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The absorption of aliphatic carbamates from the rat colon

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The colonic absorption of a series of aliphatic carbamates has been studied using an *in situ* preparation in the rat. The absorption process appeared to be passive and no metabolism by the colon membrane was detected. A linear correlation between the logarithms of the absorption rate constants and apparent partition coefficients was found. The absorption rates were not affected by concurrent water flux.

A study of the absorption of ionizable foreign compounds from the rat colon (Schanker, 1959) has revealed an absorption pattern similar to that of the small intestine. In agreement with these findings Michels, Bittighofer & Kurz (1974) reported a correlation between colonic absorption of certain drugs and their partition coefficients, although details of this correlation were not included.

Previous studies on aliphatic carbamates (Houston, Upshall & Bridges, 1974) have shown differences in absorption profiles between the stomach and the small intestine. However, nothing is known concerning the absorption of such compounds from the colon.

MATERIALS AND METHODS

Carbamates of general structure R–O–CO–NH_2

Methyl (Koch-Light Labs. Ltd., Bucks), ethyl (BDH Ltd., Poole, Dorset), n-propyl and n-butyl carbamate (Kodak Ltd., Liverpool) were obtained commercially and were reagent grade. t-Butyl (m.p. 104–106°), t-pentyl (2,2-dimethylpropyl; m.p. 80°), n-pentyl (m.p. 53–54°), t-hexyl (3,3-dimethylbutyl; m.p. 55–56°), n-hexyl (m.p. 59°), n-heptyl (m.p. 62°) and n-octyl (m.p. 67°) carbamate were synthesized by the Chemistry Division of the Chemical Defence Establishment. The purity of these chemicals was at least 99% as determined by g.l.c. The apparent partition coefficients of these carbamates were reported by Houston & others (1974).

Radiolabelled compounds

[^{14}C-carbonyl]Ethyl carbamate (specific activity 31·4 mCi mmol^{-1}), was supplied by Fluorochem Ltd., Glossop, Derby. [^{14}C-carbonyl]n-Butyl, n-hexyl and n-octyl carbamates (specific activity 1·5 mCi mmol^{-1}) were synthesized by the Chemistry Division, Chemical Defence Establishment. The radiochemical purity of each carbamate was greater than 99% as shown by t.l.c. and liquid scintillation counting of areas of the plate. [U-^{14}C]Erythritol (specific activity 2·3mCi mmol^{-1}) and [^{14}C]polyethyleneglycol 4000 (specific activity 40 mCi mmol^{-1}) were purchased from the Radiochemical Centre, Amersham.

General chemicals and solvents. Unless otherwise stated all chemicals and solvents were of analytical grade and were obtained from BDH Ltd., Poole, Dorset.

G.l.c. assay of carbamates

All aliphatic carbamates were assayed using a Pye 104 gas chromatograph fitted with a flame ionization detector. Column: glass 18″ × ¼″ i.d. packed with Porapak type P (Phase Separations Ltd., Flintshire) mesh size 100–120; gas flowrates: nitrogen 20, hydrogen 35, air 500 ml min^{-1}. Samples (1 μl) of the aqueous gut lumen contents were injected directly on to the column. No clean up of solutions was necessary. Carbamate concentration was determined by peak height measurement and by reference to a standard curve which was checked daily. The limit of detection was approximately 1 μg carbamate ml^{-1}. All carbamates gave characteristic peaks.

Procedure adopted for in situ *absorption studies*

Male adult rats of Porton strain, 200–250 g, were starved for 20 h before use but were allowed free access to water. Rats were anaesthetized with pentobarbitone (50 mg kg^{-1}; i.p.). The colon was exposed, washed out with isotonic saline and cannulated by the method of Doluisio, Billups & others (1969). Carbamate solution (5 ml; 0·5–50 mM) was intro-

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duced into the colon lumen in a weakly buffered (pH 7·2) perfusion solution containing (mmol litre^{-1}); NaCl 145; KCl 4·56; $CaCl_2$ 1·25; Na_2HPO_4 1·33 and NaH_2PO_4 0·33 (Schanker, 1959). Also included in the perfusion solution was 0·5μCi[^{14}C]polyethyleneglycol 4000 as a non-absorbable monitor for lumen water loss. The colon lumen was emptied and refilled every 3 min for sample removal. The carbamate was assayed by g.l.c. and the polyethyleneglycol 4000 determined by liquid scintillation counting. Where the binding of radiolabelled carbamate was studied, [^{14}C]polyethyleneglycol 4000 was not included in the perfusion solution and 0·5μCi of ^{14}C-labelled carbamate was added to give the same final carbamate concentration as was used in the absorption experiments. To determine the amount of [^{14}C]carbamate bound at the completion of an experiment, the whole colon was excised, washed with saline, weighed, scissor minced, 0·5 g amounts solubilized in NCS solubilizer (Amersham/Searle, High Wycombe) and the radioactivity present determined in a Packard Tricarb 3375 liquid scintillation counter (sampling efficiency 70–85% determined by external standard). A dioxan-based scintillation cocktail (10 ml per sample) was used comprising of naphthalene (BDH Ltd, Scintillation grade) 216 g, 2,5-diphenyloxazole, PPO 7·2 g, 1,4-2-(5-phenyloxazolyl) benzene, POPOP, 0·2 g ethyleneglycol monethyl ether 300 ml, 1,4-dioxan (Koch-Light Labs. Ltd.) 1500 ml.

RESULTS

The disappearance rate of each of the 11 carbamates from the colon lumen *in situ* was monoexponential. Absorption rate constants (ka) (see Table 1) were calculated from the half-lives of disappearance ($t_{\frac{1}{2}}$) using the equation (Doluisio & others, 1969):

$$ka = \frac{\ln 2}{t_{\frac{1}{2}}}$$

The absorption rate constants increased with increase in the apparent parition coefficient (from methyl to n-octyl carbamate) (Fig. 1). The best line of fit was calculated by the method of least squares and yielded the following equation,

Log ka = 0·076 log P − 1·297; n = 11, r = 0·956.

Where P is the apparent octanol/buffer partition coefficient. No hydrolysis products or other metabolites were detected by g.l.c. To investigate the possibility that the absorption rate may be dependent on concentration, studies using n-propyl carbamate were made over a concentration range 0·5 to 50 mM. The results shown in Table 1 indicate that no significant alteration in rate constant was found over this range.

Table 1. *Rate constants for colonic absorption of carbamates.*

Carbamate (concn mM)	Absorption rate constant (min^{-1}) (with s.d.)*
Methyl (10)	0·0442 (0·0020)
Ethyl (10)	0·0528 (0·0028)
n-Propyl (5)	0·0508 (0·0024)
n-Butyl (5)	0·0574 (0·0028)
n-Pentyl (5)	0·0624 (0·0052)
n-Hexyl (2)	0·0707 (0·0019)
n-Heptyl (1)	0·0774 (0·0043)
n-Octyl (0·2)	0·0841 (0·0032)
t-Butyl (5)	0·0559 (0·0016)
t-Pentyl (5)	0·0614 (0·0018)
t-Hexyl (5)	0·0629 (0·0030)
n-Propyl (50)	0·0479 (0·0019)†
n-Propyl (10)	0·0504 (0·0022)†
n-Propyl (1)	0·0491 (0·0011)†
n-Propyl (0·5)	0·0517 (0·0031)†

* Means of 4 animals with s.d.
† Results are means of 2 animals wih range.

The possibility that carbamate disappearance from the colon lumen was not true absorption but rather a reflection of binding to the colon wall or to mucus was investigated by following the distribution within the colon wall for four ^{14}C-radiolabelled carbamates. After 20 min, only small quantities of carbamate were bound (Table 2).

Absorption from solutions producing differing rates of water flux was examined using isotonic mannitol or hypotonic buffer. Since sodium ion

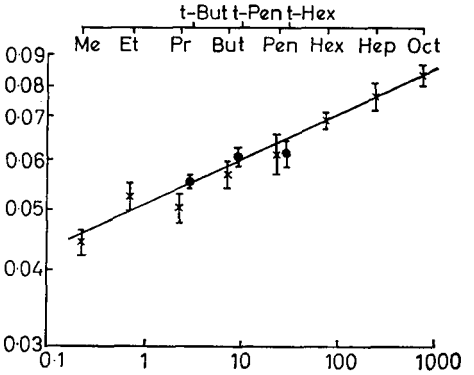

FIG. 1. Relation between *in situ* colonic absorption rate constants and apparent partition coefficient. Each point is the mean of four animals with s.d. Concentration of carbamate as shown in Table 1 (pH 7·2). Straight chain carbamates are shown by × where Me = methyl, Et = ethyl, Pr = propyl, But = n-butyl, Pen = n-pentyl, Hex = n-hexyl, Hep = n-heptyl and Oct = n-octyl. Branched chain carbamates are shown by ● where t-But = t-butyl, t-Pen = t-pentyl and t-Hex = t-hexyl. Ordinate: Absorption rate constant (min^{-1}). Abscissa: Apparent partition coefficient (octanol/buffer).

Table 2. *Distribution of ^{14}C-labelled carbamate in the colon after 20 min perfusion.*

Carbamate	% of total found in Washing	Mucus	Membrane
Ethyl	2·45 (0·25)	<0·1	0·65 (0·13)
n-Butyl	2·82 (0·53)	<0·1	0·89 (0·03)
n-Hexyl	1·71 (0·59)	<0·1	0·77 (0·08)
n-Octyl	1·99 (0·16)	<0·1	2·10 (0·22)

Results are means of 2 animals (with range).

absorption is a prerequisite for water absorption, water flux was either reduced using an isotonic mannitol solution or increased using hypotonic buffer. The results obtained (Table 3) show that the percentage of water absorbed from the perfusate after 20 min can be varied from −0·5 to +30·75% without any apparent effect upon the absorption of methyl, ethyl or n-octyl carbamate. Similar results were obtained with [^{14}C]erythritol (Table 3) which has similar molecular dimensions to the lower carbamate homologues (Fordtran & Ingelfinger, 1968).

DISCUSSION

The results suggest that the aliphatic carbamates are rapidly absorbed from the colon. With n-propyl carbamate no significant alteration in rate constant was exhibited over a 100-fold concentration range. This indicates that the absorptive process is a passive phenomenon dependent upon the concentration gradient between the colon lumen and the blood stream and that no saturable process is involved. Only small amounts of carbamate were bound to the colon wall at the end of an experiment thus confirming that the measurement of rate of disappearance from the lumen reflects true absorption through the membrane and that the rate constants obtained are true absorption rate constants.

The water flux rates reported are in good agreement with those of Kitazawa, Ito & Sezaki (1974). These authors stated however, that the small intestinal absorption rate of a number of drugs including sulphanilamide and metoclopramide, was enhanced by increasing concurrent water absorption and decreased by influx of water into the gut lumen. Their results obtained using the *in situ* rat small intestine, would seem explicable in terms of solvent drag through membrane pores. In contrast, the carbamate absorption rate from the colon proceeded independently of even rapid rates of water flux. This indicates a major difference between the rat small intestine and colon membranes presumably relating to the size and distribution of pores.

Evidence that a gradient of pore radius may exist along the intestinal tract has been furnished by Fordtran & Ingelfinger (1968), who reported the effective pore radius of the jejunum to be twice that of the ileum as measured by erythritol absorption. This conclusion is confirmed by Hollander & Truscott (1974a, b) who found a rapid transport of

Table 3. *Effect of change in rate of water absorption on carbamate and erythritol absorption rate constant.*

Carbamate concn (mM)	Isotonic buffer* $ka(min^{-1})$	Isotonic mannitol $ka(min^{-1})$	Isotonic buffer: isotonic mannitol 1:1 (v/v) $ka(min^{-1})$	Isotonic buffer: distilled water 1:1 (v/v) $ka(min^{-1})$
Methyl (10)	0·0442 (0·0020)	0·0453 (0·0009)	0·0439 (0·0011)	0·0437 (0·0019)
Ethyl (10)	0·0528 (0·0028)	0·0525 (0·0015)	0·0508 (0·0013)	0·0559 (0·0018)
n-Octyl (0·1)	0·0852 (0·0029)	0·0850 (0·021)	0·0866 (0·022)	0·0841 (0·039)
Erythritol* (10)	0·0025 (0·0002)	0·0026 (0·0002)	0·0024 (0·0003)	0·0026 (0·0002)
% Water* absorption after 20 min	6·25% (1·99)	−0·50%** (1·29)	4·50% (1·00)	30·75% (7·13)

Mean with range for 2 animals.
* Mean with s.d. for 4 animals.
** Negative value indicates net water influx into the colon lumen. Study with n-octyl carbamate carried out using ^{14}C-labelled material.

erythritol from a rat *in vitro* small intestine preparation but could not detect absorption from an *in vitro* rat colon preparation. The results from our study show that not only was erythritol absorption negligible but also that increased rates of water absorption produced no change in the absorption for erythritol or for aliphatic carbamates.

In comparing the colonic carbamate absorption data with those reported for the same series of carbamates from the rat stomach and small intestine (Houston & others, 1974) several differences were apparent. The parabolic relationship between log ka and log P found to be operative in the rat small intestine and confirmed in a subsequent study, is not found in the rat colon. Rather a linear relation between log ka, and log P is seen for the colon which is similar to that found in the stomach. The branched-chain carbamate homologues fit the linear regression line well with the colon, as was also reported for the stomach, again a different finding to that in the small intestine where the branched-chain homologues were absorbed at a much slower rate than would be predicted from their partitioning properties. These findings indicate that the structure and composition of the colon and stomach membranes may be similar, with respect to the absorption of simple unionized molecules, due to the absence of significant pores.

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