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Effect of chronic phenobarbital administration on the turnover of hepatic microsomal protein

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THE INCREASE in microsomal constituents after the administration of phenobarbital could result from an increase in synthesis, a decrease in degradation, or both. The present evidence would indicate that the increase in enzyme and protein concentrations results primarily from an increase in synthesis.[1–4] On the other hand, our previous work has indicated that the increase in phospholipids is due primarily to a decrease in catabolism with a slight increase in synthesis in male rats only.[5,6]

Schuster and Jick[7,8] have reported that the increase in both total microsomal protein and NADPH-cytochrome *c* reductase results from both an increase in synthesis and a highly significant decrease in degradation. In these studies they concurrently gave radioactive leucine and phenobarbital. Unfortunately, a large fraction of leucine is reutilized[9] so the decay curves really represent the combined turnover curves for the protein and the free-leucine pool. Further, since the label was given at the beginning of the induction period, the level of microsomal protein was not in a steady state during the period of observation and, therefore, the simple first-order kinetics should have been modified to account for the changes in the levels of protein. Finally, they did not examine the effect of the treatment on the precursor pool. This pool appears to be markedly affected.[4]

To avoid these problems, I have examined the effect of chronic phenobarbital administration on the long-term decay of microsomal protein using a non-reutilized amino acid. I have found that this drug has no effect on the catabolism of total microsomal protein and therefore must act almost solely by increasing synthesis.

METHODS

Female Sprague–Dawley rats (160–180 g) from the NIH colony were maintained in individual hanging-wire cages and received a standard laboratory chow. In the experiment in which the turnovers of glutamic- and arginine-labeled microsomes were compared, animals received either 4·5 μc of ^{14}C-glutamate (u.l.) or 3·0 μc of ^{14}C-guanido-arginine via the caudal vein. At doubling-time intervals from 45 min to 7 days, 3 animals from each group were sacrificed by decapitation. When the effect of phenobarbital on the catabolism of microsomal protein was investigated, the animals received throughout the entire experimental period a single daily intraperitoneal dose of phenobarbital (80 mg/kg/day) in saline or an equivalent volume of saline. On the eleventh day of treatment both the experimental and control groups received 5 μc of ^{14}C-glutamate (u.l.) and the animals were sacrificed as above.

At sacrifice the livers were removed, weighed, chilled on ice and homogenized in 3 vol. of KCl (150 mM)-Tris(20 mM)-(pH 7·4) per g of liver. The homogenate was centrifuged at 9000 *g* for 15 min in a Sorval RC-2B centrifuge with a SS-34 rotor. The supernatant was centrifuged at 164,000 *g* for 38 min. The pellets were frozen at − 20° until analysis.

The pellets were resuspended to give about 2 g of liver wet weight per ml of KCl-Tris and the actual volume determined.

The protein concentration was determined by the method of Sutherland *et al.*[10]

An 0·5-ml aliquot of the microsomal suspension (20–30 mg protein) was placed in a 20 ml glass counting vial, 3 ml of NCS solubilizer (Nuclear-Chicago) added and the mixture allowed to stand overnight at room temperature. After solubilization 15 ml of a 4% solution of BBOT [2, 5-2-(5-*tert*-butyl-benzoxazoyl)-thiophene] was added and the samples counted in a Tri-Carb 3375 liquid scintillation counter. The absolute activity was determined by the addition of ^{14}C-toluene as an internal standard.

The curves were fitted by inspection.

RESULTS AND DISCUSSION

Studies on the use of radioactive amino acids in the determination of the turnover of albumin demonstrated differing $T_{\frac{1}{2}}$'s for the protein depending on the amino acid used, due to recycling of some

amino acids.[9] To circumvent this problem for liver proteins, Swick[11] recommended the use of ^{14}C-guanido-arginine, since the guanido group, on degradation of the protein, becomes urea with loss of the label as CO_2 or urea. I have compared the decay curve for microsomal proteins labeled with arginine to that obtained with glutamate, another acid with a large serum pool. As can be seen in Fig. 1 after i.v. administration [4·5 μc ^{14}C-glutamate (u.l.), 190 mc/mM; 3·0 μc ^{14}C-guanido-arginine,

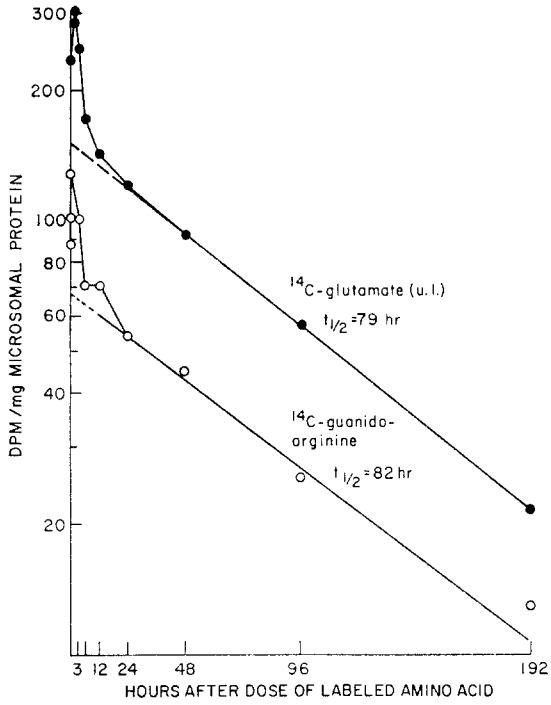

FIG. 1. Disappearance of radioactivity from microsomes of adult female rats after 4·5 μc of ^{14}C-glutamate (u.l.) or 3·0 μc of ^{14}C-guanido-arginine.

30 mc/mM], the two amino acids give essentially the same $T_{\frac{1}{2}}$ for microsomal protein ($T_{\frac{1}{2}}$ glutamate 79 hr, $T_{\frac{1}{2}}$ arginine = 82 hr) suggesting that glutamate is as valid an index of microsomal protein turnover as is arginine. Yet glutamate has a higher specific activity resulting in less perturbation of the amino-acid pool and a greater incorporation per microcurie of administered isotope (at T_0 = 0 DPM/mg microsomal protein/μc-^{14}C-glutamate = 33·8; per μc ^{14}C-guanidoarginine = 22·7).

As can be seen in Fig. 2, there is no significant difference between the decay curves for control and phenobarbital treated animals receiving ^{14}C-glutamate (u.l.) (5 μc-193 mc/mM i.p.) ($T_{\frac{1}{2}}$ control = 86 hr, $T_{\frac{1}{2}}$ phenobarbital = 80 hr) in spite of a 20 to 30 per cent increase in the total microsomal protein, suggesting that only synthesis is increased without a decrease in degradation.

A second interesting aspect of all these curves is that they are not straight lines, but rather appear to consist of at least two components ($T_{\frac{1}{2}}$ long = 80 hr; $T_{\frac{1}{2}}$ short = 2 hr). Although the absolute amount of each component increases, neither $T_{\frac{1}{2}}$ is affected by the administration of phenobarbital

There are several possible reasons for this nonlinearity. The microsomes are derived from several morphological structures as the smooth and rough endoplasmic reticulum and the plasma membrane; each may have a different $T_{\frac{1}{2}}$.

Second, the rat liver consists of both hepatocytes, reticuloendothelial cells and other assorted fibrous and hemopoetic cells. Each may well contribute components to the microsomal pellet which would have differing $T_{\frac{1}{2}}$'s. Neither of these first two possibilities was evaluated as the methods available are both very demanding and have yet to be well established. A third possibility is that the short

component represents nascent albumin, but all the immunospecific-labeled albumin has left the microsomes by 50 min.[12]

A fourth possibility is that these two components represent two separate proteins within the endoplasmic reticulum of the hepatocytes. If this is the case, the data would lend further support to the concept that the components of the endoplasmic reticulum do not turn over as little solid packets of protein and phospholipid, but rather as if they are essentially in a liquid medium.

The latter possibility is further supported by the wide range of $T_{\frac{1}{2}}$'s reported in both my own work and that of others for the various microsomal components. On the basis of our incorporation data, the phospholipid had a considerably shorter $T_{\frac{1}{2}}$ ($T_{\frac{1}{2}}$ = 10-12 hr) than did the protein ($T_{\frac{1}{2}}$ = 27 hr)

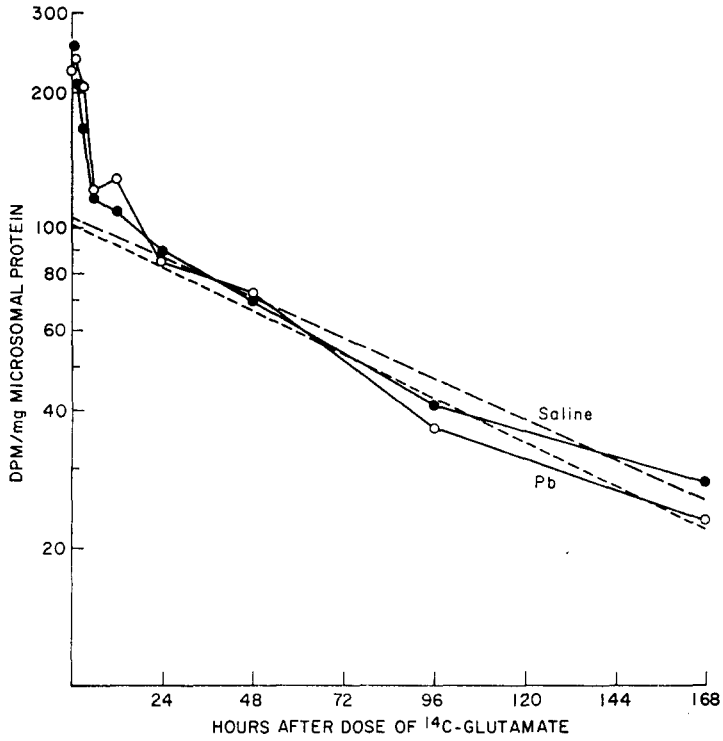

FIG. 2. Effect of phenobarbital (Pb) (80 mg/kg/day i.p.) in adult female rats on the disappearance of radioactivity after 5 μc of ^{14}C-glutamate (u.l.) 10 days after the first dose of phenobarbital.

while the aniline hydroxylase had $T_{\frac{1}{2}}$ which fell in between ($T_{\frac{1}{2}}$ = 19 hr).[6] The protein $T_{\frac{1}{2}}$ was estimated from the rate of induction and would be an average of the mixture. If in determining the average $T_{\frac{1}{2}}$ of the protein we weight them in proportion to their intercept on the ordinate (2 short/one long), we would get an overall $T_{\frac{1}{2}}$ for the protein of 28 hr. Studies now in progress* indicate that even within the microsomal phospholipids there are marked differences in $T_{\frac{1}{2}}$.

Other workers have also presented data indicating that the various components of the microsomes turn over independently. Arias and DeLeon[3] have shown that the $T_{\frac{1}{2}}$ of the hexobarbital hydroxylase is much longer ($T_{\frac{1}{2}}$ = 60 hr) than the value we observed for aniline hydroxylase. Schimke *et al.*[4] have shown that there are a multitude of microsomal components with a wide range of $T_{\frac{1}{2}}$'s. Finally, Omura *et al.*[13] presented data which suggest that the microsomal protein, glycerol and fatty acids turn over at different rates. Unfortunately, the labeled leucine and fatty acids used are both extensively reutilized. The $T_{\frac{1}{2}}$ for the glycerol portion differs from our incorporation data.

In conclusion it would appear that the microsomes consist of a number of constituents which

turn over independently of each other. Further, the mechanism of increase for each constituent after phenobarbital administration would appear to be different; the protein portions showing solely an increase in synthesis without any decrease in catabolism, while the phospholipid portions show primarily a decrease in catabolism with little or no increase in synthesis.

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* J. L. Holtzman, T. E. Gram and J. R. Gillette, unpublished observations.

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Adenyl cyclase in non-nucleated erythrocytes of several mammalian species

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EARLY reports to the effect that the nucleated avian erythrocytes, but not the non-nucleated canine variety, possessed measurable adenyl cyclase activity[1] lead to a generalization that only nucleated cells possessed this enzyme. A subsequent report that the erythrocytes of frogs and tadpoles contained an adenyl cyclase stimulated by catecholamine and fluoride while those of humans were devoid of a catecholamine-stimulated enzyme[2] supported this concept. However, the dog erythrocyte is deficient in a sodium pump and thus probably a Na^+-K^+ activated ATP-ase, making it atypical of non-nucleated erythrocytes in this respect. One must also consider that the absence of a catecholamine-stimulated adenyl cyclase in human erythrocytes does not preclude the presence of an enzyme which is stimulated by fluoride or other hormones.

The enzyme is reported to be present in the plasma membrane,[2,3] and, therefore, non-nucleated erythrocytes would yield a membrane preparation free of contamination from other cellular inclusions. Motivated by a desire to study the activation and inhibition of adenyl cyclase a decision was made to investigate the possibility that non-nucleated erythrocytes of certain species possessed a hormone-activated enzyme. It was also of interest to examine the possible positive correlation of adenyl cyclase with a sodium pump. Therefore, the high Na^+-containing erythrocytes from the cat and dog were compared with the high K^+-containing erythrocytes of the human, rat and mouse.