Chronic active liver disease occurring in adolescents who use drugs: A study of possible etiologies. IRIS F. LITT, MICHAEL I. COHEN, S. KENNETH SCHONBERG, and ILYA SPIGLAND. *Albert Einstein Coll. of Med., Montefiore Hosp. and Med. Ctr., N. Y., N. Y.*

Of 16,800 presumably well teenagers, 3181 were found to be drug abusers. Routine liver function tests in these latter patients revealed abnormalities in 1306, with SGPT elevation the most frequently noted. Forty patients, abnormal for 3 months, had a percutaneous liver biopsy. All specimens exhibited infiltration of the portal area and 75% showed hepatocyte necrosis. One half of the biopsies showed portal fibrosis. This degree of chronicity suggests that the usual course of viral hepatitis may be altered by the abuse of drugs. Alternatively, the drugs themselves may be directly responsible.

Guinea pig liver explants were grown in culture medium and shown to be metabolically active for 96 hours. Transaminase activity was assayed in the culture medium before addition of the explant and serially thereafter. Changes in enzyme activity were followed after the addition of substances commonly abused by teenagers, as well as CCl_4.

The pattern of transaminase elevation at 24 hours by CCl_4 provided the model for acute toxicity. Other drugs tested showed no evidence of acute toxicity, nor did the addition of heroin to this system result in the transaminase elevation associated with acute toxicity.

This study suggests that heroin is not acutely hepatotoxic. The abnormalities of the liver noted in the heroin-using adolescents may be the result of a modifying effect on the usual course of viral hepatitis.

Electron microscopic changes in the liver in Reye's syndrome. JOHN C. PARTIN and WILLIAM K. SCHUBERT. *Children's Hosp. Res. Found., Cincinnati, Ohio.*

Reye's syndrome is acute encephalopathy in children associated with fatty liver. Liver cells are filled with fat droplets throughout the lobule; there is no inflammation. We have examined the electron microscopic (EM) changes in 16 children with Reye's syndrome. Diagnostic Menghini needle biopsies were obtained 6 hours to 4 days after onset of central nervous system signs; follow-up biopsies were obtained 2 months later from 7 of 9 survivors. Distinctive mitochondrial changes were present in all initial biopsy specimens: The matrix was distended (greatly in severe cases) and matrix protein was disorganized. Cristae were disrupted. The swollen mitochondria assumed bizarre contours. In two cases with severely altered mitochondria in the initial biopsy who were treated by exchange transfusion, most but not all mitochondria were normal by 2 months. One of these biopsied on the 3rd day, after onset of CNS signs and 6 exchange transfusions, showed great improvement in mitochondrial morphology. In early biopsy specimens the smooth endoplasmic reticulum (ER) was hypertrophied; in well glycogenated cells extensive "glycogen body" formation was seen. In fatal cases, glycogen depletion was severe. Peroxysomes were increased in all biopsy specimens. In less severe cases there was active peroxysome proliferation from smooth ER. Peroxysome proliferation may represent a compensatory response to deficient mitochondrial respiration. In recovery, rough ER was increased and its cisternae were distended. The Golgi system was hypertrophied with lipid filled saccules. The EM changes show that potentially reversible mitochondrial injury is a main feature of the liver lesion in Reye's syndrome. The etiologic agent may be a mitochondrial toxin.

Effects of phenobarbital on bile salts in cholestasis. ADOLF STIEHL, M. MICHAEL THALER, and WILLIAM H. ADMIRAND. *Univ. of Calif., San Francisco, Calif.* (Intr. by M. M. Grumbach).

Phenobarbital (PB) reduces pruritis in children with cholestasis. Individual bile salts were determined before and during PB treatment in 3 children with intrahepatic cholestasis (2 with benign recurrent cholestasis, 1 with paucity of intrahepatic bile ducts) and in 3 with extrahepatic biliary atresia. In *intrahepatic cholestasis* the cholate/chenodeoxycholate ratio in serum, bile and urine was 2.5–10. Total serum bile salt concentration was 100–400 μg/ml. After 4 days on PB (10 mg/kg/day) total serum bile salts decreased dramatically to 1–10 μg/ml with concomitant disappearance of pruritus. Daily urinary bile salt excretion declined concomitantly from 15–40 mg to 1–2 mg. In contrast, in *extrahepatic cholestasis,* the cholate/chenodeoxycholate ratios were 0.1–0.4 in blood, bile and urine. Total serum bile salt concentration was 60–130 μg/ml. Treatment with PB did not lower serum or urinary bile salt concentrations. Thus, the ratio of cholate/chenodeoxycholate is different in intrahepatic and extrahepatic cholestasis. PB greatly enhances the removal of bile salts from blood in two types of intrahepatic cholestasis, but is ineffective in extrahepatic cholestasis. The simultaneous decrease in serum and urinary bile salt concentrations suggests that PB stimulates the biliary excretion of bile salts.

METABOLISM

Lysosomal bone disease. I. A. SCHAFER, D. W. POWELL, and J. C. SULLIVAN. *Case Western Reserve Univ. Sch. Med. at Cleveland Metro. Gen. Hosp., Cleveland, Ohio.*

For normal bone growth, matrix must be laid down and resorbed. The process of remodeling could be altered if the activity of a single lysosomal hydrolase was deficient since compounds catabolized by the enzyme might then accumulate within bone cells and matrix with resultant abnormalities in bone architecture and growth retardation. We have studied a 9 year old dwarfed white male with normal intelligence whose disease appears limited to bone. He shows no corneal infiltration, visceromegaly or mucopolysaccharidurla. His radiological diagnosis is spondyloepiphysealmetaphyseal dysplasia. Chemical studies of cultured skin fibroblasts from the patient showed decreased enzyme activity of α-L-fucosidase (controls 2.67 $\pm$ .75 vs patient 0.25 $\pm$ .05 n-moles/min/mg protein) with accumulation of fucose in his cells (controls 5.42 $\pm$ 1.52 vs patient 27 γ/mg protein). Fibroblast enzyme activities for α-L-mannosidase, acid phosphatase, β-D-galactosidase and n-acetyl glucosaminidase were comparable to normal controls. A bone specimen from the patient was compared to 7 control specimens for hydrolase activity. Fucosidase activity was decreased in the patient (controls 3.2 $\pm$ .38 vs patient 0.36 n-moles/hr/mg wet wt.) as were several other acid hydrolyses. Compositional analyses of bone are in progress to define the character of the stored material. Thus far, the data in this patient is consistent with the hypothesis that his bone disease is due to a deficiency of lysosomal hydrolase, α-L-fucosidase. Since lysosomes in

bone cells contain a variety of acid hydrolyses, the chemical bases for other chondrodystrophies may be related to the altered activity of one or more of these enzymes.

Decrease and inhibition of liver phosphorylase (LP) after fructose: An experimental model for the study of hereditary fructose intolerance (HFI). JEAN HOLOWACH THURSTON and ELIZABETH M. JONES (Intr. by Philip R. Dodge). *Wash. Univ. Sch. of Med., St. Louis, Mo.*

It has been postulated that inhibition of LP plays a significant role in the fructose-induced hypoglycemia of HFI. To test this hypothesis weanling mice were injected i.p. with 30 m-moles/kg of fructose or saline of equal osmolality. Although liver glucose levels did not fall the findings are relevant to the understanding of the pathophysiology of HFI. 20 min after fructose injection, fructose and fructose-1-P (F-1-P) levels were 21.4 ± 2.9 and 10.6 ± 0.9 m-moles/kg respectively. ATP and Pi were reduced 50%, to 1.36 m-moles/kg and 2.07 m-moles/kg, $p = 0.001$. At the same time LP was reduced 48% ($p = 0.002$). This finding suggests a shift to the inactive form of the enzyme. To mimic the *in vivo* situation LP in normal liver homogenate was measured in the presence of the concentrations of Pi and F-1-P found after fructose injection. The activity of the enzyme was reduced 88% by these additions (from 5.9 to 0.74 μ-moles/g min, p 0.001). Therefore, it appears that *in vivo* the activity of liver phosphorylase is reduced by two mechanisms, a conversion of the active to the inactive form, and an inhibition of the remaining active enzyme by the elevated F-1-P and reduced Pi levels.

X-linked hypophosphatemic rickets: A PTH-insensitive transport defect responsive to phosphate. F. GLORIEUX, C. ARNAUD, C. CLOW, H. GOLDMAN, T. READE and C. SCRIVER. *McGill Univ.-Montreal Children's Hosp. Res. Inst., Montreal, Que., Can.; and Mayo Clinic, Rochester, Minn.*

Six children (2F, 4M) have been treated up to 6 years with a phosphate supplement by mouth 5 times daily (1–4g Pi/d). This regime required 27 home visits/pt/year but yielded a serum Pi conc. of 3.75 ± 0.8 mg% for the group. This in turn achieved: 1) complete healing of rickets in all; 2) satisfactory bone density; 3) marked catch-up growth in 5 of 6 patients. Serum PTH was normal in 4 previously untreated patients (mean = 35 μl Eq/ml, normal <40), but was raised during phosphate treatment (mean = 150 μl Eq/ml; 6 patients, 11 determinations). Secondary hyperparathyroidism with bone signs and hyperaminoaciduria occurred in 3 patients, reduction of phosphate and use of vitamin D_2 (50–100,000 u/d) suppressed parathyroid hyperactivity. Net tubular reabsorption of phosphate studied intensively in one patient was constantly saturated at 39–50 μmoles/100 ml GFR (normal <90) over a serum Pi range from 1.5 to 7.3 mg%, and at endogenous serum PTH concs. varying between 28 and 490 μl Eq/ml. A primary renal disorder of phosphate reabsorption, which is insensitive to PTH and can be offset by dietary phosphate suplements, is proposed for this X-linked trait.

A new inherited defect of isoleucine catabolism. R. DAUM, E. DELVIN, H. GOLDMAN, P. LAMM, O. MAMER and C. SCRIVER. *McGill Univ.-Montreal Children's Hosp. Research Institute, Montreal, Que., Can.*

Known inborn errors of metabolism affect 4 different enzymes in the oxidation of isoleucine to succinate. A new disease involves a fifth enzyme responsible for the formation of propionate from α-methylacetoacetate. This compound and its precursor, α-methyl-β-hydroxybutyrate, are not present in significant amounts in body fluids of normal persons. Large amounts were identified by GC and mass spectrometry, at all times in the urine of a boy who experienced 3 episodes of transient but profound metabolic acidosis. Their concentration increased greatly during acute febrile illness and specifically after L-isoleucine loading. Butanone was present on these occasions while levels of amino acids and propionate were normal. The parents and one of two sibs of the proband constantly excrete modest amounts of the two organic acids, and the latter were increased by isoleucine loading in contrast to the normal response. In vitro oxidation of isoleucine-u-^{14}C by cultured skin fibroblasts incubated at 37C was 40 percent of normal in the proband; there was no further inhibition after 24 hour incubation at 40C. The study of this mutant phenotype confirms that later stages of isoleucine catabolism are analogous to oxidation of straight-chain fatty acids.

High pyridoxine diet in the rat: Implications for clinical megavitamin therapy. GERALD GAULL, PHYLLIS COHEN, KARMELA SCHNEIDMAN, FREDDA GINSBERG-FELLNER, JOHN STURMAN and JEROME KNITTLE. (Intr. by Philip Glade). *N. Y. Inst. Basic Res. Ment. Retard. and Dept. of Ped., Mt. Sinai Hospital Sch. of Med. of City Univ. of N. Y., N. Y.*

Megavitamin therapy has found increased clinical application. Although the toxicity of fat soluble vitamins is well established, little has been learned of the actions and fate of massive doses of the water soluble vitamins. Current interest in the use of vitamin B_6 in the treatment of homocystinuria due to cystathionine synthase deficiency prompted us to explore its mode of action and metabolic side effects in the rat. Large amounts of pyridoxine in the diet had no effect on appetite but resulted in a 20% increase in body weight and 40% in liver weight of *pair-fed* controls. The fat/protein ratio and the percentage of each in liver was unchanged despite the increased liver weight. The weights of brain, pancreas and kidney were unaffected. There appeared to be an increase in peritoneal fat, however, the epidyimal fat pads were significantly smaller in the high-pyridoxine group because of smaller cell size. Concentrations of free amino acids were unchanged as were the activities of cystathionine synthase and cystathionase. Incorporation of ^{35}S from both methionine and cystine into the proteins of various organs were also unchanged. In the rats on high-pyridoxine diets there was greater incorporation of ^{35}S from cystine into reduced glutathione and less into oxidized glutathione. Since the amounts of pyridoxine consumed by the experimental group was of the order of magnitude as that currently used in the treatment of homocystinuria, further studies are needed before it can be assumed that massive doses of water soluble vitamins can be used with impunity.

A potential danger of chlorpropamide therapy: Impaired excretion of a water load. MICHAEL A. LINSHAW, MARK SEY, ANGELO M. DIGEORGE, and ALAN B. GRUSKIN. *Temple Univ. Sch. of Med., St. Christopher's Hosp. for Child., Philadelphia, Pa.*

A modified Carter Robbins Test was used to diagnose central diabetes insipidus (DI) in 3 patients. After 2–3 months of oral chlorpropamide 250–500 mg/day, the test was repeated. In 2 patients CH_2O remained negative even during a water load of 20 cc/kg and a 2½% saline load of 10 cc/kg/45 min. There was no further drop in CH_2O following 0.1 units aqueous pitressin intravenously. These and one other patient with DI were then sub-