandin E_1 or other agents withc increase platelet cyclic AMP levels prevents E-PHA-induced release, although these agents have little effect on E-PHA binding to platelets. Thrombin and E-PHA produce different rates and extents of serotonin release. Throbmin (1 U/ml) causes release of 75–85% of platelet [^{14}C] serotonin, with half-maximal release occurring less than 0.5 min after thrombin addition. E-PHA, however, induces release of a maximum of 40–60% of platelet serotonin at a 10-fold lower rate. These experiments indicate that platelet aggregation and the release reaction may be triggered in part by binding of E-PHA to the cell surface and that these reactions are mediated in some manner by cAMP. (Supported by grants from NIH and ACS.)

313. In Vivo Toxicity of Cyanate in Rats. PHILLIP TOSKES,* PAUL HILDEBRANDT,* BERTIL GLADER,* THOMAS BENSINGER,* FREDERICK RICKLES,* AND MARCEL CONRAD, Washington, D. C.

Cyanate has been proposed as a potential agent for the treatment and prevention of occlusive sickle-cell disease. It is postulated that cyanate prevents sickling by carbamylating the terminal valine residue of sickle hemoglobin. This reaction is nonspecific because cynate reacts with the amino and thiol groups of many proteins and produces irreversible inactivation of G6PD activity in erythrocytes in vitro. To investigate the toxicity of NaCNO in vivo, rats were fed 50 mg/kg (116 μmoles) by gastric tube daily for 8 wk. Except for mild lethargy, this dose was tolerated well. No abnormalities were found in iron and vitamin B_{12} absorption, fecal fat excretion, or intestinal disaccharidase levels. G6PD activity in erythrocytes, intestinal mucosa, brain, kidney, and adrenal glands was normal. However, G6PD values were decreased in the livers of NaCNO-treated rats, 37.2±2.6 (mean ±SE) vs. 66.4±4.6 enzyme U/g of protein, $P < 0.05$, Histological sections showed increased glycogen deposition in hepatocytes. Larger doses of NaCNO (200 mg/kg, 497 μmoles) fed to rats for 10 days caused severe lethargy in half the animals, some of which developed hind limb paralysis. These rats had decreased hepatic G6PD activity, 37.9±3.9 vs. 73.2±4.2, $P < 0.01$, whereas the other rats which tolerated NaCNO had normal G6PD activity. Liver sections from both groups of rats showed marked glycogen deposition (PAS positive and diastase digestable) which was most severe in periportal hepatocytes. In addition, increased mitosis of parenchymal cells was seen. Electron micrographs showed severe glycogen deposition with compression and displacement of smooth and rough endoplasmic reticulum. These data suggest that large doses of NaCNO causes hepatic toxicity in rats from carbamylation of liver enzymes in vivo.

314. Radioreceptor Assay (RRA) for Human Growth Hormone (hGH). TOSHIO TSUSHIMA* AND HENRY FRIESEN, Montreal, Canada.

A RRA for hGH has been developed using the 100,000 g pellet obtained from pregnant rabbit liver tissue homogenates. However, specific receptors for hGH also were found in several other rabbit tissues (adrenal ≫ liver > kidney). In the routine assay 30–40% of [^{125}I] hGH was specifically bound to the liver receptors and 85% was displaced with 1 μg/ml hGH. Pituitary extracts or purified GH preparations from bovine, rat, and rabbit also inhibited the binding of [^{125}I] hGH, but in a nonparallel manner. HPL and hPRL displaced [^{125}I] hGH, but only at concentrations greater than 1 μg/ml. hTSH, hLH, hFSH, and insulin did not cross-react. After isoelectric focusing of human pituitary extracts, three major hGH components were detected by RIA and RRA in identical fractions. Potency estimates of GH preparations obtained by RRA and radioimmunoassay (RIA) correlated well. The sensitivity of the RRA using liver membranes was 5 ng/ml. More sensitive assays could be obtained with adrenal receptors. In six acromegalics, estimates of serum hGH by RIA and RRA were in agreement, whereas in seven hypopituitary patients the values were undetectable by RRA. In one patient with Laron type dwarfism, serum hGH by RRA as well as by RIA exceeded 100 ng/ml, suggesting that the hGH molecule secreted by this patient was biologically active. (Research supported by MRC and NIH.)

315. Diminished Thyroid-Stimulating Hormone (TSH) Reserve After Long-Term Thyroid Suppression. APOSTOLOS VAGENAKIS,* FEREIDOUN AZIZI,* GARY PORTNAY,* JACQUES DERIDDER,* LEWIS BRAVERMAN, AND SIDNEY INGBAR,** Boston, Mass., and San Francisco, Calif.

Recovery of pituitary-thyroid function after withdrawal of long-term thyroid suppression has been evaluated in six goitrous subjects, one of whom was receiving estrogen. Before discontinuing thryoxine or desiccated thyroid medication, values for serum T_4(D) were 9.2±1.3 μg/100 ml (mean ±SE), T_3(RIA) 132±21 ng/100 ml, and TSH(RIA) undectable (< 1.2 μU/ml); no TSH response to intravenous TRH (250 μg) occurred; 24-hr ^{131}I thyroidal uptakes were < 6%. These indices of pituitary-thyroid function were reassessed at weekly intervals for 8 wk after discontinuing therapy. 1–2 wk after withdrawal of thyroid medication, serum T_3 and T_4 concentrations decreased into the hypothyroid range except for the serum T_4 in the estrogen-treated patient, the nadirs averaging 30.2±9.0 ng/100 ml and 2.9±0.7 μg/100 ml, respectively. In spite of the decreased serum T_4 and T_3 concentrations during this period, basal TSH levels were not elevated and no TSH response to TRH was evident in five of the six subjects until the second or third week after thyroid withdrawal. A blunted TRH response occurred in the estrogen-treated patient at the first week. A greater TSH response to TRH occurred 1–2 wk after the initial response in four subjects, at which time serum T_4 and T_3 concentrations were below normal in three. TRH responsiveness remained stable thereafter. Thyroidal ^{131}I uptake returned to normal (> 15%) 2–3 wk after hormone withdrawal, with a peak rise 1–2 wk later. The present study represents the first systematic evaluation of pituitary-thyroid function after withdrawal of long-term thyroid suppression. The pituitary was unresponsive to TRH despite subnormal serum concentrations of T_4 and T_3. Apparent diminished TSH reserve may persist for several weeks after thyroid hormone withdrawal.

316. Evidence for In Vivo Cloning of an hCG-Secreting Tumor. JUDITH L. VAITUKAITIS,* Bethesda, Md. (introduced by Mortimer B. Lipsett**).

The origins of heterogeneity observed in hormonal peptides secreted by neoplasms remain unknown. The possibility that clonal selection in vivo might result in qualitative differences in synthesis and secretion of hormonally related peptides was studied. We therefore characterized hCG and its subunits in plasma, urine, and extracts of tumor and two metastases, obtained from a man with a mediastinal hCG-secreting tumor. These were filtered on a Sephadex G-100 column, calibrated with hCG, hCGα, hCGβ, and blue dextran. Each eluate was assayed in a homologous hCG, hCGα, and hCGβ radioimmunoassay. In addition to native hCG, the primary tumor and each metastasis contained other hCG peaks with elution volumes different from that of native hCG and hCGβ. The amount of hCG and its altered forms, as well as the quantity of hCGβ per milligram tissue, varied among the tumor extracts. Two distinct forms of hCG, one indistinguishable from

highly purified hCG, were present in the plasma along with a small amount of hCGα. The urinary pattern of hCG excretion appeared to be the resultant of the abnormal forms of hCG, hCGβ, and hCGα observed in plasma and the tumor tissue extracts, suggesting that the altered forms of hCG and the presence of subunits was not an artifact of urinary excretion. Since hCGα was present in plasma and urine but not in the primary tumor or the two metastases, it is probable that other metastases were secreting that subunit. The qualitative and quantitative differences in the altered forms of hCG and its subunits found in the primary tumor and its metastases suggest in vivo cloning of cells from the primary tumor to the metastases. This is the first evidence for spontaneous cloning of a cancer in vivo.

317. C3 Nephritic Factor (C3NeF) from Plasma of a Patient with Chronic Glomerulonephritis and Hypocomplementemia (CGH): Isolation and Characterization. ENRIQUE H. VALLOTA,* HANS L. SPIEGELBERG,* JUDITH FORRISTAL,* CLARK D. WEST,* AND HANS J. MÜLLER-EBERHARD, La Jolla, Calif., and Cincinnati, Ohio.

C3NeF has been isolated from plasma of a patient with CGH by ion-exchange and molecular sieve chromatography. This material was further treated with solidified anti-IgG antiserum. Antiserum to human IgG, IgG3, Fab, Fc and kappa or lambda chains failed to react with the purified active material. A single antiserum to normal human serum (NHS) showed three precipitin lines on Ouchterlony test which gave a pattern of nonidentity with IgG. Isolated C3NeF was found to be a protein with an s-rate of 7S and a mol wt of approximately 150,000, which on gel electrophoresis at pH 8.6 behaved as a cation. C3NeF is not a C1q precipitin. Unlike cobra factor, C3NeF failed to enter into a complex with C3 proactivator (C3PA) when incubated with NHS and then subjected to sucrose density-gradient ultracentrifugation. The isolated material produced C3 and C3PA cleavage when added to NHS or C2-deficient serum. No residual hemolytic activity was found after incubation of 100 μl NHS with 6.4 μg C3NeF at 37°C for 30 min. However, neither C3 nor C3PA cleavage occurred when C3NeF was incubated with serum which was C3PA convertase deficient but has normal hemolytic activity. C3a and C5a anaphylatoxins were consistently generated by C3NeF in serum lacking anaphylatoxin inactivator activity. The results indicate (*a*) that C3NeF is not an immunoglobulin, (*b*) that it initiates cleavage of C3 through the alternate complement pathway, and (*c*) that it is unable to activate the classic pathway. The possibility is raised that it constitutes an altered form of an early acting component of the bypass mechanism. (Supported by USPHS grant AI-07007.)

318. Circulatory Responses to Hemorrhage in Conscious Dogs. STEPHEN F. VATNER, Boston, Mass. (introduced by Eugene Braunwald).

It is generally held that hemorrhage activates the sympathetic nervous system, resulting in increases in heart rate, contractility, and intense vasoconstriction, particularly in the renal vascular bed. The left ventricular and regional vascular responses to hemorrhage were examined in eight healthy conscious dogs 2–6 wk after instrumentation with Doppler or electromagnetic flow probes on the ascending aorta, and the mesenteric, iliac, and renal arteries, and with pressure gauges in the aorta and left ventricle for arterial pressure, left ventricular pressure, dP/dt and dP/dt/P, and internal left ventricular diameter. Hemorrhage, 24±2 ml/kg, reduced mean arterial pressure from 98 to 75 mm Hg, cardiac output from 2.3 to 1.3 liters/min, left ventricular end-diastolic diameter from 38.7 to 33.3 mm, dP/dt from 3520 to 3100 mm Hg/sec; increased heart rate from 75 to 154 beats/min; and did not change dP/dt/P, while it decreased mesenteric (−56%) and iliac (−58%) flows and increased mesenteric (+78%) and iliac (+102%) resistances. In contrast, renal flow rose (+11%) and renal resistance fell (−31%). In four conscious dogs with chronic heart failure produced by tricuspid avulsion and pulmonary stenosis, hemorrhage induced a similar differential pattern in the mesenteric, iliac, and renal beds. Pretreatment with atropine, propranolol, phentolamine, and tripelennamine, singly and in combination, failed to prevent renal vasodilatation with hemorrhage. Hemorrhage after indomethacin, 4 mg/kg, a prostaglandin E blocker, decreased arterial pressure by a similar amount, but decreased renal flow (−47%) and increased renal resistance (+51%). Thus, the compensatory response to moderate hemorrhage in the conscious dog involves increases in heart rate, iliac and mesenteric resistances with little change in contractility and, surprisingly, renal vasodilation occurs. Renal vasodilation also occurs with hemorrhage in heart failure and appears to be caused by a prostaglandin E compound. Renal autoregulation may be explained, in part, by the release of such a compound.

319. Release of Vasoactive Substances from the Lung During Hypoxic Breathing. CAROL VREIM,* SATOSHI KITAMURA,* AND SAMI I. SAID, Dallas, Tex.

It has long been known that hypoxia evokes constriction of pulmonary vessels. Recent evidence suggests that lung tissue is essential to the vasoconstrictor response, but the mechanism of this interaction is poorly understood. This investigation was designed to test the hypothesis that vasoactive substances are released from the lung during hypoxia, and to characterize any which may be found. Cat lungs were isolated, ventilated mechanically with air or hypoxic (8%, 2% O_2) mixtures, and perfused with plasma or Krebs-dextran at constant flow. The perfusate was made to drip onto strips of smooth-muscle tissues, selected for their sensitivity to biologically active substances. The tissues included rat colon (RC), rat stomach (RS), and guinea pig trachea (GPT). In four experiments, the pulmonary pressor response to hypoxia was associated with contraction of RC and GPT but little or no contraction of RS. In 12 experiments in which the tissues had been previously rendered insensitive to acetylcholine, histamine, serotonin, and catecholamines, the tissue responses were generally unchanged. In two living cats, mechanically ventilated through the trachea, inhibition of prostaglandin (PG) synthesis by intravenous infusion of aspirin (> 3 mg/kg) or indomethacin (> 14 μg/kg) did not affect the pulmonary pressor response to hypoxia on any of seven applications, but reduced or abolished the associated increase in airway pressure. The data provide evidence for the release of biologically active substances from hypoxic cat lungs. These substances are detectable in perfusates of pulmonary vessels and may include PG's, but their full identity remains to be determined. (Research supported by a Center Award from NHLI.)

320. Effects of Breathing Enriched O_2 Mixtures on the Distribution of Ventilation-Perfusion Ratios in Dogs and Normal Human Volunteers. P. D. WAGNER,* R. B. LARAVUSO,* R. R. UHL,* AND J. B. WEST, La Jolla, Calif.

Continuous distributions of ventilation-perfusion ratios ($\dot{V}_A/\dot{Q}$) were measured in 17 dogs and 9 normal human volunteers breathing both air and added O_2. We used a new technique (1972: *Fed. Proc.* **31**: 347) employing the intravenous infusion of a mixture of dissolved inert gases. The dogs were studied after inducing acute respiratory failure by injection of small glass beads or oleic acid into the venous blood. O_2 breathing increased the blood flow to areas in which the $\dot{V}_A/\dot{Q}$ ratio was low (< 0.1) and also resulted in shunts ($\dot{V}_A/\dot{Q}$ of 0). These changes generally occurred within 30 min. In one

apparently normal subject, a shunt of 10.7% developed, and in the dogs, the shunt increased by up to 16%. In the dogs, changes were seen with inspired O_2 concentrations as low as 50%. On returning to room air, shunts were completely reversed in the human subjects (awake, semirecumbent) but this was not the case with the dogs (supine, anesthetized, not periodically hyperinflated). These changes with O_2 could be explained by two processes resulting from the large rise in alveolar P_{O_2} in units with low $\dot{V}_A/\dot{Q}$ ratios. First, expired ventilation falls due to the increased O_2 transfer, and this may be so marked as to result in alveolar collapse. The second mechanism is an increase in perfusion due to the release of hypoxic vasoconstriction. Two clinical implications of these studies are: (*a*) some atelectasis rapidly occurs during O_2 breathing even in health and especially in disease; (*b*) in the measurement of shunt by the traditional method of breathing 100% O_2, much of the shunt may be an artifact of the method, particularly in patients with lung disease. (Supported by NIH grants HE-13687-02, HE-14169-02, and HE-05931-02.)

321. Serial Studies on Hepatitis-Associated Antigen and Antibody in Patients Receiving Chemotherapy for Myeloproliferative and Lymphoproliferative Disorders. JACK R. WANDS,* JOSEPH ROLL,* CATHERINE CHURA,* AND WILLIS C. MADDREY,* Baltimore, Md. (introduced by T. R. Hendrix).

The effects of antitumor chemotherapeutic agents on hepatitis-associated antigen (HAA) and antibody (anti-HAA) were studied serially in patients with myeloproliferative (38 patients) and lymphoproliferative (47 patients) disorders. HAA and anti-HAA titers were determined by hemagglutination (HA) and hemagglutination inhibition (HAI). HAA was detected at some time in 17 patients and anti-HAA in 40 patients. 17 patients who had pretreatment detectable anti-HAA showed a decrease in anti-HAA titer paralleling the fall in white blood cell count. In 14 of these 17 patients a return of anti-HAA to pretreatment levels was observed paralleling an increase in white blood cell count. In five patients, however, HAA appeared when anti-HAA titers fell. In three of these five, a secondary rise of anti-HAA was observed with disappearance of HAA from the serum. In the two others HAA persisted once it appeared. In all three patients who had HAA at the time of chemotherapy, bone marrow suppression was associated with a marked increase in HAA titer. The increase in HAA titer was associated with hepato-cellular damage, as manifested by an elevation in SGPT. In one patient who underwent bone marrow transplantation after cyclophosphamide-induced bone marrow aplasia, a pretransplant anti-HAA titer fell dramatically but reappeared later with repopulation of the marrow with donor cells. No HAA was found. These observations suggest that antitumor agents transiently reduce anti-HAA titers. In some patients HAA appears after decrease in anti-HAA titers. Whether this appearance of HAA is related to an inhibition of antibody formation allowing expression of preexisting antigen or to an inhibition of cellular immunity allowing viral proliferation is uncertain.

322. Role of the Carotid Bodies in the Sensation of Breathlessness. KARLMAN WASSERMAN, J. TERRANCE DAVIDSON,* BRIAN J. WHIPP,* SANKAR KOYAL,* AND ROBERT LUGLIANI,* Torrance, Calif.

To determine the role of the carotid bodies in the sensation of breathlessness during hypoxia and hypercapnia, breath-holding studies were performed in normal subjects and ones with asymptomatic asthma who had had bilateral carotid body resection (CBR). The breath-holding times and the alveolar O_2 ($P_{A_{O_2}}$) and CO_2 ($P_{A_{CO_2}}$) tensions were determined in each subject after inspiratory vital capacity breaths of 100%, 50%, 20%, and 12% O_2 and after breathing 12% O_2 for 1 min. Breath-holding time averaged 2.2 min after a single breath of 100% O_2 in both groups. In normal subjects, breath-holding time and $P_{A_{CO_2}}$ at the breaking point progressively decreased as $P_{A_{O_2}}$ decreased, $P_{A_{O_2}}$ being approximately 10 mm Hg lower than that for the CBR group at a $P_{A_{O_2}}$ = 50 mm Hg., i.e., the lowest value reached by the normal subjects. In contrast, there was no change in $P_{A_{CO_2}}$ or breath-holding time at the breaking point until $P_{A_{O_2}}$ decreased below 50 mm Hg in the CBR group. In the latter group, breath-holding time decreased at $P_{A_{O_2}}$ values between 25 and 50 mm Hg but was still twice that of the control subjects when $P_{A_{O_2}}$ = 50 mm Hg. These studies indicate (*a*) that the carotid bodies play a role in the breaking point of breath holding, when $P_{A_{O_2}}$ is below 300 mm Hg; (*b*) that the well recognized interaction of CO_2 and O_2 tension on respiratory control appears to occur exclusively at the level of the carotid bodies; and (*c*) the sense of "breathlessness" in hypoxic states is reduced in the CBR subjects as evidenced by breath-holding times, increased $P_{A_{CO_2}}$, and decreased $P_{A_{O_2}}$ at the breaking point. (Supported by NIH grant HL 11907.)

323. Immunocompetent Cells Develop Hormone Receptors During Immunization. YACOB WEINSTEIN,* KENNETH L. MELMON, HENRY R. BOURNE,* AND G. M. SHEARER,* San Francisco, Calif., and Bethesda, Md.

Hormone receptors of mouse splenic leukocytes were studied pharmacologically, by measurement of hormone-induced increases in cellular cyclic AMP (cAMP), and by binding of cells to insolubilized hormones chemically linked to protein or peptide carriers which were in turn linked to Sepharose (S) beads. We have previously documented leukocyte receptors for histamine, beta-adrenergic catecholamines, and E prostaglandins, and have shown that insolubilized preparations of the same hormones (but not the carriers of S) were responsible for affinity binding. Since we had also shown that hormone-carrier-S columns did not subtract precursors of antibody-forming cells, we asked whether commitment of a cell to antibody formation might develop *pari passu* with hormone receptors. When splenic leukocytes from BALB/BL or BALB/c mice immunized with sheep erythrocytes were passed over columns of hormone-carrier-S, certain insolubilized hormone preparations [histamine (H), isoproterenol (I), epinephrine (E), and PGE_2] subtracted $\frac{1}{3}$ to $\frac{2}{3}$ of the 7S or 19S hemolytic plaque-forming cells (PFC). PFC were not subtracted by S-linked norepinephrine (N), $PGF_{2\alpha}$, S alone, or S-carrier. The corresponding free drugs showed a complementary pattern when tested for ability to inhibit plaque formation in vitro: H, I, E, PGE_1, and PGE_2 produced dose-dependent inhibition of plaque formation, whereas $PGF_{2\alpha}$, N, and phenylephrine were not inhibitory. The inhibition correlated with increased leukocyte cAMP, was potentiated by theophylline, and was reproduced by dibutyryl cAMP and cholera enterotoxin. Therefore, H, beta-adrenergic amines, and E prostaglandins work through cAMP to inhibit production or release of specific antibody by mouse spleen cells. Specific receptors for the same hormones are present on plaque-forming cells, but precursors of PFC do not have the same hormone receptors. It is therefore likely that the receptors develop during the course of immunization and in parallel with antibody production.

324. Intestinal Surface Membranes: Factors Common to Fetal, Intestinal Tumor, and Mitotically Active Crypt Cells. MILTON M. WEISER,* DANIEL PODOLSKY,* AND J. THOMAS LA MONT,* Boston, Mass. (introduced by Kurt J. Isselbacher).

It has been suggested that cell-surface glycoproteins and glycosyltransferases serve as determinants of cell behavior, and that fundamental changes in membrane glycoproteins and

glycolipids accompany malignant transformation. We have now demonstrated that the cell surface membranes of human fetal intestinal cells, rat intestinal tumor cells, and normal mitotically active rat intestinal crypt cells appear to have similar active glycosyltransferase enzyme activities and endogenous glycoprotein acceptors. Intestinal crypt cells were separated from villus cells as an isolated cell preparation (M. M. Weiser, *J. Biol. Chem.* In press); rat intestinal tumors were induced with 1,2-dimethylhydrazine. Upon incubation with nucleotide sugars, crypt cells, intestinal tumor cells, and human fetal cells were shown to have glycosyltransferase:endogenous acceptor activities that were at least 10-fold greater than that seen for normal, differentiated villus cells. The one exception was sialyltransferase:endogenous acceptor activity, which characteristically was higher on normal villus cells and low or absent on the undifferentiated cells. Both human fetal cells and normal rat intestinal crypt cells were preferentially agglutinated by concanavalin A. When concanavalin A was covalently attached to the surface of nylon fibers, the resultant concanavalin A-derivatized fibers were found to preferentially select crypt cells, thus giving further evidence of their apparent affinity for concanavalin A. The cells adhering to the fibers were cells that incorporated [^{3}H] thymidine and exhibited the cell-surface membrane glycosyltransferase:endogenous acceptor activities of intestinal crypt cells. These results indicate that the cell-surface membranes of malignant, fetal, and undifferentiated germinal cells hvae features in common which distinguish them from differentiated cells. This suggests the possibility that onco-fetal factors such as the carcinoma-embryonic antigen of Gold, a membrane-associated glycoprotein, may also be a membrane constituent of the normal undifferentiated crypt cell. (Research supported by NIH grants AM-03014 and ACSBC-93.)

325. Immunopathogenesis of the Vasculitis of Rheumatoid Arithritis. MICHAEL H. WEISMAN* AND NATHAN J. ZVAIFLER, San Diego, Calif.

A vasculitis of unknown etiology complicates rheumatoid arthritis. Seven patients with rheumatoid vasculitis were studied. All had peripheral neuropathy, and six had dermal infarcts and digital gangrene. As a group they had high titers of rheumatoid factor (antigamma globulins) and modest depression of serum C3. The serum of all seven contained cryoprecipitable proteins. Serum samples (5 ml) were kept at 4°C for 72 h. The precipitates that formed were washed and resuspended in 1 ml of normal saline. The protein concentration of the vasculitis cryoglobulins averaged 0.4 mg/ml. For comparison, sera of 36 RA patients without vasculitis were examined; 14 (39%) had cryoglobulins with protein concentrations $\geqq$ 0.1 mg/ml (average 0.17 mg/ml). IgG and IgM were detected in all vasculitis cryoglobulins; four contained Clq or C3. All nonvasculitis cryoglobulins had IgG, 8 IgM, and 5 C1q or C3. Antigamma globulin activity was present in all vasculitis cryoglobulins. Two had titers similar to or greater than the corresponding serum. A few (4 or 14) nonvasculitis cryoglobulins had low-titer antigamma globulins. A portion of the antigamma globulin activity of four of five vasculitis cryoglobulins resisted reduction with mercaptoethanol, but in density-gradient ultracentrifugation analysis, antigamma globulin activity was limited to the IgM region. Vasculitis cryoglobulins were not precipitated by a monoclonal rheumatoid factor that detects IgG—7s anti-IgG complexes. Cyclophosphamide therapy controlled the vasculitis of four patients. Serial studies disclosed a marked reduction of cryoprecipitable protein in two, and a significant fall in the antigamma globulin titers in three cryoglobulins. One untreated patient continues to show large amounts of cryoglobulins containing high-titered antigamma globulins. Large amounts of cryoglobulins containing antigamma globulins are regularly found in the serum of patients with rheumatoid vasculitis, and their fall with immunosuppressive therapy and clinical improvement suggests that circulating immune complexes of IgG—19s anti-IgG are pathogenetic in the vasculitis of rheumatoid arthritis. (Supported in part by funds from the USPHS.)

326. Effect of Bromodeoxyuridine (BrdU) on the Oncogenic Potential of Murine Tumor Cells. MARC E. WEKSLER* AND SELMA SILAGI,* New York (introduced by G. W. Siskind).

The addition of BrdU to cultures of murine melanoma was previously shown to reduce their oncogenic potential. Such cells regain oncogenic potential when cultured in medium without BrdU. A similar effect of BrdU on a highly oncogenic AKR murine lymphoblastic leukemia cell line has been documented. AKR lymphoblasts grown in medium supplemented with 6 μg/ml BrdU are less oncogenic than lymphoblasts grown in medium supplemented with 6 μg/ml thymidine. 28 AKR mice given 100 viable BrdU-exposed lymphoblasts intravenously had a mean survival 2.4 times that of 40 mice given thymidine-exposed lymphoblasts. Further, 23% of mice given BrdU-exposed lymphoblasts survived 120 days without apparent disease, while all mice given thymidine-exposed lymphoblasts were dead within 60 days. BrdU stimulates the expression of latent viral infection of murine cells. Tumor cells incubated with BrdU may be less oncogenic because expressed cell surface determinants may subject these cells to immunological rejection. To test this hypothesis melanoma cells incubated with BrdU were injected into C57B1/6J mice given either rabbit antithymocyte serum (ATS) or normal rabbit serum (NRS). 82% (23/28) of mice treated with ATS died after injection of BrdU-exposed melanoma cells, while only 6% (1/17) mice treated with NRS died. One-way mixed leukocyte reaction offered evidence for an immunologic response of host cells to BrdU-exposed tumor cells. AKR lymph node lymphocytes mixed for 72 h with washed, irradiated, BrdU-exposed lymphoblasts incorporate 2.4 times the amount of [^{3}H] thymidine incorporated by the unmixed cellular components. In conclusion, incubation of two murine tumor lines with BrdU results in their loss of oncogenic potential. This effect is at least in part mediated by the capacity of the recipient animal to mount an immune reaction toward these cells.

327. Immunoglobulin Synthesis in Cultured Lymphocytes from a Patient with Immune Deficiency Mediated by a Serum Factor. PETER WERNET,* FREDERICK P. SIEGAL,* HOWARD DICKLER,* SHU MAN FU,* AND HENRY G. KUNKEL,** New York.

A variety of studies were carried out on the isolated peripheral blood lymphocytes of a 64-y-old female with severe Ig deficiency, a thymoma, and seemingly intact cellular immunity. By immunofluorescence her cells were consistently negative for surface Ig staining. However, by means of another B-cell marker, staining with aggregated IgG, 3–12% of the cells showed positive staining. Since these two procedures give completely parallel results in normal blood lymphocytes, a cellular defect at the B-cell level appeared possible. Short- and long-term cultures of the patient's lymphocytes under various conditions led to the appearance of surface Ig on up to 10% of the cultured cells. This was demonstrated by three different methods including immunofluorescence, surface radioiodination, and Ig consumption. Ig was also found in the culture supernatants. With the surface radioiodination procedure, early primary synthesis of IgM was observed, whereas later cultures showed mainly IgG. A factor present in normal human serum and fetal calf serum was required for the Ig production; no synthesis was found in cultures where the pa-

tient's own serum was used. Human thymocytes or normal peripheral blood lymphocytes stimulated by conA replaced the deficiency in the patient's serum. This was demonstrated through Ig synthesis when a 0.22 μ Millipore filter separated the patient's cells and serum from the thymocytes or normal lymphocytes. A possible interpretation of these findings is that the necessary factor represents a thymus-derived B-cell differentiation factor which is absent in the patient. Culture studies with the patient's cells and serum offer a useful means of delineating this factor.

328. Small Bowel Cell Loss After Intestinal Resection. ELLIOT WESER,* TREASURE TAWIL,* AND LEONEL RODRIGUEZ,* San Antonio, Tex. (introduced by S. J. Friedberg).

After small bowel resection, mucosal hyperplasia and increased cell turnover occur in remaining intestine. At the end of their life span, epithelial cells are shed into the bowel lumen. This cell loss may be estimated by measuring the DNA loss into a gut segment. Male Sprague-Dawley rats underwent resection of 50 cm of proximal or distal intestine or sham operation. 1 and 6 months after surgery, 50 cm of the remaining proximal or distal remnant was perfused with saline in vivo for 2 h and the perfusate DNA was assayed. In addition, mucosal epithelium was isolated from bowel remnants and weighed, and DNA content was assayed. In human studies, a duodenal segment was perfused with 1000 ml of saline containing PEG as a marker in a patient with 75% bowel resection and in three control subjects. 1 and 6 months after resection DNA was significantly increased over shams in the perfusate from distal rat remnants: 5.02 vs. 2.30 ng atoms DNA-P per min ($P < 0.02$), and 2.0 vs. 0.65 ($P < 0.05$), respectively. Mucosal weight and DNA concentration per centimeter length of gut was significantly increased in distal remnants, but not proximal remnants. DNA concentration per milligram weight of mucosa remained unchanged from control shams. Similar findings were noted for distal remnants 6 months after resection. The patient with intestinal resection had 5 times more DNA in the perfusate than the control mean. We conclude that mucosal hyperplasia after intestinal resection is most striking in distal bowel remnants. Increased DNA loss into the lumen reflects a greater mucosal cell mass and/or increased cell turnover. (Research supported by grant from NIAMDD.)

329. Rapid Increase in Platelet Cyclic 3′,5′-Guanosine Monophosphate (cGMP) Levels in Association with Irreversible Aggregation, Degranulation, and Secretion. JAMES G. WHITE, NELSON D. GOLDBERG,* RICHARD D. ESTENSEN,* MARI K. HADDOX,* AND GUNDU H. R. RAO,* Minneapolis, Minn.

Physical alterations and changes in the intracellular concentrations of cGMP and serotonin in platelets were examined after exposure of stirred and unstirred samples of citrate platelet-rich plasma (C-PRP) and washed platelets to collagen, thrombin, epinephrine, and phorbal myristate acetate (PMA). The concentration of cGMP in controls averaged 1.5 pmoles/10^9 platelets. A 2- to 4-fold increase in platelet cGMP occurred at the onset of irreversible aggregation in C-PRP after addition of collagen, thrombin, or epinephrine. Small amounts of PMA, the active principle of croton oil, caused irreversible platelet aggregation and nearly complete degranulation without causing injury to the cells. A 2½-fold rise in platelet cGMP was apparent 15 s after exposure to this agent, and increased to 3½-fold at 30–40 s. Levels of cyclic 3′,5′-adenosine monophosphate (cAMP) fell by 25% over thes ame period. Serotonin release from PMA-stimulated platelets followed the same rapid time course as the rise in cGMP. Prostaglandin E_1, depending on its concentration, partially or completely inhibited PMA-induced aggregation, degranulation, and serotonin release. These findings indicate that aggregating agents which cause irreversible aggregation also stimulate a rapid increase in intracellular cGMP which is apparent at the time of the platelet release reaction. The coincidence of the rise in cGMP with release, degranulation, and irreversible aggregation suggests that this cyclic nucleotide is linked to the process of platelet secretion. (Supported by grant HE-11880 from the USPHS.)

330. Purification and Immunologic Assessment of Myocardial Myosin Chains and Na^+, K^+-ATPase Membrane Systems. J. WIKMAN-COFFELT,* RAMON A. FABREGAS,* CLAUDA FENNER,* ROBERT ZELIS,* AND DEAN T. MASON, Davis, Calif.

Improved characterization of myosin and Na^+, K^+-ATPase in cardiac muscle has been provided in this study by the development of advanced purification procedures and new immunologic techniques allowing qualitative and quantitative evaluation. Thus, pure heavy and light myosin chains were obtained from canine myocardial tissue by differential salt precipitation of the various chains and a buffer system which reduces protein interaction. The heavy (mol wt 2.1×10^5) and the two light chains (mol wt 2×10^4 and 3×10^4) give single bands on SDS-polyacrylamide gels. An antiserum to each of the chains has been developed giving single precipitin lines and showing no cross-reaction with the other myosin components in an Ouchterlony assay. The dissociated antigen-antibody complex, when electrophoresed on SDS-polyacrylamide gels, also demonstrated specificity of the antibody for its respective chain. The antiserum is used as a means of myosin purification and allows for study of turnover rates of heavy and light chains; the heavy chains have a shorter half-life by several days, as compared to the light chains. Further, a radioimmunoassay has been developed giving a sensitive quantification of myosin in small samples of tissue. In additional studies, Na^+,K^+-dependent membrane ATPase was prepared by deoxycholate-sodium iodide-treated microsomes extracted from the dog heart and identified by electrophoresis. An antibody was developed against 3.2×10^4 and 9.6×10^4 mol wt proteins. Further immunologic identification of these proteins is under analyses by lubrol solubilization and purification by column chromatography. These new myosin and Na^+, K^+-ATPase determinations, combined with our previously developed immunoassays for DNA and ribosomes, afford advancement in investigation of protein molecular properties in the normal, hypertrophied, and failing myocardium. (Supported in part by NIH HL-14780.)

331. Increase in Lysosomal Proteolytic Enzyme Activity in Hearts of Fasted Animals: Possible Role of Insulin Defficiency. KERN WILDENTHAL,* A. ROBIN POOLE,* AND JOHN T. DINGLE,* Dallas, Tex., and Cambridge, England (introduced by Daniel W. Foster).

To assess the response of the heart's lysosomal proteolytic capacity to food deprivation, mice and rabbits were starved for 1–3 days. The activity of cathepsin D in the left ventricle was increased by 14% after 1 day and by 30–45% after 3 days ($P < 0.01$ for both conditions). The increase occurred in both the "free" and the "bound" fractions of the tissue homogenate. Simultaneously, activity of acid phosphatase and beta-acetylglucosaminidase remained unchanged. Fluorescent staining of the tissue with specific anticathepsin D antiserum revealed that the enzyme was located primarily in interstitial cells in the control state; after starvation there was no apparent change in the interstitial cells, but myocytes displayed increased staining. The results indicate that cardiac muscle, like liver and unlike skeletal muscle, increases its lysosomal proteolytic capacity during brief periods of starvation. Unlike liver, however, the heart does not undergo a general stimula-

tion of lysosomal activity, and at least some lysosomal enzymes remain unaffected. To test for a possible influence of insulin deficiency in mediating the observed changes, isolated hearts of late-fetal mice were maintained in organ culture for 2–3 days, in the presence or absence of purified bovine insulin (50 μg/ml). Activity of cathepsin D was 24% greater in the insulin-deprived group than in litter-matched hearts exposed to the hormone (66.4 vs. 53.5 g tyrosine per h per mg protein, $P < 0.01$). These data demonstrate that insulin deficiency under precisely controlled conditions in vitro is accompanied by stimulation of cardiac cathepsin D activity. It is suggested that hypoinsulinemia may account for at least part of the increased catheptic activity that occurs in hearts of fasted animals. (Research supported by grants from DHA, MRC, and NHLI.)

332. The Influence of Hypertonic Mannitol on Regional Myocardial Blood Flow (RMBF) During Acute Myocardial Ischemia (AMI). JAMES T. WILLERSON,* JOHN T. WATSON,* W. L. SUGG,* AND DAVID E. FIXLER,* Dallas, Tex. (introduced by Jere H. Mitchell).

Previously hypertonic mannitol has been shown to improve left ventricular function and total coronary blood flow while reducing myocardial injury during AMI in anesthetized dogs. In the present study the influence of mannitol on RMBF during AMI was examined in 11 anesthetized dogs (chloralose) at constant cardiac outputs and heart rates as provided by right heart bypass and atrial pacing. RMBF during AMI was measured using 8-μ radioactive microspheres. A separate group of eight dogs was studied as "time-related controls." Myocardial ischemia was provided by reversibly ligating the proximal left anterior descending artery. Mannitol was infused intravenously at 3.82 ml/min for 30 min before ischemia. Dextrose and water was similarly given before the control ligation. Average serum osmolality increased 24 mOsm during mannitol; mean hematocrits were not significantly different (32.4 vs. 29.6 for mannitol). Neither mean systemic arterial pressure (MAP) nor LVEDP was significantly different between groups (MAP 70±3 SE vs. 67.5±3 for mannitol; LVEDP 10±1.0 vs. 10±1.2 for mannitol). Mannitol resulted in significant increases in collateral coronary blood flow (CCBF) to the ischemic area of 33±12% (0.07 ml/g) ($P < 0.025$). Significant increases in RMBF to the ventricular septum of 50±9% (0.32 ml/g) ($P < 0.001$), to the nonischemic portion of the LV of 46±8% (0.28 ml/g) ($P < 0.001$), and to the right ventricle of 34±13% (0.22 ml/g) ($P < 0.025$) also occurred during the mannitol ligation. Similar increases in RMBF were not found in time-related controls. It appears that mannitol results in increased CCBF during AMI to the ischemic region while also increasing RMBF to periischemic areas. It seems likely that this at least in part explains the improved ventricular function and reduced injury previously demonstrated. (Research supported by a grant from NHLI.)

333. Effect of Digitoxin on Myocardial Response To a Pressure Load. JOHN F. WILLIAMS, JR., Galveston, Tex.

The role of digitalis in the treatment of heart disease without failure remains argumentative. Therefore, 195 cats underwent pulmonary artery banding (4 mm ID clip). 64 cats, group A, received 75 μg/kg of digitoxin at banding and 15 μg/kg per day thereafter. The remainder, group B, were untreated. 9 group A and 34 group B cats died from heart failure ($P < 0.05$). Right ventricular papillary muscle mechanics were determined in 12 group A and 20 group B cats without failure after 6 wk. 19 normal cats and 9 nonbanded cats given digitoxin for 6 wk served as controls. Right ventricular weight was 56±8.9 (SEM) and 38±6.1% above normal in groups A and B, respectively (A vs. B, $P > 0.05$). Passive length-tension curves among groups were similar. In group B at peak resting tension, developed isometric tension averaged 3.0±0.3 g/mm² and maximum velocity of muscle shortening (isotonic contraction) 0.41±0.06 muscle lengths per s. Respective values in group A were 4.6±0.5 and 0.69±0.12, digitoxin controls 5.9±0.7 and 0.72±0.1, and untreated controls 4.9±0.3 and 0.74±0.05. Group B values were significantly different from values in each of the other groups; values among group A and controls were not. 8 animals in groups A and B were sacrificed after 24 wk. No differences in extent of hypertrophy, peak developed tension, or maximum velocity of muscle shortening between these groups was observed. Although "prophylactic" use of digitoxin reduced mortality from heart failure and maintained myocardial function for a short period after banding, the development of ventricular hypertrophy was not affected nor was the enhancement of myocardial performance sustained.

334. In Vitro Response of Skin Vessels in Scleroderma to Catecholamines and Temperature. RICHARD K. WINKELMANN* AND MARC E. GOLDYNE,* Rochester, Minn. (introduced by Ward S. Fowler**).

Isolated, cutaneous, vascular, smooth-muscle strips were prepared from arterioles taken from the calf of 1 patient, the volar wrist of 12 scleroderma patients with Raynaud's phenomenon, and the skin of control patients who had breast, carpal-tunnel, or amputation surgery. The strips were studied for response to norepinephrine, and the responses were compared with those of a standard dose of potassium chloride during cooling from 37° to 15°C and during rewarming. As the temperature was lowered, scleroderma vessel strips lost their responsiveness to norepinephrine before control strips did, and with rewarming, these strips regained their responsiveness at a higher temperature than control strips did. A rapid rate of cooling (0.2°–0.3°C/min) caused an earlier loss of response to norepinephrine in scleroderma vessel strips than slow cooling (0.04°C/min) did; no difference, however, was observed in control strips. Dose-response curves to norepinephrine in increasing concentrations at 37°, 27°, and 17°C revealed increased response of scleroderma strips at 27°C as compared to normal vessel strips. This study demonstrates that in vitro measurement of the pharmacologic responsiveness of scleroderma vessels indicates changes in smooth-muscle response to temperature, which possibly underly the vascular component of this disease.

335. Hyperglucagonemia: a New Mechanism for the Diabetogenic Effects of Glucocorticoids. JONATHAN K. WISE,* ROSA HENDLER,* AND PHILIP FELIG, New Haven, Conn.

Glucose overproduction induced by glucocorticoids has generally been attributed to increased availability of amino acids for gluconeogenesis or direct induction of gluconeogenic enzymes. In the present study a new mechanism has been considered by examining the effect of glucocorticoids on glucagon secretion. Dexamethasone (8 mg/day for 3 days) was administered to 14 healthy nonobese subjects. Plasma immunoreactive glucagon and glucose were measured in the basal state and after infusion of alanine (0.15 g/kg) or ingestion of beef (3 g/kg). Basal glucagon levels (90±14 pg/ml, predexamethasone) were increased by 50% after dexamethasone administration ($P < 0.02$). The maximal increments in glucagon and glucose after alanine infusion were, respectively, 94±10 pg/ml and 6±1 mg/100 ml before dexamethasone, and rose to 201±41 pg/ml and 14±1 mg/100 ml after dexamethasone ($P < 0.01$). Similarly the glucagon increment after beef ingestion (120±22 pg/ml, predexamethasone) rose by 50% after dexamethasone ($P < 0.05$). Since obesity is a characteristic of chronic hypercorticism and has been shown to suppress glucagon secretion, dexamethasone was also ad-

ministered to five obese subjects. After dexamethasone basal glucagon levels doubled in the obese, while the glucagon response to alanine increased fourfold. In four patients with Cushing's syndrome of 6 months duration or longer (three had normal glucose tolerance), basal glucagon was 50% above control levels, while the glucagon response to alanine was increased threefold. We conclude that glucocorticoids increase basal glucagon levels and enhance alpha cell responsiveness to alanine infusion and protein ingestion. This effect is rapidly induced, occurs in the face of obesity, and persists in chronic hypercorticism. The data suggest that hyperglucagonemia contributes to the glucose overproduction and diabetes induced by glucocorticoids. Furthermore, these observations may explain the greater effectiveness of glucocorticoids in increasing gluconeogenesis in the intact organism as compared to their in vitro action in isolated liver. (Supported by NIH grants.)

336. Rosette-Forming Cells and Cytotoxicity for Malignant Cells. JOSEPH WYBRAN,* INGEGERD HELLSTRÖM,* KARL E. HELLSTRÖM,* AND H. HUGH FUDENBERG, San Francisco, Calif., and Seattle, Wash. (introduced by Gilbert S. Gordan**).

Peripheral blood lymphocytes from cancer patients are cytotoxic for tumor cells. The origin of the cytotoxic lymphocytes is unknown in man. Lymphocytes that bind, in vitro, to sheep erythrocytes in a rosette formation are thymus derived. We studied nine patients with solid tumors. Their blood rosette-forming cells (RFC) were isolated by mixing and spinning lymphocytes and sheep erythrocytes over Ficoll-Hypaque. This method allows separation of two populations of cells. One population consisting of RFC (90–99%) is an almost pure T-cell preparation; the other population, when further depleted in RFC by centrifugation, contains only 6–25% RFC. These two populations were assayed in vitro for their killing activity against tumor cells in microcytotoxic assay. Control experiments were also performed using separated lymphocytes from normal volunteers, fibroblasts, or various tumors cells. All the control experiments (normal lymphocytes-tumor cells, cancer lymphocytes- fibroblasts, or nonrelated tumor cells) were negative. In the cancer patients, the RFC consistently killed the tumor cells. Furthermore, this T-cell population was still cytotoxic using 25,000 cells per well, whereas the unfractionated population requires 150,000 cells. The T-cell-depleted population showed either an absent killing activity or a lesser cytotoxicity than the RFC population. The decreased cytotoxic effect of this population was correlated with their low number of RFC. These results indicate that human T-cells from cancer patients have the property to kill tumor cells. It appears that this activity belongs to T-cells although some participation of other cells cannot be completely excluded. (Research supported by grants from USPHS and ACS.)

337. Studies with a Newly-Developed Ammonia Electrode: Parotid Salivary Ammonia Secretion in Normal Subjects. TADATAKA YAMADA* AND EDWARD W. MOORE, Richmond, Va.

No data are available on the kinetics of salivary ammonia secretion or production. Biologic membranes are believed to be freely permeable to gaseous ammonia (NH_3) and relatively impermeable to NH_4^+. Thus, according to the pH-partition hypothesis, the distribution of total ammonia (TA) between blood and saliva should vary exponentially with pH (in the absence of ammonia production). This hypothesis was investigated in studies using a recently developed (Orion Research Inc., Cambridge, Mass.) ammonia electrode. Multiple samples of pure parotid saliva were collected by Curby cup in eight normal subjects. Parotid secretion was stimulated with graded concentrations of citric acid administered sublingually, yielding flow rates of 0.01–3.15 ml/min. Simultaneous plasmas were analyzed for pH and [TA] both by the electrode and by an ion-exchange resin method. Salivary ammonia output varied linearly with flow rate over the entire observed range (up to 0.4 μmoles/min per gland). pH increased curvilinearly with flow rate and ranged from 4.93 to 7.94. At low flow rates, observed [TA] approximated [TA] predicted by the pH-partition hypothesis. With increasing flow rates, observed/predicted [TA] ratios increased curvilinearly and reached values ranging from 2 to 10. These results suggest the following model for salivary ammonia secretion: (*a*) ammonia is produced in the acinar region; (*b*) at low flow rates salivary NH_3 nears equilibrium with plasma NH_3 in the duct, in accordance with nonionic diffusion theory; and (*c*) at high flow rates, equilibrium is not reached and [TA] of secreted saliva approaches that of acinar fluid. (Supported by NIH grant.)

338. Circulating Humoral Factors: a Role in Postobstructive Diuresis? W. E. YARGER,* R. H. HARRIS,* N. W. SCHRADER,* D. D. SCHOCKEN,* AND R. R. ROBINSON, Durham, N. C.

Postobstructive diuresis is known to occur after release of bilateral ureteral ligation (BUL) in rats. It is effected, at least in part, by decreased reabsorption from the ascending limb. The factors responsible for this decreased reabsorption have not yet been clarified completely. Their nature, with special emphasis on the possible role of humoral substances, was evaluated using standard clearance techniques in six groups of rats 24 h after exposure to the following experimental maneuvers: sham-operated antidiuretic controls (AD); BUL; and four groups with unilateral ureteral ligation (UUL). In these four groups with UUL, the contralateral kidney was: left untouched (UUL); surgically removed (UUL-Nx); its urine vented into the peritoneum, (UUL-V); or its urine reinfused IV (UUL-UR) throughout the period of obstruction. 24 h later, ureteral ligation was released (on one side only in rats subjected to BUL) and postrelease function was compared to that of AD rats. GFR was less than 16% of AD in all groups. Postobstructive diuresis occurred in BUL, UUL-UR rats; urine flow and sodium excretion were 6–10 times greater than in AD rats. Postobstructive diuresis did not occur in UUL or UUL-V rats. The results suggest (*a*) that postobstructive diuresis is not mediated via obstructive damage per se (UUL, UUL-V), and (*b*) that it is not dependent on continuing contralateral obstruction (UUL-Nx, UUL-UR). It seems likely that a circulating diuretic factor may accumulate during ureteral obstruction (BUL, UUL-Nx, UUL-UR) but is excreted by a normal contralateral kidney (UUL) and does not rapidly cross the peritoneal membrane (UUL-V). Urea, comparably increased in BUL, UUL-Nx, UUL-V, and UUL-UR, does not appear to be this substance.

339. Cordycepin Sensitivity Limited to the First 15 Min of Action of Glucocorticoid Hormones on Rat Thymic Lymphocytes: Further Evidence for Involvement of a Messenger RNA. DONALD A. YOUNG,* THOMAS J. BARNARD,* STEPHEN J. GIDDINGS,* AND STEVEN L. MENDELSOHN,* Rochester, N. Y. (introduced by Christine Waterhouse**).

We have previously interpreted a brief period of sensitivity to actinomycin (during the first 5 min) as evidence for an early RNA-synthetic step that initiates the later inhibitory actions glucocorticoids exert on lymphatic tissues. Evidence that this represents mRNA, rather than other RNA species more sensitive to actinomycin, is largely circumstantial but fits well with irreversibility after 5 min, a temperature-sensitive lag period (5–15 min, possibly message transport), and a subsequent protein-synthetic event (15–35+ min) that

coincides with hormone-directed limitation of glucose penetration. In this presentation we add new evidence for mRNA involvement by demonstrating sensitivity limited to the first 10–15 min of hormone action to an antibiotic more selective than actinomycin, cordycepin, which blocks addition of polyadenylic acid to newly made message. Although for rapid block high levels of cordycepin (0.5–1 mg/ml) are required with thymus cells, probably because of slow penetration (also seen with actinomycin), its ability to block hormone action correlates with its ability to reduce adenosine incorporation into poly(A) (measured by millipore binding). Cordycepin added simultaneously with dexamethasone (10^{-7} M) or cortisol (10^{-6} M) reduces glucocorticoid action on glucose uptake (measured 20–30 min after cordycepin) by $\sim 1/2$ and appearance of cytoplasmic poly(A) at 20–30 min by $\sim 1/3$. If hormone precedes cordycepin by 10 min, a larger hormone effect ($\sim 75\%$), and by 20 min a full effect emerges. In similar experiments [^{3}H] cortisol (10^{-8} M) binding to nuclear receptors, levels of ATP, and incorporation of [^{3}H] uridine into RNA are not reduced by cordycepin, suggesting that it blocks through specific actions on poly(A). (USPHS support: AM-16177 and 5-S01-RR05403.)

340. An In Vivo Method for Monitoring Differential Effects of Chemotherapy on Target Tissues in Animals and Man: Correlation with Plasma Pharmacokinetics. ROBERT C. YOUNG* AND BRUCE A. CHABNER,* Bethesda, Md. (introduced by Vincent T. DeVita).

Potentially exploitable recovery patterns of DNA synthesis in normal and tumorous target tissues might prove of value in the design of chemotherapy regimens. We have monitored the effects of methotrexate (MTX) on DNA synthesis in L1210 ascites tumor, intestinal epithelium, and bone marrow by determining the rate of incorporation of [^{3}H] UdR into DNA at various times after MTX administration. Tumor-bearing mice were given 5, 50, and 350 mg/kg MTX, and were injected with [^{3}H] UdR 1 h before sacrifice. Plasma was obtained for pharmacokinetic analysis and tissues were analyzed for cpm/μg DNA. 1 h after treatment, profound suppression of DNA synthesis was observed at all doses in all tissues. Bone marrow recovery occurred in 9–12, 18–24, and 36–48 h for the three doses of MTX, respectively, while intestinal mucosal recovery lagged behind. Only partial recovery of DNA synthesis in tumor was seen. DNA synthesis resumed in all tissues at plasma MTX levels of 10^{-8} or less, indicating the importance of persistence of low levels of drug in extracellular fluid. Three patients with ovarian carcinoma, treated with 25 mg/M^2 MTX, showed a variable rate of plasma disappearance of MTX. Duration of suppression of DNA synthesis in bone marrow varied according to the rate of elimination of MTX, but recovery occurred when plasma MTX was 2×10^{-8} or below. Recovery of DNA synthesis in ovarian ascites tumor antedated bone marrow recovery in each instance, predicting the ultimate lack of clinical response. These studies demonstrate a method for monitoring kinetic alterations induced by chemotherapy in host and tumor in vivo, allowing a definition of relationships between plasma drug levels and DNA synthesis inhibition.

341. Hemoglobin Andrew-Minneapolis, β144 Lysine → Asparagine: a New High-Affinity Mutant. SOLOMON J. ZAK,* BERNADINE BRIMHALL,* RICHARD T. JONES,* AND MANUEL E. KAPLAN, Minneapolis, Minn., and Portland, Ore. (introduced by Paul G. Quie).

A new high-affinity hemoglobin, Andrew-Minneapolis (Hb And-Mpls), transmitted as an autosomal dominant over three generations, has been found. Blood of affected individuals contained approximately 50% Hb And-Mpls and exhibited a P_{50} of 14 mm Hg (pH 7.4, 37°C). Asymptomatic erythrocytosis was present without evidence of hemoglobin instability or hemolysis. Hb And-Mpls had a slightly faster electrophoretic mobility than Hb A at pH 8.6 and was readily isolated from hemolysates by DEAE-Sephadex chromatography. When stripped of 2,3-diphosphoglyceric acid (2,3-DPG), its P_{50} was 3.5 mm Hg (pH 6.8, 37°C) compared with 10 mm Hg for Hb A. It exhibited an impaired Bohr effect (-0.233), but interacted normally with 2,3-DPG. Chain-chain interaction was decreased, Hill's constant = 1.7. After molecular cleavage, a mutant electronegative β-chain was recognized. Purified β-chains from Hb And-Mpls were aminoethylated, digested with trypsin, fingerprinted, and chromatographed on Aminex A-5. Absence of the normal βT-15 peptide was noted. Amino acid analysis of the aberrant βT-14 peptide revealed that it contained the βT-15 peptide because asparagine had replaced lysine at the β144 position. It is postulated that a salt bridge is formed between the side-chain oxygen of β144 asparagine and the imidazole proton of β146 histidine. As a result, the histidine cannot bind normally to β94 aspartate precluding formation of the "taut," (deoxy-) configuration of the Hb molecule. This would account for the high oxygen affinity and decreased Bohr effect exhibited by Hb And-Mpls but would not prevent its interaction with 2,3-DPG. (Research supported by grants from the NIH and the VA.)

342. The Effects of Chronic Metabolic Acidosis and Alkalosis on Rat Erythrocyte (RBC) Lactate Production (LP). ANTHONY ZAPPACOSTA* AND ROBERT NARINS,* Philadelphia, Pa. (introduced by Robert Austrain**).

LP in vitro is inhibited by low pH and enhanced by high pH. In vivo chronic acidosis decreases and chronic alkalosis increases rat erythrocyte (RBC) 2,3-diphosphoglyceric acid (DPG), a metabolic intermediate known to inhibit lactate production (LP). To study the interplay between pH and DPG in the regulation of RBC LP, DPG was varied by inducing alkalosis or acidosis for 2 days in groups of rats, and then RBC LP was measured in vitro at different pH levels and compared to normal controls. Metabolic acidosis and alkalosis was induced by tube feeding rats NH_4Cl and $NaHCO_3$. Two groups of rats served as controls. One group was tube fed NaCl and the second group was tube fed NH_4HCO_3. LP at normal pH (pH 7.4) was the same in both groups of controls. Production by rats made chronically alkalotic in vivo was 20% lower than controls, while rats made chronically acidotic in vivo produced 20% more than controls. When incubated at low (7.0), the increased LP by acidotic RBC no longer occurred. DPG showed the expected changes with chronic acid-base disturbances. Thus it appears that the reduction in RBC DPG produced by sustained acidosis does indeed enhance RBC LP, but only if the independent effect of pH is neutralized. Low pH per se inhibits the enhanced LP that would otherwise be expected in sustained acidosis. Hence rapid correction of acidosis in vivo before DPG can return to normal may be expected to cause significant lactic acidosis. (Research supported by NIH grant 5R1 AM-14207-03.)

343. Abstract withdrawn.

344. Variants of Chronic Lymphocytic Leukemia with Cells Having Surface Properties of T-Lymphocytes. D. ZUCKER-FRANKLIN, J. W. MELTON III,* AND F. QUAGLIATA,* New York.

Blood lymphocytes of most patients with CLL are believed to be marrow derived (B-lymphocytes) since they possess surface Ig, receptors for complement, and a poor response to PHA. A notable exception may be the peripheral lymphocytes

of patients with variants of lymphoma cutis, particularly those with Sézary's syndrome (exfoliative erythroderma and circulating lymphocytes with "cerebriform" nuclei). The cells of four patients with Sézary's syndrome were studied. WBC ranged from 13,000 to 65,000/cmm. After isolation of mononuclear cells by Ficoll-Hypaque gradient and removal of monocytes with iron filings, the cells were (*a*) subjected to electron microscopy, (*b*) supravitally stained with fluorescent antiserum to γ-globulin, (*c*) assayed for complement binding (EAC rosettes) and erythrocyte-binding (E-rosettes), and (*d*) stimulated with PHA. On electron microscopy, 44–72% of the cells proved to be typical Sézary cells. None had ingested latex particles, eliminating the possibility that they were monocytes or histiocytes. No Ig was detected on the cells of three patients, while in one the incidence was 2%. EAC rosettes ranged from 0 to 1%. E-rosettes formed with 22–83% of the lymphocytes in three patients and with 2.5% in one patient who had received chemotherapy. Sézary cells were identified within E-rosettes. The cells of all but the treated patient responded normally to PHA. A fifth patient with CLL, Sézary cells, and skin infiltrates did not fulfill the clinical criteria of the syndrome. However, with an absolute lymphocytosis of 23,000 only 4% of the cells carried surface Ig, 3.2% formed EAC rosettes, 60% formed E-rosettes, and all cells transformed with PHA. On the basis of these studies, it seems possible that the neoplastic lymphocytes circulating in patients with skin lymphomas, particularly those with Sézary's syndrome have surface properties of T- rather than B-lymphocytes. (Research supported by NIH grant AMO 12274.)

345. Prostaglandin A Concentrations in Plasma of Normal and Hypertensive Humans. R. ZUSMAN,* D. SPECTOR,* B. CALDWELL,* L. SPEROFF,* B. FORMAN,* G. SCHNEIDER,* AND P. MULROW,** New Haven, Conn.

A number of observations suggest that naturally occurring prostaglandin A (PGA) may be involved in the regulation of sodium balance and blood pressure. In order to investigate these possibilities, plasma concentrations of PGA were measured in normal humans during changes in sodium balance and in hypertensive subjects. Seven healthy volunteers were placed on three dietary sodium intakes: (*a*) high, 230 mEq Na; (*b*) ad lib., 150 mEq Na; and (*c*) low, 10 mEq Na daily. Prostaglandin A (PGA) and plasma renin activity (PRA) were measured by radioimmunoassay. Changes in sodium intake had parallel effects on PGA and PRA. Mean PGA level on the high Na diet was 0.82 ± 0.05 SEM ng/ml. PGA levels rose 95% to 1.60 ± 0.05 ng/ml, $P < 0.001$, on the ad lib. sodium and rose 161% to 2.14 ± 0.09 ng/ml, $P < 0.001$, on low Na. PRA levels on the high, ad lib., and low Na diets were 0.61 ± 0.03 SEM ng/ml per h, 1.49 ± 0.12, and 8.49 ± 0.75, respectively. In contrast, patients with essential hypertension or renal artery stenosis (RAS) have low prostaglandin A levels. Essential hypertensives on ad lib. diet had PGA levels of 0.60 ± 0.07 ng/ml ($n = 14$), $P < 0.001$, compared with controls. In six RAS patients on 10-meq diets, mean PGA was 0.67 ± 0.11 ng/ml, $P < 0.001$, and in three RAS patients on ad lib. diets mean PGA was 0.79 ± 0.09 ng/ml, $P < 0.01$. In contrast to normals, there was no correlation between PGA and PRA levels in the hypertensive patients. In patients with RAS, renal venous samples were obtained and renal vein PGA was 2.25 times the peripheral PGA level. In six anephric patients, PGA levels were very low: 0.19 ± 0.02 ng/ml. These findings, plus the extraction of PGA from human renal tissue, suggest a renal origin for circulating PGA. In conclusion, these data suggest that (*a*) prostaglandin A may be involved in sodium homeostasis, and (*b*) a deficiency of prostaglandin A production may play a role in the pathogenesis of human hypertension.