Intestinal Sorption of Anisotropine Methylbromide in the Rat

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Abstract □ The uptake into the circulation and the binding of anisotropine methylbromide to the intestine and the residuum in the intestinal lumen were studied in rats using the acute *in vivo* intestinal loop technique. The presence of food in the intestine decreases the uptake rate but has no effect on the total uptake. The sum of drug bound to intestinal tissue and that taken into the circulation, over the concentration range studied, is a constant 48% of the dose administered. Tissue binding is describable by a Langmuir adsorption isotherm. *In vitro* everted intestinal sac experiments confirm the extent of this binding. The material bound to the intestinal wall is released into the lumen of the gut and is unavailable for circulatory uptake. There are gradients for both intestinal binding and circulatory uptake, both generally being greater in the anterior portion of the small intestine and decreasing in the posterior portion. Neither binding nor uptake is correlated with the quantity of protein in the intestinal tissue. Bile does not affect circulatory uptake but decreases binding. There is no evidence for the existence of a saturable transport process effecting the sorption of this drug.

Keyphrases □ Anisotropine methylbromide—intestinal sorption, rats □ Intestinal sorption—anisotropine methylbromide, rats □ Sorption, intestinal—anisotropine methylbromide, rats

The urinary excretion of the quaternary ammonium compound anisotropine methylbromide[1] (8-methyltropinium bromide 2-propylvalerate) by humans after oral and intravenous administration was reported previously (1, 2). The present experiments, carried out in rats, concern several aspects of the intestinal sorption and distribution of this compound.

"Drug absorbed" is not just the amount that is removed from the intestinal lumen and tissue into the circulation (3–5) but is, rather, all the drug that passes onto, into, or through the lumenal membrane. Reference to "drug bound" to intestinal tissue includes drug adsorbed onto or absorbed into intestinal tissue without prejudice as to the site of retention. Circulatory uptake of anisotropine methylbromide refers to material lost from both the lumen and intestinal tissues, whether taken into blood or lymphatic fluid. The material lost from the lumen, whether bound to or in the intestinal wall or taken into the circulation, is referred to as the "drug sorbed."

EXPERIMENTAL

Reagents—Anisotropine methylbromide originated at Endo Laboratories. Chloroform was of spectrophotometric reagent grade[2], and crystalline bovine albumin was obtained from a commercial source[3]. All other chemicals were commercial products of reagent grade.

Animals—The studies were performed on 270–330-g. male CFN rats[4].

Analytical Methods—Protein was determined by the Henry *et al.* (6) modification of the biuret method, using crystalline bovine albumin as the protein standard. Anisotropine methylbromide was determined by the previously reported picric acid assay method (1), in which a water-insoluble dye salt formed between picric acid and the quaternary ammonium compound in aqueous solution is extracted into chloroform and determined spectrophotometrically.

Drug Administration—*In vivo* studies were performed using the acute intestinal loop method of Levine *et al.* (3). A midline incision was made in the abdomen of a rat under light ether anesthesia, and a section of the small intestine was exposed. A ligature was secured at the posterior end of the chosen section of the gut; a 0.63-cm. (0.25-in.) 24-gauge hypodermic needle, mounted on a 1-ml. tuberculin syringe, was introduced into the anterior end of the section. A ligature was tied at this point around the small intestine and the needle. The solution in the hypodermic syringe was injected; and as the needle was withdrawn, the ligature was tightened to maintain the seal on the intestinal loop. To ensure reproducibility of dose, the syringe was fitted with an automatic stop. The intestinal section was returned to the abdominal cavity, and the incision was sutured closed. After the rat regained consciousness, it was not allowed access to food or water. At the end of the relevant time interval, the animal was reanesthetized, the incision was reopened, and the loop was stripped of mesentery, severed on the distal side of each ligature, and removed.

The loops always were made long enough to include four mesenteric blood vessels between the ligatures. The anterior ligature was located approximately 8 cm. distal to the pylorus. This particular loop location was used throughout the studies of the effects of contact time, feeding, fasting, drug concentration, *etc.*, on sorption of anisotropine methylbromide and is referred to as the standard loop location. In studies of the anisotropine methylbromide sorption and binding gradients in the small intestine, successive loops, each including four mesenteric blood vessels, were isolated along the length of the small intestine from the pylorus to the ileocecal junction. Sorption in the loop immediately posterior to the pylorus was determined with the bile duct both ligated and nonligated. The rats were fasted for a period of time sufficient to clear solid matter from the desired section of the gut (16–48 hr.). All experimental groups contained five animals. All fiducial limits presented on experimental data are standard errors of the means (*SEM*).

In vitro studies of sorption, to determine whether active transport was occurring, were carried out with five everted intestinal sacs prepared from 3-cm. sections of rat duodenum, according to the method of Wilson and Wiseman (7). There were 2 ml. of serosal solution (inside the everted sac) and 25 ml. of mucosal solution (outside the everted sac). Both the mucosal and serosal solutions were Krebs improved mammalian Ringer III solutions (8), containing 20 mcg. anisotropine methylbromide/ml. The mucosal solution was oxygenated and the system was incubated, with shaking, in a Dubnoff metabolic incubator for 1 or 2 hr. at 37° in a 50-ml. conical flask under a compressed air flow of 10 ft.^{3}/hr. The serosal and mucosal solutions and the intestinal tissues were separately analyzed for methylanisotropinium cation as indicated later.

Sample Preparation—To determine circulatory uptake by difference, an entire loop, both tissue and lumenal contents, was analyzed as a whole and the value was subtracted from the amount originally injected into the loop. To determine the residual drug in the lumen of the loop and the bound drug in the intestinal wall, an excised loop was cut open and the lumen contents were carefully drained, collected, and measured. The mucosal surface was washed with 20 ml. of isotonic saline solution, and the washings were combined with the drained lumen contents. Examination of the mucosal surface at 60× magnification revealed no surface damage.

The intact loop or washed intestinal wall was then homogenized in 25 ml. of distilled water using a power-driven Teflon pestle tissue grinder. The homogenates were deproteinized by adding, sequentially, 5.0 ml. each of 7.2% $ZnSO_4 \cdot 7H_2O$ and 0.5 *N* NaOH. The volume was adjusted to 50 ml. and the samples were filtered and analyzed by the method already indicated. The lumen contents were

[1] Valpin, Endo Laboratories, Inc.
[2] J. T. Baker Chemical Co.
[3] Nutritional Biochemicals Corp.
[4] Carworth Farms.

Table I—Effect of Feeding and Fasting on Circulatory Uptake of Anisotropine Methylbromide by the Rat[a]

Intestinal Contact Time, hr.	—Circulatory Uptake, % of Dose ± *SEM*— Fed Rats	Fasted Rats
0.25	—	4.0 ± 0.6
1	0	14.2 ± 3.3
2	2.8 ± 2.2	21.3 ± 5.1
3	8.8 ± 0.7	17.4 ± 1.3
4	15.8 ± 1.9	—
5	16.0 ± 1.3	—
6	17.6 ± 1.0	—

[a] Anisotropine methylbromide dose: 0.50 mg./loop in 0.50 ml. of solution; five rats per experimental group; standard loop location.

treated with 1.5 ml. each of 7.2% $ZnSO_4 \cdot 7H_2O$ and 0.5 *N* NaOH and brought to a final volume of 15 ml. before analysis.

The *in vitro* everted sac experiment was analyzed similarly. The sac was removed from the mucosal solution, and the surface was washed with isotonic saline solution which was pooled with the mucosal solution. The serosal solution was withdrawn by hypodermic syringe and the sac was opened. Washings of the serosal surface of the sac were pooled with the serosal solution. The intestinal tissue was prepared for analysis by the method outlined for determination of drug bound in the intestinal wall. The entire serosal solution and 5 ml. of the mucosal solution were analyzed by the same method used for the lumen contents.

Anisotropine methylbromide tissue recovery standards were run through all these procedures. The average recovery was 99.8 ± 0.5%. Tissue extraction blanks were negligible.

RESULTS

Because circulatory uptake is determined by difference, *i.e.*, by disappearance from the loop, it had to be ascertained that there was no metabolic destruction of anisotropine methylbromide in the loop during the time period of the experiments. Any such metabolism would result in a decrease of drug levels in the loop, which could mistakenly be attributed to circulatory uptake.

Mesenteric blood vessels were stripped away from sections of small intestine of fasted rats at the standard loop location and acute intestinal loops were established, containing 0, 0.125, 0.50, and 1.00 mg. of anisotropine methylbromide, each in quintuplicate. The loops were excised and incubated for 1 hr. in 15 ml. of Krebs improved mammalian Ringers III solution in 50-ml. conical flasks in a Dubnoff metabolic incubator at 37° under compressed air at a flow rate of 10 ft.^{3}/hr. The intact loops were then homogenized in the incubation solutions, and the homogenates were processed and analyzed using the technique indicated in the *Experimental* section for a tissue-containing sample. The recovery of anisotropine methylbromide was

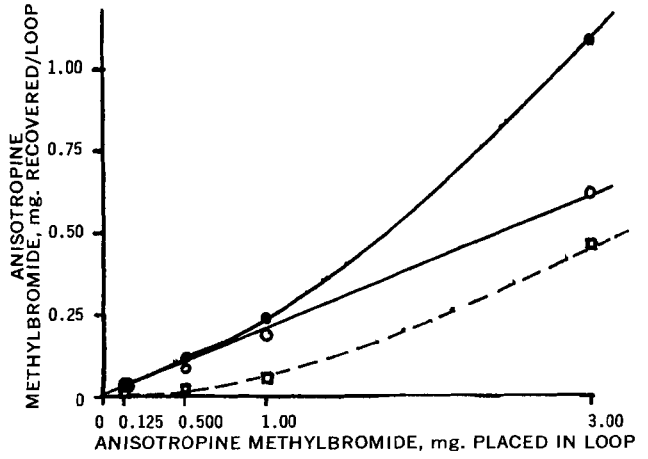

Figure 1—*Sorption of anisotropine methylbromide from acute intestinal loops after 15 min. Key:* —●—, *total sorption;* —○—, *tissue binding; and* --□--, *circulatory uptake.*

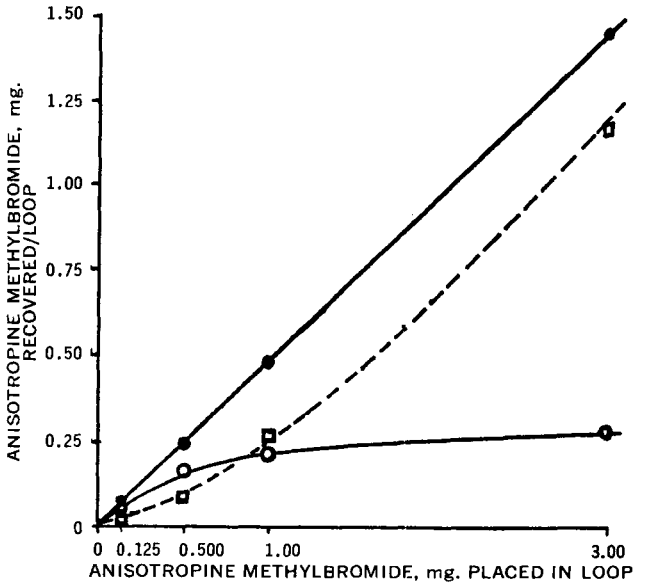

Figure 2—*Sorption of anisotropine methylbromide from acute intestinal loops after 2 hr. Key:* —●—, *total sorption;* —○—, *tissue binding; and* --□--, *circulatory uptake.*

100 ± 0.3%. Thus, there does not appear to be any drug loss due to metabolism or destruction during analysis.

The effect of feeding on circulatory uptake was studied with rats fasted for 18 hr. prior to experimentation and with rats fed normally until experimentation. Standard acute loops containing 0.5 mg. anisotropine methylbromide each were analyzed, in the fasted rats, at 15 min. and 1, 2, and 3 hr. and, in the fed rats, at 1, 2, 3, 4, 5, and 6 hr. (Table I). The total circulatory uptake was the same in fed and fasted animals, but the uptake was complete at 2 hr. in fasted animals in contrast to the 4 hr. required in fed animals.

A study was performed to determine the effect of intestinal preincubation on absorption. Loops that incorporated eight mesenteric blood vessels (*i.e.*, double length) were established at the standard loop location in 12 fasted rats. Six milligrams of anisotropine methylbromide in 1 ml. of distilled water was introduced into each loop. After 2 hr. of incubation *in vivo*, the fluid in the lumen was withdrawn and a portion of the pooled lumenal fluid was analyzed. The anisotropine methylbromide concentration in this solution was 2.31 mg./ml. Fifty percent of the original content remained, but osmotic pressure had caused water to enter the lumen and increased the volume of liquid 1.3-fold. Ten 0.5-ml. portions of the pooled, loop-incubated solution were reintroduced into 10 fresh single-length standard acute loops. Circulatory uptake of the drug from these loops was compared to circulatory uptake from 10 loops containing 0.5 ml. each of freshly prepared anisotropine methylbromide solu-

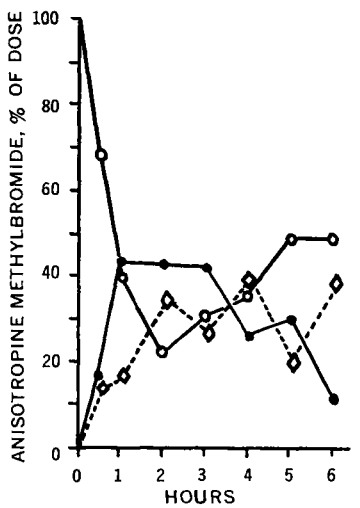

Figure 3—*Time course of anisotropine methylbromide distribution in an acute intestinal loop. Key:* —○—, *residue in intestinal lumen;* —●—, *intestinal tissue binding; and* --◇--, *circulatory uptake.*

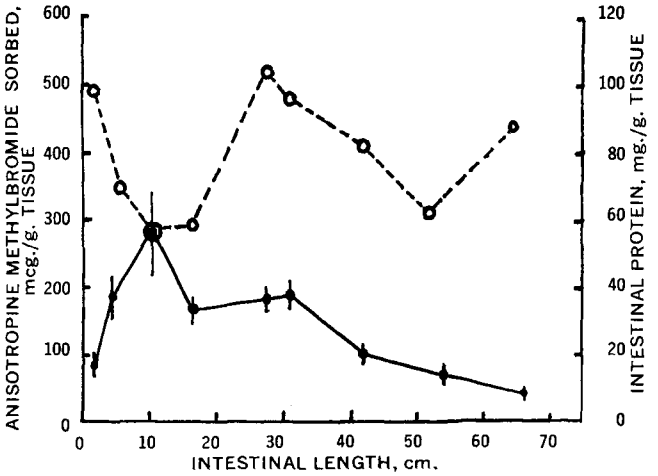

Figure 4—*Gradient of binding of anisotropine methylbromide to rat intestinal tissue. Key: ——, tissue sorption (values on left axis); and ---, tissue protein content (values on right axis); vertical bars represent standard errors of the means. The pylorus is at 0 cm.; the ileocecal junction is at 70 cm.*

tion, 2.31 mg./ml. The mean uptake per loop from the loops containing fresh drug solution was 367 ± 25 mcg., and from the loops containing loop-incubated drug solutions, it was 91 ± 21 mcg. Thus, approximately 75% of the residual loop-incubated anisotropine methylbromide was unavailable for uptake. This could be the same type of quaternary ammonium compound unavailability noted by Levine *et al.* (3) and attributed to binding to intestinal mucin.

The distribution of anisotropine methylbromide between gut tissue and the circulation was examined in loops at the standard location while the uptake was proceeding (15 min.) and at the cessation of uptake (2 hr.). The anisotropine methylbromide concentrations were 0.125, 0.50, 1.00, and 3.00 mg./loop at both time periods. Tissue binding was determined in one set of experiments and circulatory uptake in a repeat set (Figs. 1 and 2).

At 15 min. (Fig. 1), the amount of anisotropine methylbromide bound to intestinal tissue followed no isotherm, was consistently greater than the amount taken into the circulation, and represented a constant 20.1 ± 1.0% of the total dosage per loop. At dosages above 1.1 mg./loop, the amount in the tissue was greater at 15 min. than at 2 hr. These findings probably represent drug in transit through the intestinal tissue.

The total sorption at 2 hr. (Fig. 2) remained constant at 48.0 ± 0.5% of the dose per loop over the entire dosage range studied. The intestinal tissue binding curve in Fig. 2 conforms to the Langmuir adsorption isotherm (10). The calculated maximum retention capacity is 1.3×10^2 μmoles of methylanisotropinium cation/g.

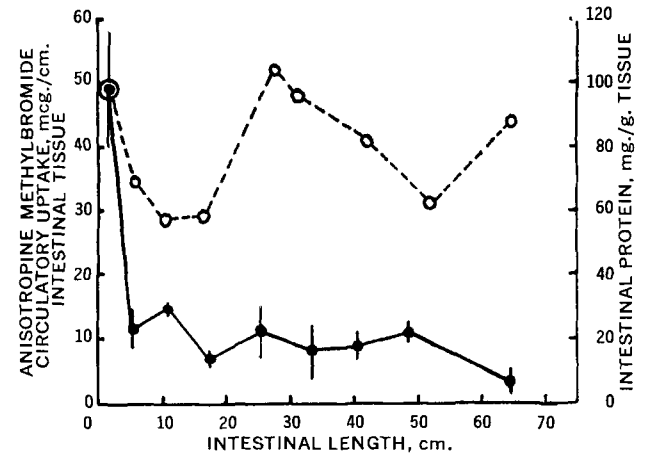

Figure 5—*Gradient of circulatory uptake of anisotropine methylbromide from the rat small intestine. Key: ——, circulatory uptake (values on left axis); all other symbols are as indicated for Fig. 4.*

Table II—Effect of Ligation of the Bile Duct on Tissue Binding and Circulatory Uptake of Anisotropine Methylbromide in the Immediately Postpyloric Acute Loop[a]

Bile Duct Treatment	Circulatory Uptake, mcg./cm. ± *SEM*	Tissue Binding, mcg./g. ± *SEM*	Lumen Residue, mcg./cm. ± *SEM*
Ligated	48.5 ± 7.7	83.1 ± 17.0	88.8 ± 1.3
Nonligated	49.0 ± 9.4	<0.28	98.8 ± 0.4

[a] Anisotropine methylbromide dose: 0.50 mg./loop in 0.50 ml. of solution; five rats per experimental group; time: 2 hr.

tissue, wet weight (2.4 μmoles/g. protein). Paton and Rang (11) reported the existence of three species of binding sites for methyltropinium cation in guinea pig intestinal muscle which have maximum retention capacities of 0.93×10^{-4}, 2.4×10^{-4}, and 1.1×10^{-1} μmole/g. protein. Therefore, most of the methylanisotropinium cation that was found bound to rat gut was almost certainly not bound to muscle. The compound may be adsorbed to material exterior to, but intimately associated with, the intestinal tissue as well as to intestinal tissue *per se*.

The results of the everted sac experiment confirmed the degree of binding to intestinal tissue. The distribution had reached equilibrium by 1 hr. and was essentially identical to the 2-hr. results. The final concentrations in mucosal and serosal solutions were 17.1 ± 0.8 and 13.2 ± 0.4 mcg./ml., respectively, both lower than the initial 20 mcg./ml. in both the mucosal and serosal solutions. If there were a transport process, one might expect the serosal concentration to be greater than the mucosal concentration once the tissue was saturated, but the reverse was found. The binding was 30.4 ± 1.7 mcg./g. tissue (wet weight), which explains the lowered concentrations in the final solutions compared to the initial values.

The relatively large amount sequestered in the intestinal tissue raised the question of the fate of this material. A longer experiment was carried out to follow the time course of distribution of 0.50 mg. of anisotropine methylbromide in standard loops in fasted rats for 6 hr. Circulatory uptake was determined in one set of experiments, and tissue binding and residual lumenal anisotropine methylbromide were determined in another identical set of experiments. The results are plotted, as percent of total dose *versus* time, in Fig. 3. The results of the shorter term circulatory uptake experiment were confirmed; *i.e.*, the uptake continued until 2 hr. and thereafter ceased. Binding achieved its maximum level at 1 hr., remained constant until 3 hr., and then fell. It was still falling at 6 hr. The lumenal content fell for the first 2 hr. and rose concomitantly with the decrease in bound material. It can be concluded that the anisotropine methylbromide which remained bound was unavailable for circulatory uptake and could only be released back into the gut lumen. The decrease in lumenal content of anisotropine methylbromide proceeded exponentially, with a 54-min. half-life, during the first 2 hr. of contact, indicating that total sorption (binding plus circulatory uptake) proceeds according to first-order kinetics. The decrease in the drug content of the entire acute loop (lumen plus tissue) was tested for conformity to a kinetic description but was not found to fit any up to a 12th-order reaction.

Figures 4 and 5 present the respective gradients for tissue binding and circulatory uptake throughout the rat small intestine. The points on the abscissa represent the midpoints of the successive loops. Since the segments were chosen to include four mesenteric blood vessels, they were necessarily of unequal lengths. The data in Fig. 5 were normalized by reporting them in terms of micrograms per centimeter of segment length. The circulatory uptake (Fig. 5) was quite high in the first 3–4 cm. of the small intestine and then fell to a lesser, relatively constant level. The values given in both figures for the first segment represent the situation when the bile duct was ligated. The protein content of the intestinal segments is included in both figures. No correlation is apparent between binding to intestinal tissue or circulatory uptake of anisotropine methylbromide and the protein content of the segments.

The tissue binding and circulatory uptake in the loop immediately posterior to the pylorus, at 2 hr., were compared with the bile duct ligated and nonligated (Table II). Circulatory uptake was the same whether or not the bile duct was ligated, but tissue binding dropped below the level of detectability when the bile duct was nonligated.

Table III—Effect of Ox Bile on Tissue Binding and Circulatory Uptake of Anisotropine Methylbromide from an Acute Loop[a]

Exogenous Bile[b] Present	Circulatory Uptake, mcg./cm. ± *SEM*	Tissue Binding, mcg./g. ± *SEM*	Lumen Residue, mcg./cm. ± *SEM*
No	16.0 ± 1.0	282 ± 60	46.2 ± 2.9
Yes	14.4 ± 3.3	151 ± 26	59.5 ± 7.2
t	0.46	3.76	—
p	>0.5	<0.01	—

[a] Anisotropine methylbromide dose: 0.50 mg./loop in 0.50 ml. of solution; five rats per experimental group; standard loop location; time: 2 hr. [b] "Bile for bacteriological use" (Nutritional Biochemicals Co.) reconstituted as a 10% (w/v) aqueous solution. Each 0.5 ml. of drug solution in a loop contained 0.05 ml. of this bile solution.

When ox bile was added to an intestinal loop in the standard location for 2 hr. (Table III), there was no significant difference in circulatory uptake in the presence and absence of bile but tissue binding was again significantly greater ($p < 1\%$) in the absence of bile.

The addition of an exogenous phosphatidopeptide fraction to acute intestinal loops in rats was reported (12) to increase the circulatory uptake of the quaternary ammonium compound, and it has been suggested that endogenous phosphatidopeptide fraction may normally serve to enhance the transport of quaternary ammonium compounds. A quaternary ammonium compound carrier such as phosphatidopeptide fraction could only be present in finite amounts, and it is characteristic of such saturable systems that, whether or not active transport is occurring, their behavior is describable by Michaelis–Menten kinetics (14, 15). The data for circulatory uptake and total sorption at 15 min. are presented as a double reciprocal plot in Fig. 6. The plot for total sorption is linear, while the plot for circulatory uptake is hyperbolic. Neither indicates any limiting maximum level of anisotropine methylbromide uptake or sorption.

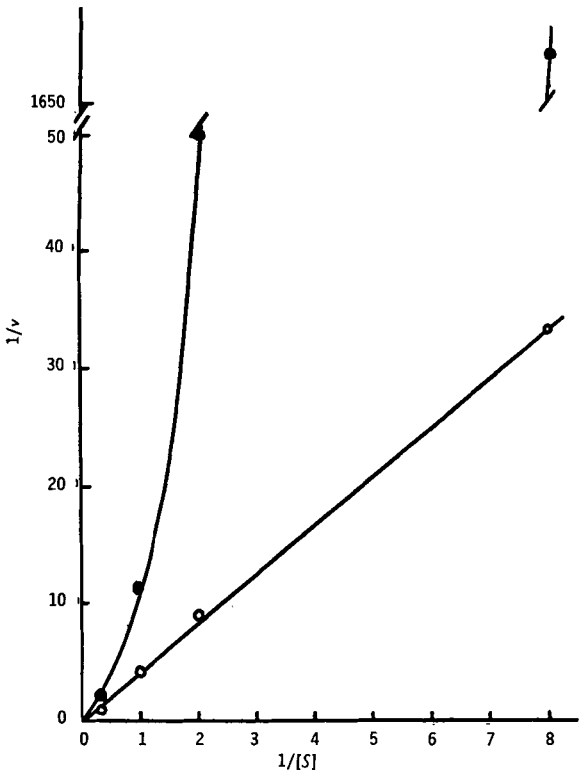

Figure 6—*Lineweaver–Burk (double reciprocal) plot of circulatory uptake and total sorption of anisotropine methylbromide from rat acute intestinal loops. Key:* S, *milligrams per loop;* v, *rate of uptake or sorption, milligrams per minute;* —●—, *circulatory uptake; and* —○—, *total sorption.*

DISCUSSION

Response to quaternary ammonium compounds in humans is less in fed than in fasted subjects (16, 17), which would imply either less absorption or a slower rate of absorption in the fed individuals. A slower rate of absorption in fed subjects would be consonant with the findings in the rat. A slower uptake rate in the fed human could cause a slower rise to effective levels at the ultimate active receptor site, or the prolongation of uptake time in fed subjects could be sufficiently long so that potentially absorbable drug passes away from the absorptive sites in the intestine.

The observation that the overall binding capacity of the rat intestinal tissues for methylanisotropinium cation is at least 1000-fold greater than the maximum binding capacity of guinea pig intestinal muscle for methyltropinium cation (11) is suggestive of a great deal of nonspecific binding. The most direct evidence bearing upon this is the effect of bile which, when incorporated into acute loops, has no effect on circulatory uptake but does significantly decrease the total intestinal sorption because of the decreased degree of binding. These results are in contradiction to those of Cavallito and O'Dell (18), according to whom coadministration of cholic acid (a bile acid) and bisquaternary hypotensives in dog intestine enhanced pharmacological response to the hypotensives, implying an increase in total circulatory uptake or in the rate of uptake. Our results conform more to the finding of Levine and Pelikan (19) that the absorption of benzomethamine into the circulation is not enhanced by rat bile. The likeliest explanation for the decrease in binding seen with bile is the solubilization of material normally adhering to the lumenal surface of the intestine which could bind the quaternary ammonium compound, probably mucus. It is also possible that anisotropinium cation could combine with anionic bile constituents with a greater affinity than it has for binding sites in or on the intestine. It appears likeliest that most of the anisotropine methylbromide is bound to material adherent to, but exterior to, the intestinal tissue. This would account for the release of bound material back into the intestinal lumen. Presumably, the presence of bile hastens this release.

The data on anisotropine methylbromide uptake into the circulation cannot be fitted to any extant model of absorption by facilitated or active transport but seem to conform to the hypothesis of Kakemi *et al.* (20–22) that binding to the intestinal mucosa is the determining step in the absorption of drugs from the small intestine; only bound material could be absorbed. The sorption would occur as a first-order, presumably passive, process. At 15 min., the amount of bound anisotropine methylbromide in a loop was a constant 20% of dose at all dose levels investigated. While some portion of the bound material would then be further taken up, again by a presumably passive process, to complete the absorption process, another portion would be bound to acceptor sites with relatively high affinity for quaternary ammonium compounds and remain at those sites. Eventually, as indicated by the data, most of this tightly bound material would be released back into the lumen in a nonbinding, nonabsorbable form. The circulatory uptake of anisotropine methylbromide could be described as the constant proportion of dose initially bound less the tightly bound amount, which can, in turn, be described by an adsorption isotherm. This can be seen in the fact that the tissue-bound anisotropine methylbromide at 15 min. was a constant 20% of administered dose, *i.e.*, it did not fit an adsorption isotherm. At 2 hr. the amount of tissue-bound material decreased and did fit an adsorption isotherm. Also, circulatory uptake ceased at 2 hr. Only material which remained tissue bound at 2 hr., at the cessation of circulatory uptake, was released back into the gut lumen. Therefore, a portion of the material tissue bound at 15 min. must be finding its way into the circulation.

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Antitumor Alkaloids from *Cephalotaxus harringtonia*: Structure and Activity

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Abstract □ Cephalotaxine and several of its esters were isolated from *Cephalotaxus harringtonia* K. Koch var. *harringtonia*. Although cephalotaxine is inactive, harringtonine, isoharringtonine, homoharringtonine, and deoxyharringtonine have shown significant activity against experimental P388 leukemia and against L-1210 leukemia in mice.

Keyphrases □ *Cephalotaxus harringtonia* alkaloids—structure, antitumor activity □ Harringtonine, isoharringtonine, homoharringtonine, deoxyharringtonine—antitumor activity □ Antitumor alkaloids from *Cephalotaxus harringtonia*—structure, activity □ NMR spectroscopy—identification, *Cephalotaxus* alkaloids

In a search for tumor inhibitors of plant origin, an alcoholic extract of the seed of *Cephalotaxus harringtonia* var. *drupacea* (Sieb. & Zucc.) Koidzumi[1] showed activity against lymphoid leukemia L-1210 and P388 leukemia in mice[2]. Subsequent fractionation of the seed extract and of an extract obtained from *C. harringtonia* (Forbes) K. Koch var. *harringtonia* cv. Fastigiata (entire plants) revealed four alkaloids with significant antitumor activity (1). The active *Cephalotaxus* alkaloids are esters of cephalotaxine (I); these include harringtonine (II), isoharringtonine (III), homoharringtonine (IV), and deoxyharringtonine (V).

DISCUSSION

Paudler *et al.* (2) first isolated cephalotaxine, and their work indicated that two partial structures were possible. Subsequent investigations by other workers, using a combination of NMR (3) and X-ray crystallographic (4) techniques, revealed that cephalotaxine has the structure indicated here (I). We have now characterized the active antitumor alkaloids II–IV and report test data for these and several related alkaloids[3].

The NMR spectra of alkaloids II–V yielded initial evidence that these compounds are esters of cephalotaxine. This conclusion was based primarily on a comparison of their NMR spectra with the NMR spectra of cephalotaxine and acetylcephalotaxine (VII, Table I). If one disregards signals attributed to the R group, the NMR spectra of the cephalotaxine esters are nearly identical. The number and nature of free hydroxyl groups in alkaloids I–IV were indicated by NMR spectra of dimethyl sulfoxide-d_6 solutions before and after deuterium oxide exchange (5). In the mass spectra of these alkaloids, the strongest ion (base peak) is at m/e 298 ($C_{18}H_{20}NO_3$). This ion corresponds to cephalotaxine minus the appropriate R group.

Transesterification of alkaloids II–IV (sodium methoxide–methanol or sodium ethoxide–ethanol) gives alkaloid I, along with the corresponding dimethyl or diethyl esters (VIII–X or XII–XIV). Structures of Compounds VIII–X were deduced from NMR and mass spectral data.

Significant features of the NMR spectra of dimethyl esters VIII and X (see *Experimental* section for chemical shift assignments) are

[1] *Cephalotaxus* plant materials were received from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture (USDA), Beltsville, Md., under a program developed with USDA by Drug Research and Development, National Cancer Institute (formerly the Cancer Chemotherapy National Service Center).

[2] Assays performed under Drug Research and Development auspices. Procedures are described in *Cancer Chemother. Rep.*, **25**, 1(1962).

[3] The previously used numbering for the cephalotaxine ring system was revised. The revised numbering corresponds closely to that commonly used for the erythrina series of alkaloids.