is 2 μM). All of these mitchondrial reactions are membrane linked. Electron microscopy confirms the biochemical data of swelling.

Lysosomes of rat liver and L-929 cells demonstrate increased permeability within one hour of exposure to bilirubin using acid phosphatase activity as a monitor of membrane fragility. This effect is seen at relatively high bilirubin levels (500 μM) but preliminary evidence suggests concentration of bilirubin by lysosomes.

Influence of early malnutrition on drug metabolism and effect. C. S. CATZ, J. A. BRASEL, M. WINICK, and S. J. YAFFE. *Sch. of Med., State Univ. of New York at Buffalo and Cornell University, Ithaca, N. Y.*

Male Sprague-Dawley rats were raised in litters of 10 (controls, C) and 18 (malnourished, M) until 21 days of age (weaning). Total body and liver weights were decreased by 30% in the M group. As a consequence liver weight remained at the same percentage of body weight (4.89 $\pm$ 0.1) as in the controls (4.75 $\pm$ 0.2). The microsomal components of the electron transport chain for oxidative pathways were similar in both groups. Metabolism for several different oxidative substrates (aminopyrine, aniline, and benzpyrene) was increased significantly in liver homogenates in the M group. Since drug metabolism is the most important determinant of drug effect, hexobarbital was used in correlative *in vivo* studies. The duration of sleep (the major action of hexobarbital) was surprisingly longer in the M animals (160 $\pm$ 9 minutes) than in the controls (112 $\pm$ 6 minutes) at a dose of 100 mg/kg. Since hexobarbital metabolism was not decreased in the M group these findings strongly suggest that brain sensitivity is altered. This may have important consequences for drug usage in malnourished children.

Pharmacologic modification of bilirubin toxicity in tissue culture. RICHARD P. WENNBERG and L. FRASER RASMUSSEN (Intro. by David Baum). *Univ. of Wash. Sch. of Med., Seattle, Wash.*

While several areas of bilirubin toxicity have been identified, the mechanism of bilirubin entry into cells and the mode of cell death are not understood. Several substances which have been associated with membrane functions were examined to determine if they would influence bilirubin toxicity. Strain 929 L-cells were washed four times in protein free media and incubated one hour with the test drug before adding bilirubin. Ten μM bilirubin killed >90% cells in four hours as determined by cell penetration of erythrocin B. Hydrocortisone totally protected the cells from bilirubin toxicity; prednisolone was slightly less effective. The rate of cell death was retarded by insulin, but only in very high concentrations (0.2 units/ml). Theophylline and caffeine, which inhibit the breakdown of cyclic AMP by the enzyme phosphodiesterase, offered partial protection. Paradoxically, epinephrine and glucagon, which stimulate adenyl cyclase, and cyclic AMP and dibutyrl cyclic AMP either failed to protect or even accelerated cellular death with bilirubin. These effects could be blocked with theophylline and caffeine, suggsting that AMP or phosphodiesterase itself may be involved in bilirubin toxicity.

These studies reveal additional parameters of bilirubin toxicity and suggest the possibility of altering susceptibility for kernicterus with pharmacologic agents.

A six year prospective controlled study of neonatal hypoglycemia. ROSITA S. PILDES, IRVINA S. WARREN, SALVATORE DIMENZA, EDWARD PAGE-EL, SANDRA NEUWELT and MARVIN CORNBLATH. *Univ. of Illinois, Abraham Lincoln Sch. of Med., Chicago, Ill., and Univ. of Maryland, Sch. of Med., Baltimore, Md.*

Between 1961–1964, thirty-nine newborns with transient neonatal hypoglycemia (Group I) were matched with 41 controls (Group II) on the basis of 9 weighted clinical criteria. On-going medical and social service care was provided and yearly EEG's, neurological and psychological examinations were done. Computer analysis indicated the infants to be well matched according to medical criteria as well as socio-economic background. The incidence of R.D.S., sepsis, hyperbilirubinemia, polycythemia and C.N.S. problems was similar in both groups. Nevertheless, the clinical course of Group I was more severe due to the manifestations of hypoglycemia. Recurrent hypoglycemia was seen in 4 children; there were no deaths in either group. The follow-up data on physical development indicate that Group I showed a significant lag in height and weight until 3 years of age, after which both groups were in the 25th percentile. Head size, significantly smaller at birth in Group I, remained below the 3rd percentile at age 6. An analysis of 214 EEG's failed to reveal any significant differences in abnormalities between the groups. Stanford-Binet scores at age 5 showed a mean IQ of 87 $\pm$ 4 in Group I (22) vs 94 $\pm$ 4 in Group II (20) children. At age 6, the mean IQ was 88 $\pm$ 4 in Group I (14) and 96 $\pm$ 3 in Group II (18) children. These differences are not significant. W.I.S.C. scores at age 5 and 6 were similar in both groups. To date, the prompt and vigorous treatment of symptomatic neonatal hypoglycemia would appear to obviate marked differences in development.

GENETICS

Normal, Duarte Variant, and galactosemic alleles code for immunologically identical gal-1-P uridyl transferase enzyme protein. THOMAS A. TEDESCO and WILLIAM J. MELLMAN. *Univ. of Pennsylvania Sch. of Med., Philadelphia, Pa.*

Human galactose-1-phosphate uridyl transferase was purified from post-mortem liver to a preparation having a single band in polyacryamide gel electrophoresis. This preparation was used successfully to produce a rabbit antibody that precipitates transferase activity from solution and that forms a precipitin band in double immunodiffusion. Hemoglobulin-free erythrocyte preparations from homozygous normal (Gt^+/Gt^+), Duarte Variant (Gt^D/Gt^D), and galactosemic (Gt^G/Gt^G) individuals show immunoprecipitin bands in double immunodiffusion against this antibody that are identical with that of the purified transferase preparation. The results indicate that the three alleles code for immunologically similar enzyme proteins suggesting that the functionally less active Duarte Variant and inactive galactosemic enzyme proteins have resulted from "point" mutations.

Fabry's disease: Evidence for structural mutation of α-galactosidase. GIOVANNI ROMEO and BARBARA R. MIGEON, *Johns Hopkins Hosp., Baltimore, Md.*

Fibroblasts from a patient with Fabry's disease have an α-galactosidase activity corresponding to 10–20% of control values, and the same difference has been found between the 2 clonal populations derived from the patient's mother and sister (Science 170: 180, 1970). The α-galactosidase present in fibroblasts of 2 unrelated patients and in "negative" clones of 2 heterozygotes shows a slower rate of heat inactivation than the enzyme of

"positive" clones and controls. The α-galactosidase from uncloned fibroblasts of a heterozygote shows a rate of inactivation indicative of a mixture of 2 enzymes. Moreover a small reproducible difference in the apparent K_m value between the wild-type and the mutant enzyme from one patient adds evidence for the structural character of this mutation. The specific activity of both mutant and wild-type α-galactosidase increases 10 and 4 times respectively, if the fibroblasts are maintained in a stationary phase of growth, while the activity of β-galactosidase increases fivefold in normal and α-galactosidase deficient cells. This has made possible electrophoretic analysis of the mutant α-galactosidase which, in the one family examined, does not migrate differently from the wild-type enzyme on Cellogel at pH 5.0.

Demonstration of a defect in the matrix fraction of keratin in Clouston's ectodermal dysplasia. R. J. M. GOLD and C. SCRIVER. *McGill Univ.-Montreal Children's Hosp. Res. Inst., Montreal, Que. Canada.*

Clouston's hydrotic ectodermal dyplasia is caused by a single dose of an autosomal gene whose effects are confined to the skin and its appendages. ED hair contains more tyrosine and phenylalanine and less serine and proline than normal hair, suggesting a defect in the matrix protein. Further investigation of this protein has confirmed this hypothesis. Electrophoresis of the S-carboxymethyl derivative of the matrix protein (SCMK-B) on starch gel at pH 2.4 generates an abnormal pattern of bands. Moving boundary electrophoresis yields an abnormal peak not present in this protein fraction from normal hair, and sedimentation velocity studies at 360,000 x g show an abnormally high proportion of low molecular weight protein. Hydrolytic cystine yields suggest, and polarography confirms, that the disulphide content in ED hair is abnormally low. This corresponds with an abnormally low-S-carboxymethyl cysteine yield when the fibrillar fraction (SCMK-A) is hydrolysed. The physical and chemical properties of the mutant hair can be explained by these findings which also give considerable insight into the structure of normal keratin.

Genetic heterogeneity in acid phosphatase deficiency. HENRY L. NADLER. *Northwestern Univ. Med Sch., Children's Memorial Hosp., Chicago, Ill.*

Lysosomal Acid Phosphatase Deficiency (LAPD) is characterized by vomiting, lethargy, opisthotonus, terminal bleeding and death in early infancy (Nadler & Egan, NEJM: *282:* 302, 1970). Acid phosphatase (AcP) activity in fibroblast from patients with this disorder is decreased to 30% of normal in original homogenates (OH) and to less than 2% in the lysosomal fraction (Lys). Recently we have observed a patient with similar clinical manifestations but who expired at 36 hours of age. AcP in his fibroblasts was reduced to 2% of normal in OH and was not detectable in Lys. Therefore, this was considered to represent a total acid phosphatase deficiency (TAPD).

In comparing the AcP activities of the two patients with normal, several differences were noted. Addition of 1 ug/ml of prednisolone to these cultures induced AcP to 50% of normal in LAPD cells but had no effect on TAPD cells. This induction was inhibited by actinomycin D, puromycin and chloramphenicol. After mixing and hybridization of LAPD and TAPD cells an increase in AcP activity up to 50% of normal could be observed in OH and Lys after one week. Addition of prednisolone could not induce any further AcP activity in the mixed or hybrid cultures.

These studies clearly indicate genetic heterogeneity for acid phosphatase deficiency. The stimulation of AcP activity by prednisolone suggests that the basic defect in LAPD is an altered regulatory mechanism in contrast to that of a structural gene defect in TAPD. The potential usefulness of prednisolone therapy in patients with LAPD is suggested.

Electron microscopy of uncultured amniotic fluid cells: *In utero* diagnosis of type II glycogenosis. GEORGE HUG, WILLIAM K. SCHUBERT, and SHIRLEY SOUKUP. *The Children's Hosp. Res. Found., Cincinnati, Ohio.*

Electron microscopy (EM) of uncultured amniotic fluid cells has been performed on 14 specimens. Of these, 10 specimens served as "normal controls" while the other 4 were from women who had previous babies with type II glycogenosis (type II GSD). EM of "normal controls" indicated the presence of two cell types: (1) frequent squamous epithelial cells with varying amounts of *cytoplasmic* glycogen but without membrane bounded accumulations of glycogen (i.e., without *lysosomal* glycogen); and (2) rare ciliated cells that may derive from the fetal trachea. Of the 4 high risk pregnancies, two had amniotic fluid cells indistinguishable from "normal controls" and produced clinically and biochemically healthy children. Amniotic fluid cells of the remaining two pregnancies contained glycogen accumulations surrounded by membranes (lysosomal glycogen) that are the hallmark of type II GSD. Upon termination of one of these pregnancies at 21 weeks of gestation, the fetus had type II GSD by biochemical and EM criteria. The other pregnancy resulted in the delivery at term of a boy who apparently was healthy clinically but who at birth had the abnormal lysosomes in skin, liver and muscle and who died of type II GSD at age 4 months. Eight obligatory heterozygotes for type II GSD did not have abnormal lysosomes in hepatic biopsy specimens. We conclude that direct EM of uncultured amniotic fluid cells may help with the in utero diagnosis of type II GSD. A major advantage of this method is diagnosis within three days of amniocentesis.

Evidence for dominant transmission of chronic granulomatous disease from leukocyte oxygen uptake studies. STELLA B. KONTRAS, JOANN G. BODENBENDER, GRAIG B. LIDEN, and SOMASUNDARAM ADDANKI. *Ohio State Univ., Coll. of Med., Children's Hosp., Columbus, Ohio.*

Chronic granulomatous disease (CGD), an X-linked disorder of males, may also occur as an autosomal trait. Detection of the heterozygote by nitroblue tetrazolium dye tests (NBT) and bactericidal studies has not been successful in these families. Leukocytes of patients with CGD fail to show normal increments in respiration (O_2 consumption) during phagocytosis of latex particles. A GME Oxygraph Model KM (Gilson) was used to determine phagocytic and basal rates of leukocyte O_2 uptake in picomoles O_2/min/million cells. A ratio of phagocytic to basal rate (P/B) for leukocytes from a series of normal males, females and children has been reported previously. Two families with clinical CGD non X-linked were studied by NBT dye tests, bactericidal studies and O_2 consumption. In one family, 3 girls were affected; the father and brother were not affected and had normal studies but the mother had intermediate bactericidal capacity in one of 3 determinations. She had consistently abnormal O_2 uptake (P/B of 2) as compared to normal females who show P/B of 13.3 ± 2.4. In the other family, a male propositus had typical clinical and lab findings of CGD. A male sib and both parents were normal clinically and by usual lab tests, however, the