Microsomal Pentobarbital Hydroxylase Activity in Acute Viral Hepatitis (36487)

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Patients with liver disease exhibit marked variations in tolerance to sedatives and other drugs that are metabolized by hepatic microsomal enzyme systems. This could occur due to hypo- or hyperfunction of the hepatic smooth endoplasmic reticulum (SER), the principal site for metabolism of exogenous drugs and chemicals. Several factors may influence drug metabolism in liver disease: (a) drugs such as phenobarbitone or diphenylhydantoin cause hyperplasia of hepatic SER and an increase in the microsomal enzymes which govern drug disposition (1); (b) protein depletion inhibits production of microsomal enzymes and thereby alters biotransformation and hepatic toxicity of certain chemicals (2); (c) major loss of hepatic functional mass by severe cirrhosis, autolytic necrosis or surgical resection interferes with drug metabolizing ability (3, 4). The influence of reversible liver cell necrosis and regeneration on drug metabolizing enzymes is not known. Availability of a simple and accurate method of measuring pentobarbital hydroxylase (PH) activity in percutaneous liver biopsy specimens now permits such assessment (5). The present study was undertaken to determine activity of this enzyme during various stages of acute uncomplicated viral hepatitis.

Materials and Methods. Studies were conducted on 5 volunteers without hepatic disease and 17 nondrug addicts with clinical features of acute viral hepatitis (14 sporadic, 2 posttransfusion and 1 posttattoo). None of the patients selected for study had a history of oral or parenteral receipt of drugs, smoking, consumption of ethanol or exposure to other known environmental factors which increase microsomal enzymes during a 3-week period prior to admission. Hepatitis-associated antigen (6), serum bilirubin (7), alkaline phosphatase (8), glutamic pyruvic and oxalacetic transaminases (9) and protein electrophoresis were obtained on each of the subjects. Percutaneous liver biopsy specimens were obtained at the time of admission using the Vim Silverman needle. Repeat biopsy specimens were obtained in nine patients during recovery phases of hepatitis. The biopsy specimen was split and one-half used for morphologic studies and the other half for biochemical analyses. Morphologic studies consisted of light microscopy, electron microscopy and studies of *in vitro* DNA synthesis (10). Biochemical analyses were performed on liver homogenate. Pentobarbital hydroxylase activity was estimated with ^{14}C-labeled pentobarbital by the method of Kuntzman *et al.* (5). Total soluble protein was estimated by a modification of the method of Lowry *et al.* (11) and used as a reference base for hepatic pentobarbital hydroxylase activity.

Results. Hepatitis-associated antigenemia was present in nine patients. Liver biopsy specimens were read blindly and classified into three groups: acute necrotic phase, regenerative phase, and recovery phase of hepatitis. Ten biopsy specimens initially exhibited necrosis, seven with localized acidophilic necrosis and diffuse inflammation (12) and three with subacute autolytic necrosis (13). Regeneration, reflected in Kupffer cell proliferation, hyperplasia of the SER and increased incorporation of ^{3}H-thymidine into hepatic DNA was initially present in seven biopsy specimens (10). Recovery phase hepatitis, characterized by normal histology or

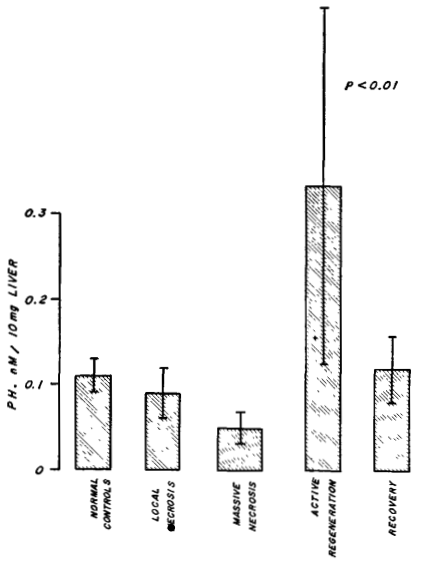

FIG. 1. Hepatic microsomal pentobarbital hydroxylase activity in normal controls and during various phases of acute viral hepatitis.

focal inflammation, was present in seven of the repeat biopsy specimens. Mean hepatic microsomal pentobarbital hydroxylase activity showed no significant difference between control biopsy specimens, biopsy specimens with localized acidophilic necrosis, and biopsy specimens with focal inflammation. Pentobarbital hydroxylase activity was decreased below control values in each of the specimens exhibiting subacute autolytic necrosis; marked increase in enzyme activity occurred in biopsy specimens which were the site of active regeneration (Fig. 1).

There was no correlation between microsomal pentobarbital hydroxylase activity and standard liver function tests during necrotic and regenerative phases of hepatitis. Serial study revealed a concomitant increase in pentobarbital hydroxylase activity with a reduction in bilirubin and transaminase (Fig. 2), although in some instances, pentobarbital hydroxylase activity decreased with continued elevation of serum bilirubin and transaminases. A patient with persistent hepatitis-associated antigenemia exhibited no significant change in pentobarbital hydroxylase activity with conversion of acute hepatitis to cirrhosis. Electron microscopy in this patient revealed hyperplasia of the endoplasmic reticulum, but *in vitro* DNA synthesis did not show the expected increase in labeling (10). Administration of 180 mg phenobarbital daily for 15 days caused an increase in enzyme activity from 0.07 m*M*/mg protein to 0.30 m*M*/mg protein.

Discussion. In the past, clinical evaluation and clearance techniques have been primarily used to investigate drug metabolism in liver disease in man. These studies demonstrated that drug tolerance usually remains intact except for severe liver disease (14), although the diseased liver may exhibit a decrease in drug removal capacity (15). A sensitive radiochemical assay for microsomal pentobarbital hydroxylase now makes it possible to quantify activity of this enzyme in a 20–25 mg specimen of liver and thereby obtain further information on drug metabolizing capacity in liver disease. Enzyme activity may be related to total protein, microsomal protein, DNA or wet weight of the specimen. In our experience, total protein has provided the best reference when percutaneous needle biopsy specimens are used (11).

Our observations indicate that despite fragmentation and vesiculation of the endo-

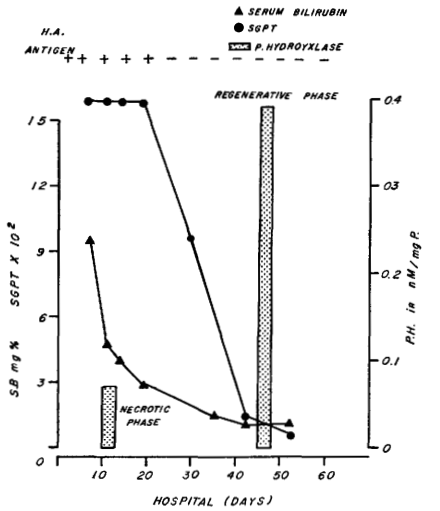

FIG. 2. Relationship of microsomal pentobarbital hydroxylase activity to hepatitis "B" associated antigenemia and biochemical liver function tests in a patient studied during necrotic and regenerative phases of hepatitis.

plasmic reticulum in acute viral hepatitis, the functional state of the SER, as reflected in pentobarbital hydroxylase activity, is not significantly altered unless there is autolysis of liver cells. Hyperplasia of the endoplasmic reticulum during regenerative phases of acute viral hepatitis is associated with a marked increase in activity of this enzyme. In contrast, hyperplasia of the SER was not associated with the expected increase in microsomal enzyme activity in a patient with persistent hepatitis "B" associated antigenemia who eventually developed cirrhosis. Hyperplastic, hypoactive SER has been described in experimental animals; in our patient while there was no spontaneous increase in enzyme activity, administration of phenobarbital produced the expected increase in pentobarbital hydroxylase.

Depletion of hepatocyte enzymes is expected with massive autolytic necrosis; a significant reduction in cytoplasmic enzymes may also occur with localized necrosis (16). An increase in pentobarbital hydroxylase activity during active regeneration was not expected since microsomal enzyme activity decreases during active regeneration after partial hepatectomy (17), and in rapidly replicating neonatal (18), or neoplastic liver cells (19). The difference may be related to availability of protein to increase microsomal enzymes with marked cell turnover. Patient factors including retention of bilirubin or other metabolites, viral particles or tissue products could be responsible for increments in pentobarbital hydroxylase activity encountered in our study. Marked elevations of pentobarbital hydroxylase were observed in one patient during regenerative phases of nonicteric hepatitis; this makes it unlikely that bilirubin *per se* is important.

Microsomal drug-metabolizing enzyme activity determines in part, the clearance rates of various drugs metabolized by the hepatic SER. Serial study of pentobarbital clearance in one of the patients with subacute massive necrosis and reduced pentobarbital hydroxylase activity demonstrated an increase in drug half-life. Repeat study at the peak of regeneration revealed an increase in pentobarbital hydroxylase activity and a decrease in drug half-life. These observations may account for the marked somnolence or coma produced occasionally by sedatives in patients with hepatitis, and explain further the remarkable tolerance for drugs in most instances of reversible liver injury.

Summary. Microsomal pentobarbital hydroxylase activity was estimated by a radioisotope dilution technique in liver biopsy specimens from patients in various stages of acute uncomplicated viral hepatitis. Results indicate that liver cell necrosis and regeneration may markedly alter drug-metabolizing enzymes independent of drug therapy. No significant difference was noted in mean pentobarbital hydroxylase activity in biopsy specimens from volunteer normal controls and those with localized acidophilic necrosis or focal inflammation. A significant decrease in enzyme activity occurred with subacute autolytic necrosis and a four-fold increase was noted during active regeneration, reflected in Kupffer cell proliferation and increased *in vitro* hepatic DNA synthesis. There is a variable relationship between pentobarbital hydroxylase activity, abnormal liver function tests, and morphologic evidence of hyperplasia of the endoplasmic reticulum.

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