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Induction of rat hepatic glutathione *S*-transferase B by phenobarbital and 3-methylcholanthrene

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Glutathione *S*-transferase activity of rat liver cytosol reflects the cumulative actions of a number of discrete transferases, many of which have been purified to homogeneity [1–3]. There is a substantial overlap among the various transferases with regard to both substrate specificity and ligandin-like activity [4]. Cumulative catalytic activity and the capacity to bind ligands, such as sulfobromophthalein, are increased by exposure of rats to PB* or 3-MC [5–12], and, indeed, there is immunological evidence for the induction by PB of at least one of the transferases, glutathione *S*-transferase B or ligandin [8]. The fact that PB and 3-MC induce microsomal enzyme systems in different and characteristic fashions [13, 14] prompted us to compare their actions on glutathione *S*-transferase B in relation to certain of the other glutathione *S*-transferases. Most earlier reports have focused on the effects of xenobiotic induction on substrate specificity (e.g. aryl, alkyl and epoxide *S*-transferases), but interpretation of these results in terms of the individual glutathione *S*-transferases is complicated by the overlap in substrate specificity [1–3].

Male Sprague–Dawley rats (150–200 g) obtained from the Canadian Breeding Laboratories (St. Constant, Quebec) were housed on pine chips in the McIntyre Animal Center (McGill University, Montreal, Quebec) and given Purina rat chow and water *ad lib.* for at least 1 week before study. Four rats were housed on Ab-sorb-dri in place of pine chips. One group of rats received PB (Merck, Sharp & Dohme, Kirkland, Quebec) intraperitoneally, 75 mg/kg daily, for 8 successive days before sacrifice 24 hr after the last dose. Control rats received identical volumes of vehicle, 0.15 M NaCl. Another group of animals received single intraperitoneal injections of 3-MC (Sigma Chemical Co., St. Louis, Mo), 20 mg/kg, in corn oil 72 hr before sacrifice. Control rats received corn oil alone.

*The following abbreviations are employed throughout: PB, phenobarbital; and 3-MC, 3-methylcholanthrene.

Rats were killed by decapitation. Homogenates of the excised livers, 20% (w/v), were prepared in ice-cold 1.15% (w/v) KCl in 0.1 M potassium phosphate buffer, pH 7.4. Each homogenate was centrifuged at 10,000 *g* for 20 min, and the pellet was discarded. The supernatant was centrifuged at 105,000 *g* for 60 min to prepare the cytosol.

Conditions for the assay of cytosol for glutathione *S*-transferase catalytic activity toward 1-chloro-2,4-dinitrobenzene (Eastman Kodak Co., Rochester, N.Y.) and 1,2-dichloro-4-nitrobenzene (Eastman Kodak Co.) were identical with those of Habig *et al.* [2]. The spectrophotometric assays were conducted at room temperature (22°) with a Beckman Acta III. Immunologic assays of glutathione *S*-transferase B were conducted with use of an undiluted antiserum to transferase B as described previously [15]. The rabbit antiserum does generate a single line of precipitation with liver cytosol on Ouchterlony immunodiffusion; this line fuses with that generated by homogeneous transferase B. Where indicated, cytosol preparations were diluted to obtain comparable catalytic activities before immunoprecipitation. The decrease in catalytic activity toward 1-chloro-2,4-dinitrobenzene after specific immunoprecipitation was attributed to glutathione *S*-transferase B. Computations of the amount of transferase B in liver were based on the maximal loss of catalytic activity after immunoprecipitation and the specific activity of the homogeneous enzyme toward 1-chloro-2,4-dinitrobenzene (11 μmoles min^{-1} mg^{-1}).

Most of the glutathione *S*-transferases have substantial specific activity toward 1-chloro-2,4-dinitrobenzene. Most also exhibit some activity toward 1,2-dichloro-4-nitrobenzene, but the ratio of activity toward these two substrates varies widely among the individual transferases. Since this ratio is highest with glutathione *S*-transferase B, the relative proportion of transferase B in a mixture of transferases is indicated by the magnitude of this ratio [15]. Animals housed on Ab-sorb-dri exhibit the same ratio and level of transferase activity toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene as animals housed on pine

Table 1. Effect of phenobarbital and 3-methylcholanthrene treatment on glutathione *S*-transferase activity in rat liver*

Treatment group	Glutathione *S*-transferase activity (μmoles/min/g wet wt)	
	1-Chloro-2,4-dinitrobenzene	1,2-Dichloro-4-nitrobenzene
Saline control	122.37 ± 6.49	3.67 ± 0.27
Phenobarbital (% control)	252.25 ± 21.69 (206)	7.83 ± 0.73 (213)
Corn oil control	109.73 ± 3.87	4.44 ± 0.20
3-Methylcholanthrene (% control)	183.92 ± 5.77 (168)	4.97 ± 0.10 (112)

* Each treatment group consisted of three to six animals. Values are expressed as means ± S.E.M. Male (150–200 g) rats received daily intraperitoneal injections of PB, 75 mg/kg body wt, in 0.15 M NaCl for 8 days and were killed 24 hr after the last injection. Another group of rats received an intraperitoneal injection of 3-MC, 20 mg/kg body wt, in corn oil, and the rats were killed 72 hr later. Control animals received 0.15 M NaCl or corn oil. Glutathione *S*-transferase activities toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene were determined as described in the text.

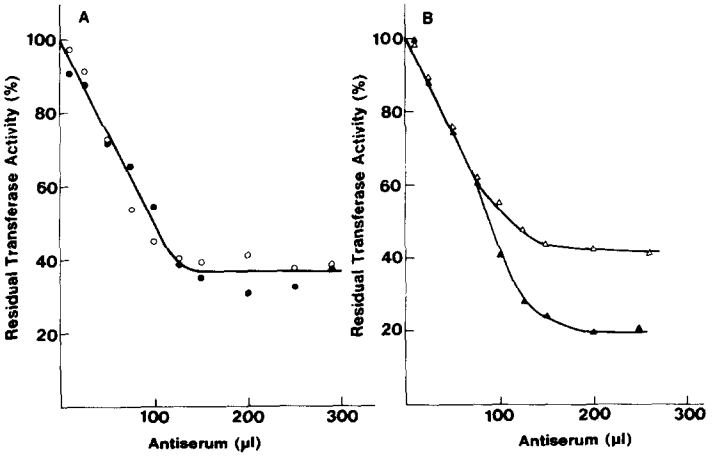

Fig. 1. Anti-glutathione *S*-transferase B immunoprecipitation of catalytic activity toward 1-chloro-2,4-dinitrobenzene. Catalytic activities of hepatic cytosol obtained from animals treated with 0.15 M NaCl (○) or phenobarbital (●) (panel A) or corn oil (△) or 3-MC (▲) (panel B) were immunotitrated with undiluted antiserum to glutathione *S*-transferase B.

chips. Administration of PB for 8 days likewise results in no change in the ratio of activity toward the two substrates (Table 1), but there is a 2-fold increase in glutathione *S*-transferase activity toward each substrate. The tentative conclusion that PB induces glutathione *S*-transferase B in parallel with the other transferases that contribute appreciably to these catalytic activities is supported by immunotitration experiments with anti-transferase B (Fig. 1A). These data reveal that the percentage of transferase activity toward 1-chloro-2,4-dinitrobenzene attributable to transferase B does not change upon induction with PB.

Contrary to the results with PB, administration of 3-MC is associated with a significant increase in the ratio of activity toward these two substrates. There is a 68 per cent increase in transferase activity toward 1-chloro-2,4-dinitrobenzene but only a 12 per cent increase in activity toward 1,2-dichloro-4-nitrobenzene. Immunotitration of liver cytosol from 3-MC-treated rats with anti-transferase B does reveal the expected increase in the percentage of activity toward 1-chloro-2,4-dinitrobenzene attributable to transferase B (Fig. 1B).

With the assumption that the turnover number of glutathione *S*-transferase B from control male rat liver applies to the enzyme from PB and 3-MC-induced animals, the concentration of transferase B can be calculated. Administration of PB increases the concentration of transferase B 3-fold from 4.26 ± 0.54 to 13.43 ± 1.06 mg/g wet wt of liver. Administration of 3-MC increases the amount of transferase B from 4.53 ± 0.48 to 11.45 ± 1.36 mg/g wet wt of liver.

The sum of these data suggests that 3-MC induces glutathione *S*-transferase B more specifically than does PB, at least with respect to the other transferases measured by this methodology. The data do not contradict the possible induction by 3-MC of other transferases, such as glutathione *S*-transferase E [5, 7], which exhibit low specific activity toward the substrates used in this study.

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