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.