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COMPARISON OF γ-GLUTAMYL TRANSFERASE INDUCTION BY PHENOBARBITAL IN THE RAT, GUINEA PIG AND RABBIT

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Abstract—Serum and hepatic γ-glutamyl transferase (GGT) activities were correlated with the microsomal markers cytochrome P-450 and aminopyrine *N*-demethylase after i.p. injection of phenobarbital (PB) to rats, guinea pigs and rabbits. The response to PB in the regimen employed was greatest in the rabbit and least in the guinea pig. Great disparities were observed in the microsomal protein contents following PB administration to the three species, masking the responses of the other indices when these were related to protein contents rather than to tissue weights. The increased hepatic GGT activities in PB-treated guinea pigs and rabbits were reflected in increased serum activities of this enzyme; the hepatic and serum GGT activities showed an excellent correlation with cytochrome P-450 and aminopyrine *N*-demethylase activities, supporting the view that the changes in GGT activity were related to enzyme induction. Although hepatic GGT activity in PB-treated rats also showed good correlation with enzyme induction indices, activity of this enzyme in rat serum was undetectable in control and PB-treated animals. Analysis of ribosome-free microsomal proteins by sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis confirmed the marked increase in three bands in the PB-treated rat, but quite different changes were noted in the guinea pig and the rabbit. Our results extend knowledge about the heterogeneous response to PB shown by different animal species. The data provide further evidence that GGT is a PB-inducible enzyme, and suggest that the rabbit is the best model for elucidating the relationship between enzyme induction and GGT activity occurring in several human clinical situations.

γ-Glutamyl transferase (GGT, EC 2.3.2.2) catalyzes the transfer of a γ-glutamyl group from γ-glutamyl peptides to other peptides or amino acid receptors. Clincal studies indicate that the GGT activity of serum is increased in human subjects receiving enzyme-inducing drugs [1–3]. This increased GGT activity has been proposed as a convenient index of hepatic microsomal enzyme induction in man [4–7]. Support for this view has come from experiments demonstrating increased GGT activity in liver homogenates and microsomes of animals to whom enzyme-inducing drugs were administered [8–12]. The results of these experiments, however, indicated much variability, and the changes in GGT activity of the serum and the hepatic microsomes did not always run parallel.

This problem has become more complex with evidence that, in the rat, the liver parenchymal cell is relatively poor in GGT compared with biliary and Kupffer cells [13–15]. In the rat and, also, in the guinea pig, it has been shown recently that GGT is probably localized in the plasma membrane of hepatocytes rather than in endoplasmic reticulum, although its activity was increased by administration of phenobarbital (PB) to both species [16, 17].

Because of the well known variation in response to enzyme-inducing drugs shown by different animals [18, 19], we have compared the effects of PB in rats, rabbits and guinea pigs. The relationships between serum and hepatic GGT activities were analyzed and correlated with the responses shown by the classical microsomal markers cytochrome P-450 and aminopyrine *N*-demethylase in three animal species.

MATERIALS AND METHODS

Male Wistar rats (100 g, average body weight) were purchased from the Canadian Biobreeding Laboratory, Ottawa, Ontario. Male albino guinea pigs (250 g) were from High Oak, Goderich, Ontario, and male New Zealand albino rabbits (2 kg) were from Reiman's, Kitchener, Ontario. They were housed in clean separate wire mesh cages at 22 ± 2° and 55–60° relative humidity, and were fed laboratory chow and tap water *ad lib*. PB was dissolved in 0.15 M NaCl and injected i.p. at a dose of 100 mg/kg body weight for 5 days in rats, 50 mg/kg for 7 days in guinea pigs, and 50 mg/kg for 10 days in rabbits. The control animals were injected with saline alone.

On the day after the last injection following a 15-hr fast, animals were anesthetized briefly with

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chloroform; blood was withdrawn by cardiac puncture and allowed to clot; and serum was removed by centrifugation to determine GGT activity. The abdominal cavity was opened, and the livers were perfused, removed, washed thoroughly, blotted dry, weighed and homogenized in 3 vol. of solution A [0.2 M sucrose containing 0.05 M *n*-2 hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.0, 1 mM EDTA, and 2 mM dithioerythritol]. After a measured aliquot was removed, the homogenate was centrifuged at 10,000 *g* for 10 min at 4°. A measured aliquot of the supernatant fraction was removed, and the remainder was centrifuged at 105,000 *g* for 60 min to precipitate the microsomes that were resuspended by homogenization in a measured volume of solution A, after washing and resedimenting as previously. All of the above operations were performed quantitatively to allow calculation of all constitutents per g of liver.

Determination of GGT activity in liver homogenate and microsomes. The method of Orlowski and Meister [20] with γ-glutamyl-*p*-nitroanilide as substrate was modified. The sample (0.5 ml) was incubated with 1 ml of the substrate mixture (4 mM γ-glutamyl-*p*-nitroanilide, 40 mM glycylglycine and 11 mM $MgCl_2$ in 185 mM Tris buffer, pH 8.25) at 37°. After 10 min, 1 ml of 25% (w/v) trichloroacetic acid (TCA) was added and mixed. The solution was centrifuged, and the absorbance of the supernatant fraction was read at 405 nm. The molar extinction coefficient of free *p*-nitroaniline in the above buffer/TCA mixture at 405 nm was experimentally determined to be 1.74×10^3 for a 1-cm light path. This was used to calculate activity as moles of product formed per minute after substracting an appropriate zero-time blank.

Determination of GGT activity in serum. The continuous spectrophotometric assay of Szasz [21] was employed at 30°.

Determination of cytochrome P-450 content. Cytochrome P-450 content was determined in the microsomal fraction (protein concentration 2 mg/ml) according to the method of Omura and Sato [22]. The use of the term cytochrome P-450 in relation to the data in this paper is defined by this method of assay. A molar extinction coefficient of 91 $l \cdot mmole^{-1} \cdot cm^{-1}$ was used for the optical density difference (450–480 nm) of the spectrum in dithionite and carbon monoxide.

Solubilization of hepatic and microsomal GGT. Since GGT is a membrane-bound enzyme, its activity in rat and guinea pig liver was determined, after solubilization with sodium deoxycholate (DOC) and Triton X-100, and compared with untreated samples. DOC in final concentrations ranging from 0.1 to 2% (w/v) was incubated with homogenate and microsomal preparations for 10 min at room temperature. The highest GGT activity occurred in the presence of 1% DOC, and the increase varied with the species and with the fraction, but it was not affected by PB treatment except in guinea pig microsomes, where 1% DOC enhanced GGT activity by 50 per cent in the controls and by only 25 per cent in the treated animals (Table 1). Apart from the data in Table 1, all values for GGT activity in this paper were based on assays that did not utilize DOC. Triton X-100 interfered with the GGT determination, as it gave a turbid solution after adding TCA, and was not used further.

Determination of aminopyrine N-demethylase activity. This was determined in the 10,000 *g* supernatant fraction according to the method of Nash [23].

Sodium dodecylsulfate (SDS)–polyacrylamide gel electrophoresis. SDS electrophoresis was carried out on flat plate 10% polyacrylamide separating gels and 5% acrylamide stacking gels by the system of Laemmli [24] as described by Cameron *et al.* [25]. The microsomal fractions were stripped of ribosomes by three successive washes in pyrophosphate citrate buffer, pH 7.4, at 0° for 30 min [26] prior to electrophoresis, and 50 μg protein was applied to the gel. The tracking dye (bromophenol blue) was allowed to migrate 18 cm from the origin, at which time (6 hr) electrophoresis was terminated and the gels were stained with Coomassie blue.

Determination of protein concentration. Protein was determined according to the method of Lowry *et al.* [27] as modified by Miller [28], using bovine serum albumin as standard.

Statistics. Student's *t*-test was used to assess the significance of the differences between test and control animals. Fisher's transformed correlation coefficient [29] was used to assess the degree of association between various biochemical procedures. Results were obtained using the HP Programmable Calculator (Hewlett Packard, Loveland, CO, U.S.A.).

RESULTS

PB, in the dose schedule employed in rats, guinea pigs and rabbits, brought about marked increases in cytochrome P-450 content and aminopyrine *N*-demethylase activity over the control animals. Simultaneously, we observed significant increases in GGT activity in liver homogenates and microsomal fractions. These increments, expressed as a percentage of the values in control animals, are shown in Figs. 1 and 2. The increase in cytochrome P-450 per mg protein was highest in rats, intermediate in rabbits, and lowest in guinea pigs. These changes in cytochrome P-450 were even more dramatic when the results were expressed per g of liver, being greatest in rabbits and least in guinea pigs. The largest increment in aminopyrine *N*-demethylase activity per mg protein and per g liver occurred in rabbits and the least occurred in rats.

Microsomal protein electrophoresis. Further evidence of induction was obtained by analyzing the stripped (ribosome-free) hepatic microsomal membrane polypeptides from control and PB-treated animals on SDS–polyacrylamide gel electrophoresis (Fig. 3). We confirmed that the principal effect of PB on rat microsomal polypeptides was the marked increase in bands *a*, *b* and *c* [30]. In guinea pigs, the intensity of only one protein band, designated *a* in Fig. 3, was increased with PB treatment. In rabbits treated with PB, the intensity of the bands designated *a* and *c* in Fig. 3 was increased, and a new band, designated *b*, was generated between *a* and *c*. Our results in PB-treated rabbits were qualitatively

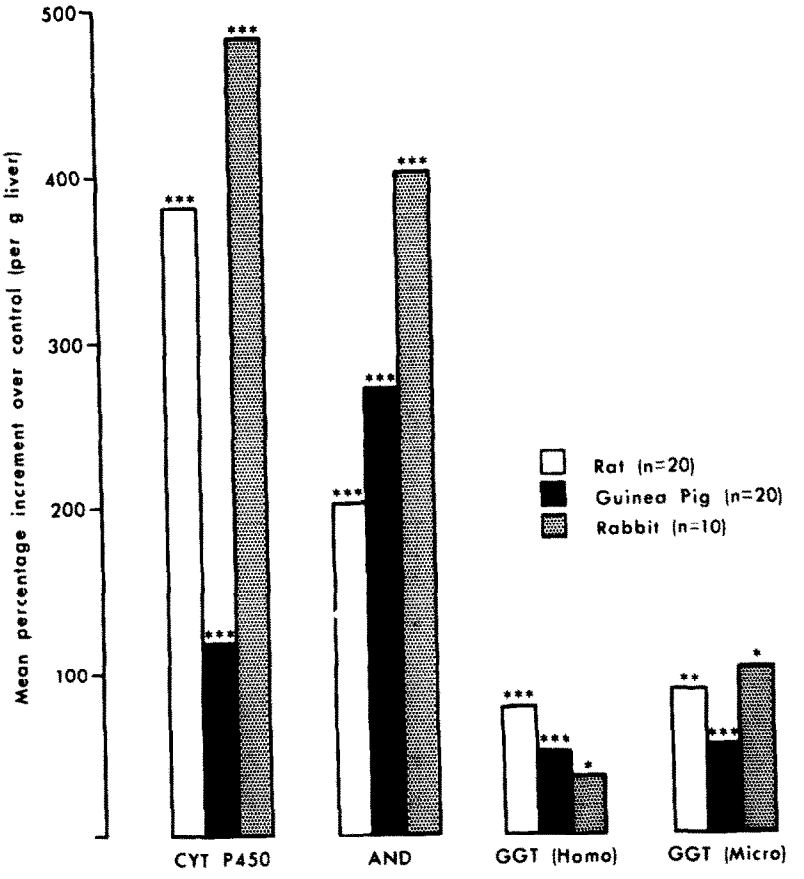

Fig. 1. Mean percentage increment in microsomal enzyme induction indices per g liver weight of PB-treated animals over values in control animals. The following abbreviations are used: CYT P-450, cytochrome P-450 AND, aminopyrine *N*-demethylase; and GGT, γ-glutamyltransferase in whole homogenate (Homo) and microsomes (Micro) of liver. Asterisks indicate the significance of differences between treated and control animals as follows: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ (N = number of animals in each test and control group).

similar to those reported previously [31]. The guinea pig was more refractory than the rat and rabbit to changes in the microsomal membrane polypeptide pattern after PB, and it showed a similar lack of sensitivity to methylcholanthrene compared with the rat and the rabbit [32].

Changes in GGT activity. A significant increase was observed in GGT activity of the liver homogenates of PB-treated animals per mg protein and per g of liver. These increments were highest in rats and least in rabbits. GGT activity per g of liver was significantly increased in the microsomes of all three species treated with PB; the increment was highest in rabbits and least in guinea pigs. Only in guinea pigs, however, was there an increased microsomal GGT content per mg protein after PB treatment. This could be explained by the fact that total microsomal protein per g liver increased by 123 per cent in rabbits and by 63 per cent in rats, but by only 40 per cent in guinea pigs. Concurrently, the mean serum GGT activity was 40 per cent higher than the control group in PB-treated guinea pigs ($P < 0.001$), and 150 per cent higher in rabbits ($P < 0.001$). GGT activity was too low to measure accurately in the serum of rats, whether treated on untreated. In identical experiments with female rats, no change in hepatic GGT occurred after PB treatment, although cytochrome P-450 content increased by 206 per cent. This refractory behaviour of GGT in the female rat accords with the reduction in its activity caused by estrogens and pregnancy [33], and with the lower values for serum GGT activities in human females [1, 2].

The GGT data in Figs. 1 and 2 refer to assays performed without DOC. Treatment of liver homogenate and microsomal fractions of rats and guinea pigs with 1 per cent DOC increased GGT activity by 20–50 per cent (Table 1). The increments in the same fraction were very close in control and PB-treated animals with the exception of guinea pig liver

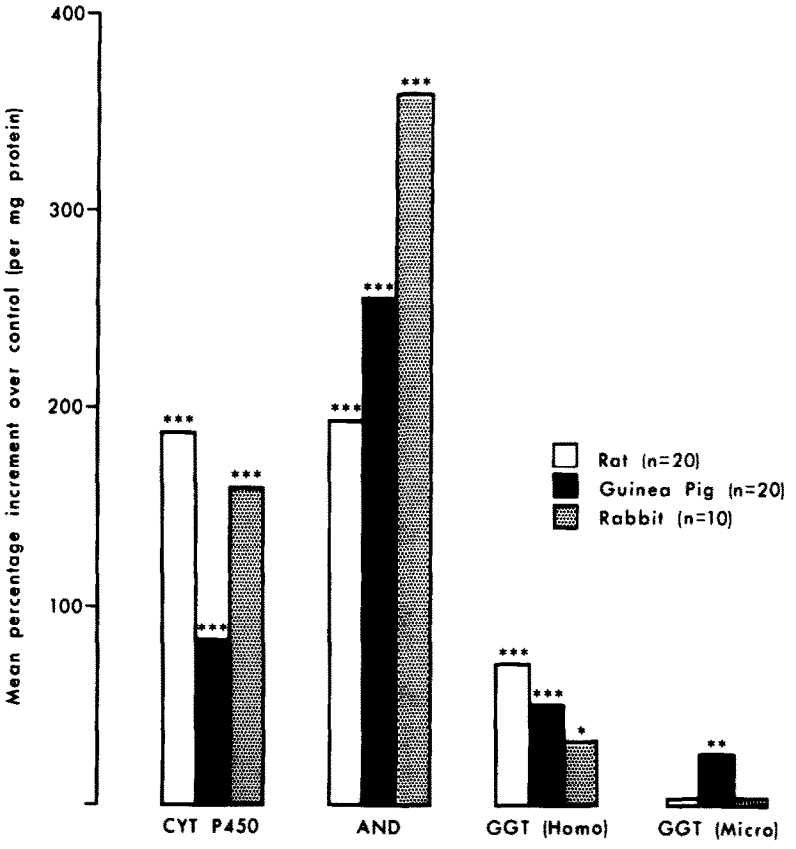

Fig. 2. Mean percentage increment in microsomal enzyme induction indices per mg protein of PB-treated animals over values in control animals. The following abbreviations are used: CYT P450 cytochrome P-450; AND, aminopyrine *N*-demethylase; and GGT, γ-glutamyltransferase in whole homogenate (Homo) and microsomes (Micro) of liver. Asterisks indicate the significant of differences between treated and control animals as follows: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ (N = number of animals in each test and control group).

homogenate, where the increments in GGT activity per mg protein and per g liver after DOC treatment approximated 50 per cent in controls and 25 per cent in the treated group. Nevertheless, all statistically significant increases in GGT activity of the PB-treated animals occurred whether or not DOC was used in preparing the tissue fraction.

GGT activity per mg protein was 2- to 3-fold greater in microsomal fractions than in liver homogenates in control animals from all three species, indicating enrichment of this enzyme in microsomes. In PB-treated animals, the differences between microsomes and whole homogenates in this regard were significantly less pronounced (Fig. 4) due to

Table 1. Percentage increase in GGT activity of rat and guinea pig liver after treatment with 1% (w/v) DOC*

	Homogenate		Microsomes	
	Activity/mg protein	Activity/g liver	Activity/mg protein	Activity/g liver
Rat (N = 20, each group)				
Control	28.8 ± 3.4	24.0 ± 2.9	21.1 ± 3.5	42.2 ± 5.6
PB-treated	26.5 ± 4.2	23.1 ± 3.3	27.0 ± 5.1	40.8 ± 4.9
Guinea pig (N = 20, each group)				
Control	51.6 ± 7.4	56.9 ± 6.5	17.5 ± 2.2	18.3 ± 1.9
PB-treated	24.2 ± 3.1†	27.3 ± 3.6†	23.4 ± 3.3	22.4 ± 3.7

* DOC treatment was for 10 min at room temperature. All data are means ± S.E.
† Difference between groups was statistically significant ($P < 0.01$).

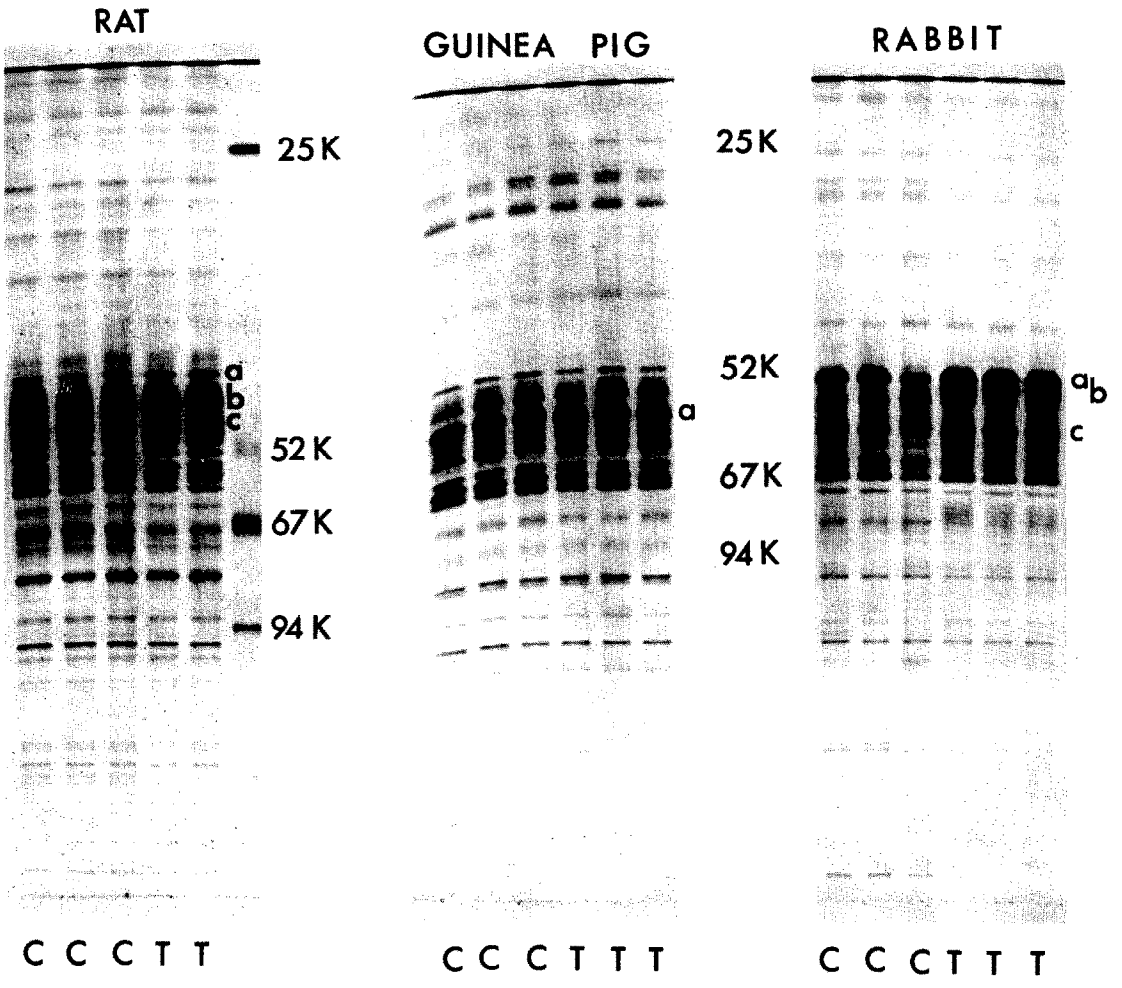

Fig. 3. Flat-plate polyacrylamide–SDS gel electrophoresis of the stripped (ribosome-free) microsomal membrane proteins, from individual control (designated C) and PB-treated (designated T) animals, stained with Coomassie blue. Bands that showed consistent and marked increases in PB-treated animals are indicated by lower-case letters, and the values for molecular weight were derived from the following protein markers: 25K, chymotrypsinogen A; 52K, heavy chain of γ-globulin; 67K, rat serum albumin; and 94K, rabbit muscle phosphorylase *a*.

Table 2. Correlation analysis of hepatic GGT activity and microsomal enzyme induction indices per mg protein

	Rat (N = 40)*		Guinea pig (N = 40)*		Rabbit (N = 20)*	
Variables analyzed	*r*	P	*r*	P	*r*	P
Cytochrome P-450 vs GGT (Homo)	0.380	< 0.02	0.615	< 0.001	0.474	< 0.025
Cyochrome P-450 vs GGT (Micro)	0.440	< 0.01	0.472	< 0.01	0.454†	< 0.05
Aminopyrine *N*-demethylase vs GGT (Homo)	0.400	< 0.02	0.930	< 0.001	0.502	< 0.02
Aminopyrine *N*-demethylase vs GGT (Micro)	0.623	< 0.001	0.465	< 0.05	0.518†	< 0.2
Aminopyrine *N*-demethylase vs Cytochrome P-450	0.713	< 0.001	0.808	< 0.001	0.922	< 0.001

* Control and PB-treated animals pooled for analysis.
† Data per g liver.

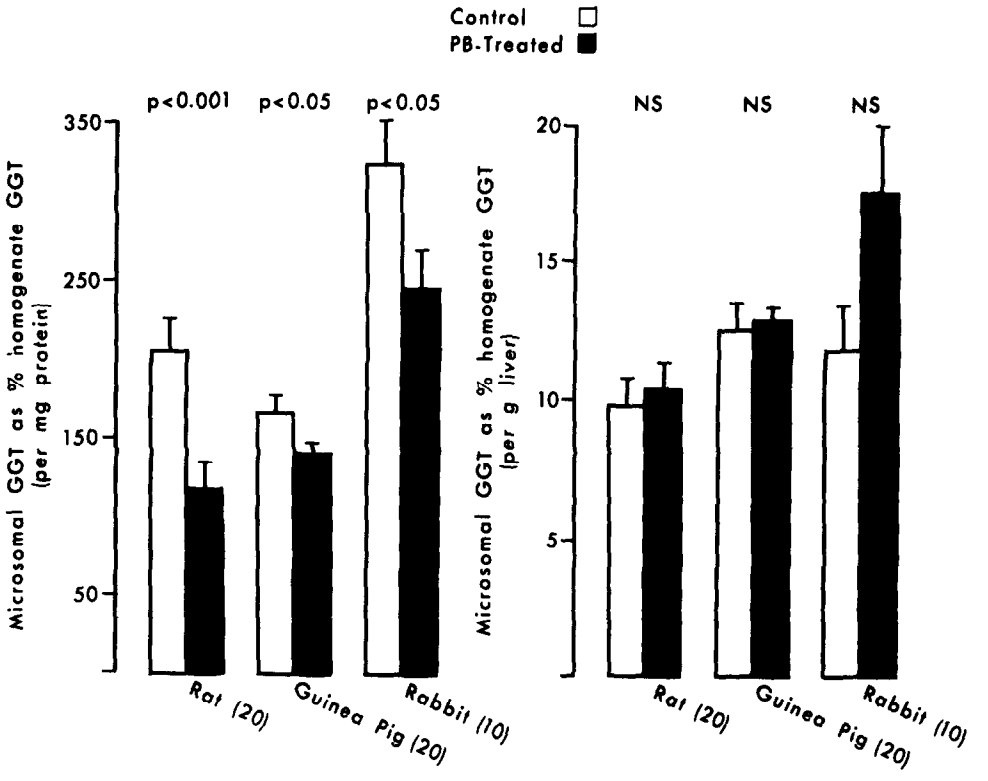

Fig. 4. Microsomal GGT expressed as a percentage of GGT in whole liver homogenate per mg protein and per g of liver in control and PB-treated animals. The significance of the differences between these groups is indicated at the top of the figure; NS = not significant. The number of animals in each test and control groups is indicated in parentheses.

the PB-mediated increase in microsomal protein content described above. GGT activity of microsomes per g liver was only 10–17 per cent that of the whole homogenate, and there were no statistically significant differences between PB-treated and control animals (Fig. 4). Thus, microsomal GGT was a minor component of total hepatic GGT, and PB did not seem to alter this relationship.

Correlation of GGT and enzyme induction indices. Total hepatic and microsomal GGT showed a significant positive correlation with both cytochrome P-450 and aminopyrine *N*-demethylase in the rat, guinea pig, and rabbit (Table 2). An excellent correlation was also obtained between aminopyrine *N*-demethylase activity and cytochrome P-450 content in all three species.

Serum GGT in guinea pigs and rabbits was significantly correlated with hepatic and microsomal GGT, and with the various indices of microsomal enzyme induction (Table 3).

DISCUSSION

The most dramatic increments in cytochrome P-450, aminopyrine *N*-demethylase, and microsomal GGT activity per g of liver occurred in rabbits (Fig. 2), and the 150 per cent increase in serum GGT activity far exceeded that in the other two species following PB treatment. These data suggest that the increased GGT activities in the hepatic microsomes and serum of PB-treated rabbits are consequences of hepatic enzyme induction, although they are not as sensitive to induction as cytochrome P-450 and aminopyrine *N*-demethylase under the conditions employed. In support of this proposal, the relationships in rabbits between serum GGT activity and the hepatic indices of enzyme induction and GGT content were highly significant (Table 3), and a good correlation was obtained between hepatic GGT content and the other two indices of microsomal enzyme induction (Table 2).

The overall response to PB treatment (Fig. 1) was more pronounced in rats than in guinea pigs. Only with aminopyrine *N*-demethylase did the increment in the guinea pig exceed that of the rat. It is therefore surprising that serum GGT activity showed no measurable increase in the rat, but in guinea pig serum it was increased 40 per cent by PB under our experimental conditions. Serum GGT activity of guinea pigs showed highly significant correlations with the hepatic indices of microsomal enzyme induction and GGT content (Table 3). In rats and

Table 3. Correlation analysis of serum GGT activity with hepatic GGT activity and microsomal enzyme induction indices

Variable correlation with serum GGT activity	Guinea pig (N = 40)*		Rabbit (N = 20)*	
	r	P	r	P
Cytochrome P-450 per mg protein	0.652	< 0.001	0.770	< 0.001
Aminopyrine *N*-demethylase per mg protein	0.898	< 0.001	0.822	< 0.001
GGT (Homo) per g of liver	0.900	< 0.001	0.926	< 0.001
GGT (Micro) per g of liver	0.496	< 0.01	0.634	< 0.005

* Control and PB-treated animals pooled for analysis.

guinea pigs, significant correlations between GGT content of homogenate and microsomes and the two other enzyme induction indices were generally obtained (Table 2). These findings suggest that the increased hepatic and serum GGT content of PB-treated guinea pigs and the increased hepatic GGT content of PB-treated rats are consequences of enzyme induction, but in view of recent data [16, 17] it seems likely that the main source of PB-induced GGT is the plasma membrane and not the endoplasmic reticulum.

The data in Fig. 2 relating the hepatic indices of microsomal enzyme induction and GGT content to protein content lead to different conclusions regarding the relative PB sensitivity of the three species. These species, however, showed different responses to PB in protein content, especially in the microsomal fraction. The increment in microsomal protein per g of liver after treatment was 63 per cent in the rat, 40 per cent in the guinea pig, and 123 per cent in the rabbit. The superior sensitivity of the rabbit to the inductive effects of PB was reflected only in aminopyrine *N*-demethylase activity when data were expressed per mg protein. Because increases in enzyme induction indices may be masked by even greater increases in protein content, expression of the data per unit weight of liver is more reliable in assessing the relative sensitivities of different animal species to enzyme induction by PB. This also explains the data in Fig. 4, demonstrating that in all three species GGT activity per mg protein of microsomes relative to that of the whole homogenate fell significantly after PB treatment.

Other workers, using different enzyme-inducing regimens, reported much higher increments of GGT activity in microsomes and serum than of cytochrome P-450 in the rat (Table 4). Hexachlorobenzene-induced rabbits showed, after 20 days, a 570 per cent increase in cytochrome P-450, an increase in aminopyrine *N*-demethylase activity of only 56 per cent, and in microsomal GGT activity of 85 per cent [10]. Alvares *et al.* [18] administered PB in a dose of 75 mg/kg for 3 days to rats and guinea pigs and in a dose of 40 mg/kg for 4 days to rabbits; the increment of cytochrome P-450 per mg protein was 87 per cent in the rat, 64 per cent in the guinea pig, and 249 per cent in the rabbit. Huseby [16] administered PB to guinea pigs at a dose of 80 mg/kg for 4 days and reported a 42 per cent increase in GGT activity per mg protein in "heavy microsomes", but the activity in this fraction was only twice that of the homogenate, whereas the "light nuclear fraction", presumed to be enriched in plasma membranes, had an activity which was 36-fold that of the homogenate. Serum GGT activity increased 70 per cent in the PB-treated group. The control animals were "untreated" and no allowance was made for the effect of handling the animals during drug administration. No other indices of microsomal enzyme induction were measured by this author.

Ratanasavanh *et al.* [17] gave PB in a dose of 100 mg/kg to rats for 4 days and of 50 mg/kg to rabbits for 4 days, but they provided no information about their controls. They did not measure other indices of microsomal enzyme induction or serum GGT activity, but they reported an increase in microsomal GGT activity per g liver of 59 per cent in the rat and 47 per cent in the rabbit after PB treatment. The homogenate GGT activity increased by 10 per cent in the rat and by 319 per cent in the rabbit, and plasma membrane GGT activity increased by 344 per cent in the rat and 449 per cent in the rabbit. They could not detect any change in microsomal protein per g of liver in the PB-treated animals. Yates *et al.* [34] administered PB in a dose of 40 mg/kg to guinea pigs for 4 days and noted an

Table 4. Responses of heaptic cytochrome P-450 and GGT activity of liver and serum to enzyme-inducing drugs in male rats

Ref.	Drug and dose	Duration (days)	Increase in biochemical indices (%)		
			Cytochrome P-450	GGT (Micro)*	GGT (serum)
8	Phenytoin (300 mg/kg)	17–25	19	548	282
9	Aminopyrine (600 mg/kg)	18–25	26	724	457
12	Ethanol (36% of calories)	42	70	207	44

* Expressed as activity per unit of microsomal protein.

increase of 223 per cent in microsomal protein per g liver, and an increase in cytochrome P-450 of 219 per cent per mg protein.

The discrepancies between our results and those described may be explained in part, by differences in the ages of the animals in the various experiments. The dose and duration of PB administration in our experiments were somewhat higher than those of other authors, and they were adequate to cause maximal microsomal enzyme induction. We verified this directly in the rabbit in preliminary experiments over a 20-day period after PB administration. Technical features also account for these discrepancies. Many of these authors used the kinetic assay of Szasz [21] to measure GGT activity in tissue preparations as well as in serum. We found that the turbidity of tissue preparations is too great to obtain reliable results with this technique and elected to use a fixed-time incubation procedure, followed by protein precipitation, having validated the linearity of activity with time and with volume of tissue fraction used. With the method of Szasz [21], serum GGT activities in the control and PB-treated rat were not significantly different from enzyme blanks. Others have reported data with this technique around 1 unit/l, which in our experience could not be measured reliably [8, 9, 11, 12].

We obtained increased hepatic activity of GGT in the presence of 1% DOC ranging from 20 to 50 per cent in the rat and guinea pig. Teschke *et al.* [11] reported that DOC increased GGT activity in rat liver microsomes by 35 per cent in control and ethanol-treated animals, but Morland *et al.* [35] and Ratanasavanh *et al.* [17] found no effect of 1% DOC upon GGT activity in their experiments. Tazi *et al.* [36] found that PB enhanced the *in vitro* solubilization of GGT from rabbit liver plasma membranes, and speculated that facilitation by PB of bile salt-mediated solubilization of membrane-bound GGT, as well as enzyme induction, could account for the increased activity of this enzyme in the serum of PB-treated rabbits. We tested the effect of DOC only in rats and guinea pigs and found no difference in the enhancement of GGT activity by DOC in PB-treated or control animals, except for guinea pig homogenates where greater enhancement occurred in the controls (Table 1). In contrast to Tazi *et al.* [36], however, we measured total GGT activity in these preparations and did not separate the activity into soluble and non-soluble fractions.

Recent findings require reconsideration of the role of GGT as a parameter of microsomal enzyme induction. In rat liver, GGT activity in parenchymal cells is much less than that of bile ductule and Kupffer cells [13–15]. On the other hand, the activity of GGT in guinea pig hepatic parenchymal cells was comparable to that of non-parenchymal cells, and it accounted for more than 90 per cent of the total hepatic GGT activity [16]. Different sub-populations of hepatocytes, especially those located near the terminal hepatic venule, show a selective sensitivity to induction by PB [37–39]. This makes it imperative to use the whole liver or the identical portion of the liver from all test and control animals as in our experiments. PB may increase the activity of cytoplasmic enzymes [40, 41], but it does not affect the distribution of marker enzymes between different cell fractions [16]. Thus, the drug can cause perturbations in hepatocytes beyond those restricted to endoplasmic reticulum and plasma membranes. The responses of enzyme induction indices in rat liver during a longitudinal study with PB were heterogeneous; some remained elevated throughout 59 days of treatment, while others declined after reaching a peak [42].

In conclusion, the heterogeneous responses to PB shown by GGT and other indices of microsomal enzyme induction in the rat, guinea pig, and rabbit accord with previous information, and with the heterogeneity in the response of microsomal proteins separated by gel electrophoresis (Fig. 3). PB caused increases in GGT contents of liver homogenate and microsomes which in all three species correlated with changes in cytochrome P-450 content and aminopyrine *N*-demethylase activity and were accompanied by an increase in serum GGT activity in guinea pigs and rabbits. Thus, consistent behaviour among enzyme induction indices cannot be expected in all species. This poses difficulties in deciding whether increase in a chemical constituent after drug administration is due to enzyme induction or to an unrelated phenomenon such as hepatic damage. The rabbit was the most sensitive of the three animals studied to induction by PB, and provides the best model for elucidating the relationship between enzyme induction and GGT activity that may have relevance to human clinical situations. Only a minor component of hepatic GGT however, is located in endoplasmic reticulum. Therefore, in those species showing PB-mediated increases in hepatic or serum GGT activity, the increments cannot be due to microsomal enzyme induction *per se* although they correlate well with other changes due to microsomal enzyme induction.

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