The Binding of Aliphatic Carbamates to Bovine Serum Albumin

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Hydrophobic bonding (Kauzmann, 1959) has been suggested to play an important role in the interaction of drugs with plasma proteins (Bird & Marshall, 1967; Helmer *et al.*, 1968; Nagwekar & Kostenbauder, 1970).

The interactions between a series of alkyl carbamates of general structure $R-O-CO-NH_2$ and bovine serum albumin have been investigated. These compounds are stable and not ionized in aqueous solution, and show a progressive increase in lipophilicity as R is increased from methyl to *n*-hexyl. A 4% (w/v) bovine serum albumin (Cohn fraction V; Sigma Chemical Co., St. Louis, Mo., U.S.A.) solution and the carbamate solutions (2–20 mM) were prepared in 0.154 M-phosphate buffer, pH 7.4. Carbamate-albumin solutions were placed in Visking dialysis sacs contained in modified Toribara ultrafiltration tubes (Toribara *et al.*, 1957) and centrifuged for 1 h at 2500 g at 37°, 20° and 10°C. The amounts of unbound and bound carbamate were determined by g.l.c. on a Porapac P column. There appeared to be no significant binding of the carbamates to the dialysis sac.

The results show a progressive increase in percentage bound with increasing hydrocarbon chain length at 37°C. The percentages bound were, for a 1.0 mM-carbamate solution: methyl, 5.47 ± 0.15 ; ethyl, 10.47 ± 0.15 ; propyl, 19.03 ± 0.13 ; butyl, 33.2 ± 1.7 ; pentyl, 41.8 ± 1.33 ; hexyl 64.33 ± 0.33 . Apparent association constants, K , for the interaction were analysed by means of the Scatchard (1949) plot for carbamate/albumin molar ratios ($r = 0$ to 8). A linear Scatchard plot for methyl, ethyl, propyl and butyl carbamate indicated one class of binding sites with $K_1 0.24 \times 10^3 M^{-1}$ and $n_1 = 1 \rightarrow 7$. The plot for pentyl carbamate gave $K_1 0.85 \times 10^3 M$ and n_1 approx. 4, with some evidence of curvature indicating the possibility of a second, lower-affinity, class of binding site. Data for the higher analogue (hexyl) indicated the presence of two classes of binding site with $K_1 5.8 \times 10^3 M^{-1}$ and n_1 approx. 3 and with $K_2 0.11 \times 10^3 M^{-1}$ and n_2 approx. 12. An increase in the apparent association constant of this series of carbamates is correlated with an increase in lipophilicity. This is consistent with the view that hydrophobic bonding is of considerable importance in the carbamate-albumin interaction.

Previous work in this laboratory (Houston *et al.*, 1972) with the same series of carbamates has shown that butyl carbamate possesses optimal lipophilicity for absorption through the intestine and skin of rat.

The possible correlation between the lipophilicity requirements for absorption and plasma-protein binding is under investigation.

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Bird, A. E. & Marshall, A. C. (1967) *Biochem. Pharmacol.* **16**, 2275

Helmer, F., Kiehs, K. & Hansch, C. (1968) *Biochemistry* **7**, 2858

Houston, J. B., Upshall, D. G. & Bridges, J. W. (1972) *Biochem. J.* **128**, 141 P

Kauzmann, W. (1959) *Advan. Protein Chem.* **14**, 1

Nagwekar, J. B. & Kostenbauder, H. B. (1970) *J. Pharm. Sci.* **59**, 751

Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660

Toribara, T. Y., Terepka, A. R. & Dewey, P. A. (1957) *J. Clin. Invest.* **36**, 738

Effect of Inducers on the Metabolism and Excretion of Disodium Cromoglycate in Rats

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Disodium cromoglycate [disodium salt of 1,3-bis-(2-carboxychromon-5-yloxy)-2-hydroxypropane] is used in the treatment of bronchial asthma (Howell & Altounyan, 1967) and has recently been introduced in the treatment of rhinitis (Taylor & Shivalkar, 1971). The metabolism of disodium cromoglycate has been studied in rats (Moss *et al.*, 1970), rabbits, dogs and primates (G. F. Moss, unpublished work) and humans (Walker *et al.*, 1971). No evidence of metabolism was detected in any of these studies. It was therefore decided to investigate whether enzyme inducers would stimulate the formation of any metabolites.

Bile and urine were collected under pentobarbitone anaesthesia from young male rats that had been dosed intravenously with disodium [3H]cromoglycate (40 or 200 mg/kg body wt.) after pretreatment with unlabelled disodium cromoglycate (50 to 200 mg intraperitoneally/kg body wt. twice daily for 4 days), phenobarbitone sodium (40 mg intraperitoneally/kg body wt. twice daily for 4 days) or methylcholanthrene (10 mg intraperitoneally/kg body wt. daily for 2 days). Extensive t.l.c. of bile and urine showed only one peak of radioactivity, which in each case corresponded in R_f to reference disodium cromoglycate.

Liver microsomal preparations from rats pretreated similarly were incubated *in vitro* with disodium [3H]cromoglycate for various times. Subsequent analysis by t.l.c. showed no detectable metabolites.

Metabolism and excretion patterns of intravenously administered disodium [^3H]cromoglycate (100mg/kg body wt.) were examined in a further four groups of rats: (1) controls; (2) phenobarbitone-pretreated; (3) disodium cromoglycate + phenobarbitone-pretreated; (4) disodium cromoglycate-pretreated. As before, no metabolites could be detected in bile or urine. Biliary excretion of radioactivity in rats in groups 2 and 3 was significantly lower than in the controls; this was accompanied by an increase in the urinary excretion of radioactivity. Disodium cromoglycate pretreatment alone caused no significant differences from controls. The overall elimination rate (i.e. urinary plus biliary) was similar in all groups.

It has been shown (Hargreaves & Lathe, 1963) that many compounds that are actively secreted into the bile can inhibit each other's excretion. It is suggested that phenobarbitone decreases the active secretion into the bile of disodium cromoglycate by competing for uptake into the hepatic cell from the plasma.

- Hargreaves, T. & Lathe, G. H. (1963) *Nature (London)* **200**, 1172
 Howell, J. B. L. & Altounyan, R. E. C. (1967) *Lancet* **ii**, 539
 Moss, G. F., Jones, K. M., Ritchie, J. T. & Cox, J. S. G. (1970) *Toxicol. Appl. Pharmacol.* **17**, 691
 Taylor, G. & Shivalkar, P. R. (1971) *Clin. Allerg.* **1**, 189
 Walker, S. R., Richards, A. J. & Paterson, J. W. (1971) *Biochem. J.* **125**, 27P

Induction of Aryl Hydrocarbon Hydroxylase in Various Tissues of the Rat by Methylenedioxyphenyl Compounds

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Safrole (4-allyl-1,2-methylenedioxybenzene) has been shown to produce liver tumours in rats and mice (Homburger *et al.*, 1961; Long *et al.*, 1963; Epstein *et al.*, 1970). Further work has shown that methylenedioxyphenyl compounds produce both induction (Parke & Rahman, 1970; Wagstaff & Short, 1971) and inhibition (Anders, 1968; Freidman *et al.*, 1971) of hepatic drug-metabolizing enzymes. An important detoxification system of potential carcinogens present in many mammalian tissues is aryl hydrocarbon hydroxylase (Conney *et al.*, 1957; Wattenberg *et al.*, 1962). The effects of some methylenedioxyphenyl compounds, namely safrole, isosafrole and piperonyl butoxide (an insecticide synergist), on aryl hydrocarbon hydroxylase, with 3,4-benzopyrene as substrate, have been determined in various tissues.

Young male Wistar albino rats were fed on a diet containing 0.25% isosafrole (1,2-methylenedioxy-4-propenyl-benzene), and at various intervals after administration the activities of benzopyrene hydroxylase were determined in the liver, lung, kidney, gut and spleen. In all tissues except the spleen considerable induction (between 200 and 1200% of control values) of benzopyrene hydroxylase was observed. Maximum enzyme activities were reached within 14 days. In addition, there were large increases in hepatic biphenyl (a type I substrate) 2-hydroxylase and 4-hydroxylase activities, microsomal protein, liver weight, cytochrome P-450 content and apparent cytochrome b_5 content. In contrast, hepatic aniline (a type II substrate) 4-hydroxylase activity was inhibited. The redox difference-spectral absorption peak at 455nm observed by Parke & Rahman (1971) with liver microsomal preparations after pretreatment with safrole or isosafrole was also observed in microsomal preparations from kidney of the rats treated with isosafrole. This could be due to the interaction of isosafrole or a metabolite with the kidney microsomal haemoprotein (Lake & Parke, 1972). Polycyclic hydrocarbons have been shown to give extrahepatic induction of aryl hydrocarbon hydroxylase activity, whereas phenobarbitone produces no increase in the activity of this enzyme (Wiebel *et al.*, 1971) or other extrahepatic drug-metabolizing enzymes (Feuer *et al.*, 1971). The increase in extrahepatic aryl hydrocarbon hydroxylase activity after treatment with safrole or isosafrole correlates well with the reported carcinogenicity of these compounds.

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- Anders, M. W. (1968) *Biochem. Pharmacol.* **17**, 2367
 Conney, A. H., Miller, E. C. & Miller, J. A. (1957) *J. Biol. Chem.* **228**, 753
 Epstein, S. S., Fujii, K., Andrea, J. & Mantel, N. (1970) *Toxicol. Appl. Pharmacol.* **16**, 321
 Feuer, G., Sosa-Lucero, J. C., Lumb, G. & Modell, G. (1971) *Toxicol. Appl. Pharmacol.* **19**, 579
 Freidman, M. A., Arnold, E., Bishop, Y. & Epstein, S. S. (1971) *Experientia* **27**, 1052
 Homburger, F., Kelley, T., Jr., Freidler, G. & Russfield, A. B. (1961) *Med. Exp.* **4**, 1
 Lake, B. G. & Parke, D. V. (1972) *Biochem. J.* **119**, 53P
 Long, E. L., Nelson, A. A., Fitzhugh, O. G. & Hansen, W. H. (1963) *Arch. Pathol.* **75**, 595
 Parke, D. V. & Rahman, H. (1970) *Biochem. J.* **127**, 23P
 Parke, D. V. & Rahman, H. (1971) *Biochem. J.* **123**, 9P
 Wagstaff, D. J. & Short, C. R. (1971) *Toxicol. Appl. Pharmacol.* **19**, 54
 Wattenberg, L. W., Leong, J. L. & Strand, P. J. (1962) *Cancer Res.* **229**, 1120
 Wiebel, F. J., Leutz, J. C., Diamond, L. & Gelboin, H. V. (1971) *Arch. Biochem. Biophys.* **144**, 78