*ORGANIC ANION-BINDING PROTEIN IN RAT LIVER: DRUG INDUCTION AND ITS PHYSIOLOGIC CONSEQUENCE**

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Abstract.—The administration of phenobarbital to rats enhanced hepatic uptake of an organic anion, bromsulphalein, *in vivo* and simultaneously increased the amount of Y, a hepatic cytoplasmic organic anion-binding protein. This study supports the postulate that Y is a major determinant in the selective hepatic uptake of certain organic anions from plasma. Induction of Y may contribute to the enhanced hepatic uptake and metabolism of various organic anions (drugs, hormones, etc.) produced by phenobarbital and other agents.

Introduction.—Gel filtration of 100,000 × *g* supernate of liver homogenate reveals two protein fractions that bind several organic anions, such as bilirubin and bromsulphalein, whether they are injected *in vivo* or added *in vitro*. These protein fractions are selectively found in mammalian liver. The two proteins, Y and Z, responsible for organic-anion binding, have been purified and partially characterized and their turnover and development have been studied.[1–3] Based upon these studies, we have proposed that Y and Z proteins are major determinants in the transfer of various organic anions from plasma into the liver and account for the selective hepatic extraction of these anions from blood.

This report concerns the effect of phenobarbital on Y and Z hepatic cytoplasmic proteins and their relationship to the plasma disappearance rate of injected bromsulphalein used as a measure of hepatic uptake of the dye.

Materials and Methods.—Adult male Sprague-Dawley rats weighing 250 to 450 gm received daily subcutaneous injections of sodium phenobarbital, 8 mg/100 gm body weight, in 0.9% NaCl. Control animals received similar amounts of saline. After periods of treatment which ranged from 2 to 40 days, the livers were removed under ether anesthesia and perfused with cold saline. A 25% homogenate was prepared in 0.25 *M* sucrose-phosphate buffer, pH 7.4, and centrifuged at 100,000 × *g* for 2 hr. Aliquots of supernate equivalent to 1 gm of liver were mixed with 3.75 mg of bromsulphalein for 5 min at 25°C. The amount of bromsulphalein bound by Y and Z protein fractions when eluted in a standardized gel-filtration system was used to quantitate the Y and Z proteins.[1]

Results.—In untreated rats, Y bound 0.59 ± 0.04 (SE) and Z bound 0.33 ± 0.02 mg bromsulphalein per 100 gm body weight. These values were unaffected by saline injection in control animals.

Y increased following administration of phenobarbital (Fig. 1) and approached a new steady-state level of approximately 120 per cent over the mean basal value ($p < 0.005$) at 6 days. Z did not change in rats treated for 14 days. With prolonged treatment, Z was reduced to 58 per cent of the mean basal value ($p < 0.025$). These changes were confirmed by electrophoresis in 5 per cent acrylamide gel using aliquots with similar protein content obtained from the peak

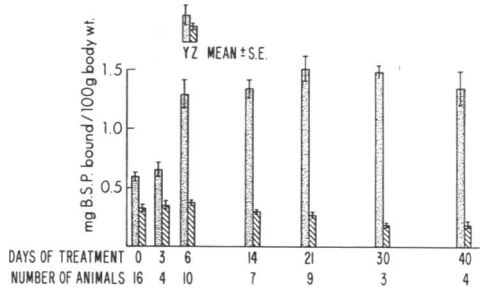

FIG. 1.—Effect of daily phenobarbital administration (8 mg/100 gm body weight) on Y and Z hepatic cytoplasmic proteins as measured by bromsulphalein binding *in vitro*.

tubes of Y and Z fractions in control rats and animals treated 14 and 30 days with phenobarbital (Fig. 2).

To study the effect of Y induction by phenobarbital on the hepatic uptake of bromsulphalein, we determined K_1, the first-order rate constant for removal of bromsulphalein from plasma after a single intravenous injection of the dye (Fig. 3).[4] In untreated rats, mean K_1 was 0.192 ± 0.005 (SE) and was unchanged in saline-injected animals. In phenobarbital-treated rats, K_1 increased rapidly and was 30 per cent over the mean basal value after six days ($p < 0.01$) and, after prolonged treatment, remained at 36 to 42 per cent over the mean basal value.

This effect was also seen in 14 additional rats in which K_1 was serially estimated before, during, and after phenobarbital or saline administration. In the nine drug-treated rats, K_1 was 24 per cent greater than the mean basal value after two days ($p < 0.025$) and 48 per cent after 12 days ($p < 0.01$). After discontinuing phenobarbital administration, K_1 returned to normal by nine days, at which time normal amounts of Y and Z were found.

Discussion.—These experiments demonstrate that phenobarbital administration increased Y protein as determined by bromsulphalein binding. Theoretically, this change could result from altered affinity of the protein for the dye; however, gel electrophoresis demonstrated an increase in Y protein (Fig. 2). Analysis of the full time-course response of Y before, during, and after phenobarbital administration suggests that its half-life is approximately three to five

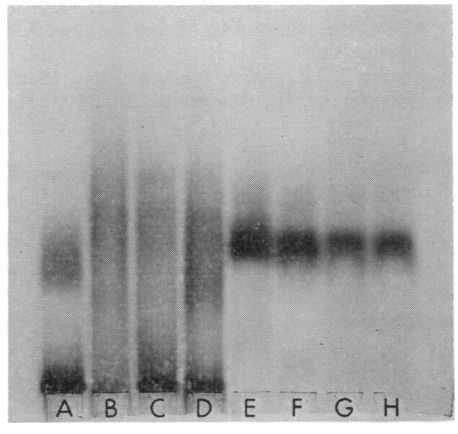

FIG. 2.—Vertical acrylamide gel electrophoresis (0.1 *M* barbital, pH 8.6) of Y and Z protein fractions from control and phenobarbital-treated rats. Amido-Schwarz stain. (*A*) Purified Y (200 μg), standard; (*B*) Y fraction (200 μg), control rat; (*C*) Y fraction (200 μg), phenobarbital-treated rat, 14 days; (*D*) Y fraction (200 μg), phenobarbital-treated rat, 30 days; (*E*) purified Z (100 μg), standard; (*F*) Z fraction (100 μg), control rat; (*G*) Z fraction (100 μg), phenobarbital-treated rat, 14 days; (*H*) Z fraction (100 μg), phenobarbital-treated rat, 30 days. Purified Y and Z standards were obtained after sequential Sephadex G-75 gel filtration and DEAE and CMC ion exchange chromatography of 100,000 × *g* supernate of rat liver.[1]

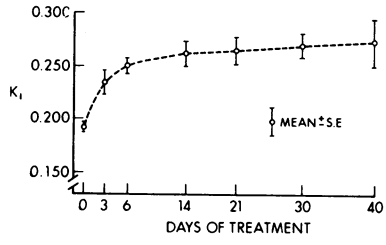

FIG. 3.—Effect of daily phenobarbital administration (8 mg/100 gm body weight) on K_1, the first-order rate constant for bromsulphalein removal from plasma. 24 hr after the last injection of phenobarbital, a single dose of 5 mg sodium bromsulphalein/100 gm body weight was injected intravenously. Blood samples were obtained in heparinized capillary tubes at 30 to 60-sec intervals from 2 to 7 min after BSP injection. Aliquots of 20 μl plasma were alkalinized with 1 ml 0.05 N NaOH. Optical density was determined at 580 mμ in a Beckman DU spectrophotometer using 20 μl of plasma diluted with 1 ml 0.0012 N HCl as a blank. Optical density was plotted semilogarithmically against time; the half-time of BSP disappearance was determined, and K_1 was calculated $K_1 = \frac{1n2}{t\,1/2}$. Each value is the mean in a group of 4–21 animals.

days and that phenobarbital increases Y synthesis[5, 6]; however, partial stabilization of Y has not been excluded.

Induction of hepatic microsomal enzymes responsible for the metabolism of bilirubin and many drugs has been regarded as the explanation for the faster plasma disappearance rate of bilirubin, dyes and drugs,[7, 8] as well as reduction of unconjugated hyperbilirubinemia in neonates[9] and older individuals[10–12] following phenobarbital administration. These changes could also result from induction of Y protein and consequent enhancement of hepatic uptake of bilirubin or drugs.

Y may be identical to one of the proteins responsible for the binding of cortisol metabolites in liver cytoplasm[13] and, therefore, induction of Y may alter the hepatic removal of cortisol and other steroids.

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