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ABSORPTION AND SECRETION OF MONOQUATERNARY AMMONIUM COMPOUNDS BY THE ISOLATED INTESTINAL MUCOSA*

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Abstract—The transepithelial permeation and the cellular uptake of *N*-methylscopolamine (NMScop), N^1-methylnicotinamide (NMN), and tetraethylammonium (TEA) were studied in the isolated jejunal mucosa of the guinea pig. (1). The permeation rate, corrected for the fraction passing through the mucosa via an extracellular route (corr. permeation), was significantly higher in the direction blood-to-lumen than in the direction lumen-to-blood with concentrations below 500 μM. The corr. permeations' ratio at concentrations of 0.1 and 1 μM NMScop was 12, the corresponding values for 1 μM NMN and 50 μM TEA were 2.5 and 2, respectively. The corr. permeation blood-to-lumen decreased with increasing concentrations administered, and with the highest concentrations (1000–2000 μM) the differences in permeation in the two directions were insignificant. During anaerobiosis the corr. permeation ratios approached 1 with all concentrations, and the corr. permeations were not dependent on the concentrations administered. (2). The kinetics of cellular uptake displayed similar characteristics: the uptake from the blood side was significantly larger than that from the lumen side of the mucosa. This difference decreased with increasing concentrations or anaerobiosis without being abolished completely. (3). The three monoquaternary ammonium compounds were transported uphill, creating a concentration ratio lumen-side/blood-side greater than 1, which was highest with NMScop (1.65 within 180 min). The establishment of this gradient was prevented by anaerobiosis. (4). Rising concentrations of NMScop progressively inhibited the corr. permeation from blood to lumen and the cellular uptake of NMN from the blood side of the mucosa. The results demonstrate the existence of an active secretory system for monoquaternary ammonium compounds in the jejunal epithelium. The experimental data are consistent with a cell model, which has two transport systems in series, one in the basal and one in the luminal cell membrane and both directed towards the intestinal lumen, plus a non-saturable pathway in each of the two cell membranes.

The intestine is usually considered to be an absorptive organ with numerous transport systems for the transfer of nutrients against a concentration gradient from the lumen into the circulation. In contrast, during the last few years it has been demonstrated that the mucosal epithelium is capable of transporting cardiac glycosides in the reverse direction and concentrating them in the intestinal lumen [2–5], thus revealing an excretory function of the intestine. Furthermore an intestinal transport system for cardiac glycosides constitutes an exception to the theory proposed by Brodie, Hogben, Schanker and Shore that drugs and other foreign compounds permeate the intestinal wall by simple diffusion of the unionized forms through the lipoid membranes of the mucosal cells [6, 7; reviews: 8, 9].

The quaternary ammonium ions are another class of compounds whose absorptive behaviour does not conform to the theory of non-ionic diffusion. These substances are absorbed to a considerable extent [10–17] in spite of the fact that they mostly have an extremely low lipoid solubility because of their permanent positive charge. Therefore passive permeation of these compounds through aqueous pores has been suggested as their mechanism of absorption [18–20]. However this concept cannot explain the time course or the dose-dependence of the absorption of quaternary ammonium compounds. Levine and coworkers observed that after a rapid initial phase, absorption came to a complete halt although 70–90 per cent of the administered dose still remained in the intestine [10–14], and that there was a non linear correlation between the dose administered and the amount absorbed [21].

On the basis of the previous experiments with cardiac glycosides it has been proposed that the peculiar absorptive behaviour of quaternary ammonium compounds may be due to the existence of a secretory mechanism in the intestinal epithelium, specific for quaternary ammonium compounds [5, 22]. Preliminary experiments *in vivo* showed that following i.v. administration to rats and guinea pigs tetraethylammonium ions were concentrated in the intestinal lumen substantiating this hypothesis [5, 22].

In order to study the permeation mechanism of quaternary ammonium compounds, the isolated mucosa of guinea pig small intestine seemed especially well suited. It allows the determination of tissue uptake and transepithelial fluxes not only in the absorptive but also in the secretory direction [4, 23]. The transepithelial permeation and the

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cellular uptake of *N*-methylscopolamine bromide (NMScop), N^1-methylnicotinamide chloride (NMN) and tetraethylammonium bromide (TEA) were studied over a concentration range of 10^{-7} M to 2×10^{-3} M.

MATERIALS AND METHODS

Materials. Guinea pigs of both sexes (inbred strain, Fa Rost, D 581 Witten), ranging in weight from 335 to 580 g and fed with a diet (Altromin 3022/MS®, Altromin GmbH, D 4910 Lage/Lippe) enriched with vitamins (Multivitaminsaft Lappe, Lappe-Arzneimittel, D 5060 Bensberg), were used in the experiments. [1-^{14}C]Tetraethylammonium bromide (1–5 mCi/m-mole) and N^1-[^{14}C]methylnicotinamide chloride (1–5 mCi/m-mole) were obtained from New England Nuclear Corp. (N.E.N.), Boston, Mass. *N*-[^{3}H]methylscopolamine bromide (107 mCi/m-mole) was supplied by C. H. Boehringer Sohn, D 6507 Ingelheim. The radiochemical and chemical purity of the substances was tested by thin-layer chromatography on Silica gel and aluminium oxide using *n*-butanol–water–acetic acid (4:5:1, by vol). All other chemicals were analytical grade. [Carboxy-^{14}C]inulin (1–3 mCi/g) and [methoxy-^{3}H]inulin (50–150 mCi/g) were obtained from N.E.N.

Experimental procedure. The method of isolating the mucosa has been described in detail in another paper [23], and therefore only the principles of the procedure will be discussed.

Guinea pigs were anaesthetized with 130 mg/kg phenobarbital sodium intraperitoneally and anaesthesia was sustained with ether given intermittently. Pieces of jejunum, 1–2 cm length, were excised starting from the flexura duodeno-jejunalis and opened along the mesenterial insertion. The sheet of gut was gently spread out, serosal side down, on a glass plate and the entire epithelium was scraped off with a razor blade held at the appropriate angle. The isolated jejunal mucosa was floated in isotonic buffered saline and placed on a thin nylon meshwork, which was then mounted between two polyvinyl chloride sheets, where it occluded a window of 5 mm diameter. The mucosa and the sheets together served as a separating membrane between two methyl methacrylate (Plexiglas®) chambers, which were firmly locked together with a clamp. The chamber was then submerged halfway in a water bath at 37°; the edge of both the sheets and the chambers had been coated with a thin layer of vaseline to keep the compartments watertight. Both chambers were filled with 0.2 ml of buffered physiological salt solution (composition in mM: 98.3 NaCl, 7.0 KCl, 3.0 $CaCl_2$, 1.0 $MgSO_4$, 0.9 sodium phosphate buffer (pH 7.4), 29.4 tris-(hydroxymethyl)-aminomethane buffer (pH 7.4), 14.0 glucose, 14.0 mannitol) and oxygenated through holes in the chamber wall. The Tris-buffer did not influence the transfer characteristics of the compounds used, as was shown in control experiments with bicarbonate buffered saline.

In the standard experiments the labelled monoquaternary ammonium compound was administered to one side of the mucosa and the other side served as the countercompartment in which the permeated amount was determined after a given period of incubation. One hundred μl of the incubation medium of both sides were counted in 9 ml of Bray's scintillator for aqueous solutions [24] with a Packard Scintillation Spectrometer, Tri-Carb, Model 3380. Thin-layer chromatography was performed with the remaining portion of the incubation medium to examine the possible formation of metabolites of the compound. At the end of the experiment the mucosa was cut out, weighed and soaked in 0.2 ml of distilled water for 2 hr at room temperature to recover the radioactivity in the tissue. Control experiments with mucosae containing labelled quaternary ammonium compounds and solubilized with Soluene 100 (Packard) revealed that the extraction with water was complete. The extracellular space and the leakiness of the mucosa were determined from one side of the mucosa with ^{14}C- or ^{3}H-labelled inulin, depending on the radionuclide of the quaternary ammonium compound. All those experiments with an inulin permeation greater than 1 per cent within 45 min were rejected as not being intact; this was the case in approximately 10 per cent of all mucosae mounted.

Calculations. Total permeation was defined as the amount in the countercompartment at the end of the experiment in per cent of the amount of drug originally administered. Since the volumes of the incubation media on both sides of the mucosa were identical, the calculated total permeation equals the concentration in the countercompartment in per cent of the concentration offered.

For the investigation of a transport mechanism the transcellular permeation is of prime interest; therefore the fraction passing through the epithelium via an extracellular route has to be subtracted from the total amount translocated. The existence of an extracellular pathway is apparent from the fact that the isolated jejunal mucosa was not completely impermeable to inulin, as observed previously [4, 23]. Within 45 min a mean of 0.34 per cent (S.E.M. $\pm$ 0.02; n = 131) of the inulin administered permeated in the direction lumen-to-blood during aerobic conditions, and 0.52 per cent ($\pm$0.02; n = 143) in the direction blood-to-lumen and this difference is significant ($P < 0.001$). This asymmetry of the inulin permeation in the two directions has been discussed elsewhere [23]. These 'inulin leaks' apparently become more numerous or widen during anaerobiosis, since the inulin permeation from lumen to blood rose significantly ($P < 0.001$) to 0.54 per cent ($\pm$ 0.03; n = 113), and that from blood to lumen increased to 0.65 per cent ($\pm$ 0.02; n = 111), when the incubation media were equilibrated with N_2. However the significance of the difference between the inulin permeations in the two directions was not abolished ($P < 0.005$).

In the case of equal percentage permeations of inulin and substrate through the extracellular pathways, the correlation between inulin permeation and total substrate permeation should yield a regression line with a slope of 1. But a regression coefficient of 1 was obtained only with relatively high inulin permeation values, whereas with small inulin permeations the slope of the regression increased (Fig. 1), suggesting that the extracellular permeation of the small substrate molecules was higher than the inulin permeation, when the number or the size of the inulin-permeable leaks was small. This phenomenon, which is

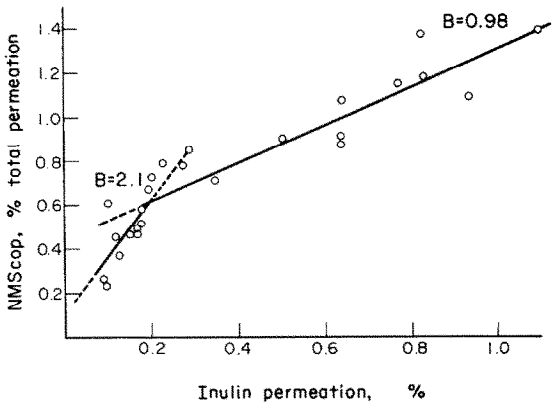

Fig. 1 Correlation of NMScop and inulin permeation across the isolated mucosa. [^{3}H]NMScop and [^{14}C]inulin were administered on the luminal side. Incubation time 45 min; O_2; 37°. Concentration on the blood side in per cent of the concentration initially administered for inulin (abscissa) and NMScop (ordinate), respectively. Each point represents one single experiment. B = slope of the regression line.

similar to that of 'restricted diffusion' described by Pappenheimer *et al.* and Renkin for capillary walls and porous membranes [25, 26], may be explained by molecular sieving and/or differences in the free diffusion coefficients of the large inulin molecules and the small substrate molecules. Thus, the substrate permeation through extracellular shunt pathways was calculated as the product of the inulin permeation times the slope of the regression line between the inulin and the total substrate permeations, determined for low, high, and in some cases intermediate inulin permeation values separately, under both aerobic and anaerobic conditions. The fraction of the substrate transferred through the cells (total substrate permeation minus extracellular substrate permeation) will be termed corrected permeation (corr. permeation).

The cellular amount of a drug was defined as the difference between total amount in the wet tissue and the amount in the extracellular space (inulin space) on the side of administration.

The mean extracellular space from the lumen side of the mucosa was 14.48 per cent (± 0.03; n = 244) and that from the blood side 12.49 per cent (± 0.04; n = 254). Therefore, the cellular concentrations of the compounds tested were calculated by dividing the cellular amount by 75 per cent of tissue wet weight. Because of the high degree of constancy of the inulin spaces and of the wet weights of the punched out mucosae—12.01 mg (± 0.01; n = 276) during aerobiosis, and 13.68 mg (± 0.01; n = 228) during anaerobiosis—there were only negligible differences between the cellular amounts and concentrations. Therefore only the cellular concentrations, expressed in per cent of the concentration administered, will be dealt with as the parameter of cellular uptake.

RESULTS

Permeation and cellular uptake of monoquaternary ammonium compounds during aerobiosis and anaerobiosis

In the first set of experiments the permeation through the jejunal mucosa and the cellular uptake of monoquaternary ammonium compounds were studied as a function of the concentration of substrate used, the side of the epithelium on which the individual substances were administered and the availability of oxygen.

Permeation during aerobiosis. The changes in corr. permeation with increasing concentrations administered during aerobiosis and anaerobiosis are presented in figures 2–4. The transepithelial fluxes per unit area and unit time have been calculated from the total permeation and are given for the aerobic as well as for the anaerobic experiments in Table 1. Both the total and the corr. permeation of the three monoquaternary ammonium compounds tested were characterized by several common features:

1. The permeation from blood to lumen was significantly larger than that in the reverse direction with concentrations lower than 500 μM.

2. The permeation from blood to lumen was dependent on the concentrations administered: With increasing concentrations the corr. permeation from blood to lumen of the three substances decreased and at the highest concentrations administered (2000 μM NMScop, 1000 μM NMN, and 1600 μM TEA) the corr. permeations in both directions through the mucosa were not significantly different from one another.

3. The permeation, both total and corrected, in the direction lumen-to-blood did not exhibit nearly as marked a concentration dependence as was observed with the permeation from blood to lumen. Only with NMN and TEA there was a small, statistically insignificant increase in corr. permeation with rising concentrations.

Aside from these common features there were some quantitative differences among the corr. permeations of the three monoquaternary ammonium compounds tested. NMScop had the highest transport rate in the direction blood-to-lumen at low concentrations: 3 per cent corr. permeation with 0.1 and 1 μM, as compared to 2.4 per cent with 1 μM NMN and 0.8 per cent with 50 μM TEA. The corr. permeation from lumen to blood on the other hand was lowest with NMScop, ranging between 0.16 and 0.26 per cent; the corresponding values for NMN were 0.92 to 1.09 per cent and for TEA 0.37 to 0.44 per cent. Consequently a ratio of permeation from blood to lumen to that from lumen to blood of 12 was calculated for 0.1 and 1 μM NMScop (5.5 for the total, uncorrected permeation). The corr. permeation ratios were lower with the other two compounds: 2.5 with 1 μM NMN and 2 with 50 μM TEA.

Thin-layer chromatography revealed that NMScop and TEA appeared metabolically unchanged in the countercompartment, whereas up to 30 per cent of the transported NMN was metabolized to a chromatographically faster-moving substance.

In order to examine whether the decreased permeation from blood to lumen with the highest concentrations of the monoquaternary ammonium compounds was due to a toxic effect on the epithelial cells, the intracellular concentrations of Na^+ and K^+ were measured with a flame photometer (Eppendorf) after the mucosal preparations had been incubated for 45 min in the usual manner (aerobic conditions) with the same drug concentrations as were used for

Table 1. Dependence of the fluxes of NMScop, NMN, and TEA on the concentration given, the side of administration, and the gas supply

	Side of administration			
	Lumen		Blood	
Gas supply	O_2	N_2	O_2	N_2
NMScop (μM)				
0.1	0.016 ± 0.001 (4)	0.024 ± 0.007 (4)	0.081 ± 0.008 (8)	0.028 ± 0.004 (6)
1	0.156 ± 0.027 (6)	0.316 ± 0.069 (4)	0.855 ± 0.113 (7)	0.267 ± 0.036 (5)
10	1.69 ± 0.27 (5)	1.89 ± 0.29 (3)	7.18 ± 0.87 (5)	3.20 ± 0.16 (4)
100	16.2 ± 3.8 (7)	24.0 ± 6.4 (4)	66.4 ± 12.7 (6)	26.9 ± 5.6 (3)
500	92 ± 51 (2)	107 ± 47 (2)	257 ± 117 (2)	131 ± 12 (2)
1000	162 ± 27 (4)	275 ± 76 (5)	367 ± 53 (6)	387 ± 44 (5)
2000	227 ± 120 (2)	471 ± 44 (2)	635 ± 173 (2)	853 ± 13 (2)
NMN (μM)				
1	0.344 ± 0.073 (5)	0.631 ± 0.096 (5)	0.731 ± 0.044 (5)	0.715 ± 0.096 (6)
10	4.00 ± 0.31 (12	6.16 ± 0.64 (11)	6.58 ± 0.42 (11)	6.73 ± 0.47 (10)
100	36.9 ± 5.1 (9)	62.7 ± 2.7 (9)	55.3 ± 4.0 (12)	65.8 ± 5.1 (10)
1000	293 ± 44 (3)	515 ± 136 (5)	529 ± 24 (5)	635 ± 66 (6)
TEA (μM)				
50	13.4 ± 1.3 (10)	21.2 ± 4.1 (8)	22.8 ± 2.2 (13)	22.2 ± 0.9 (6)
100	28.4 ± 3.8 (16)	43.1 ± 7.3 (9)	48.4 ± 2.0 (19)	43.1 ± 3.1 (10)
200	52.4 ± 3.1 (9)	78.7 ± 15.6 (6)	84.9 ± 8.0 (9)	88.4 ± 16.9 (5)
400	96 ± 11 (9)	160 ± 31 (9)	164 ± 10 (9)	172 ± 20 (10)
1600	380 ± 39 (9)	786 ± 82 (9)	501 ± 32 (8)	892 ± 39 (9)

Isolated intestinal mucosae were incubated 45 min at 37°. Fluxes are calculated from the total permeation and are expressed in units of pmole cm^{-2} min^{-1}. Mean values ± S.E.M.; number of experiments in parenthesis.

the permeation study. The intracellular concentrations of both electrolytes were unaffected even by the highest concentrations of the organic bases administered. Therefore, it may be concluded that the decrease in permeation, both total and corrected, from the blood to the lumen side of the mucosa was not brought about by a deterioration of the cells but rather by saturation of a transport mechanism of the intact epithelium.

Cellular uptake during aerobiosis. The aerobic cellular uptake of the three monoquaternary ammonium compounds is depicted in the lower left portion of Figs. 2–4.

The cellular uptake exhibited a pattern similar to that of the corr. permeation: the uptake of all three ammonium compounds was significantly higher from the blood side than from the lumen side. Again a dependence of the cellular uptake on the external concentration was common to all three substances, as was observed in the permeation data. When increased concentrations were administered from the blood side the cellular uptake decreased. Although the difference in uptake from the two sides of the mucosa decreased with increasing concentrations administered, it was not abolished completely even with the highest concentrations.

The cellular uptake from the lumen side did not display as striking a concentration dependence as was observed with the uptake from the blood side. The luminal uptake seemed to decrease somewhat with higher concentrations of NMN and TEA, while that of NMScop remained practically unchanged. Quantitatively, the uptake from the blood side was 79 per cent with 1 μM NMScop and 50 μM TEA, but only 21 per cent with 1 μM NMN. Similarly, the uptake from the lumen side was higher with NMScop (11–14 per cent) and TEA (13–18 per cent) than with NMN (4–8 per cent). Evidently there is no parallelism between the cellular uptake and the corr. permeation. None of the compounds tested was accumulated above 100 per cent of the concentrations administered under these conditions.

Permeation and cellular uptake during anaerobiosis. In order to investigate whether the observed differences in permeation in the two directions and the discrepancy in cellular uptake from the two sides of

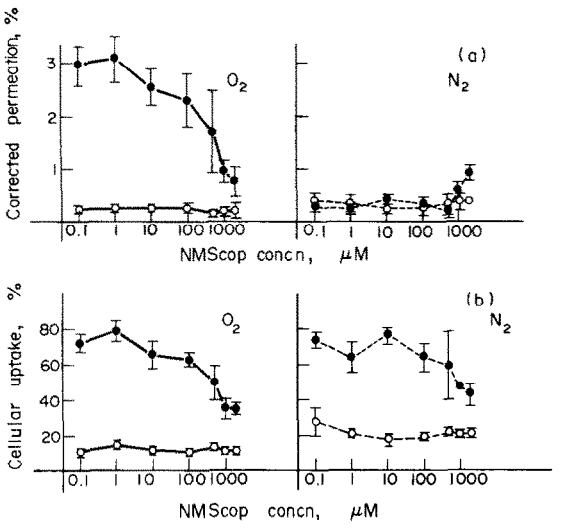

Fig. 2 Corr. permeation (a) and cellular uptake (b) of NMScop from the bloodside (●) and from the lumen side (○) during aerobiosis (O_2) and anaerobiosis (N_2). Abscissa: concentration of NMScop administered (logarithmical scale); ordinate: corr. permeation (a) and cellular uptake (b) within 45 min. Each point represents the mean ± S.E.M.

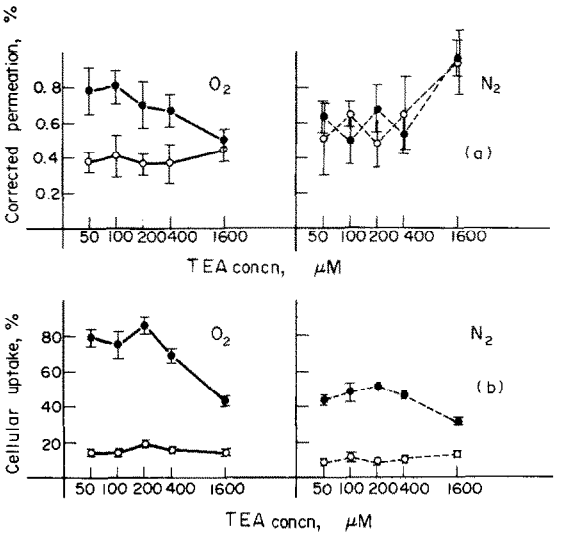

Fig. 4 Corr. permeation (a) and cellular uptake (b) of TEA from the blood side (●) and from the lumen side (○) during aerobiosis (O_2) and anaerobiosis (N_2). Abscissa: concentration of TEA administered (logarithmical scale); ordinate: corr. permeation (a) and cellular uptake (b) within 45 min. Each point represents the mean ± S.E.M.

the mucosa were dependent on aerobic metabolism, the same experiments as described above were repeated under anaerobic conditions by bubbling the incubation media with N_2. It is apparent from the upper right portions of Figs. 2–4 that the permeation ratios for the three tested substances approached 1 at all concentrations during anaerobiosis. Additionally, the dependence of the permeation on the concentration was abolished. The increase in permeability of the mucosa observed with the highest concentrations of TEA and NMScop may be due to a toxic effect of the administered bases in combination with the oxygen lack.

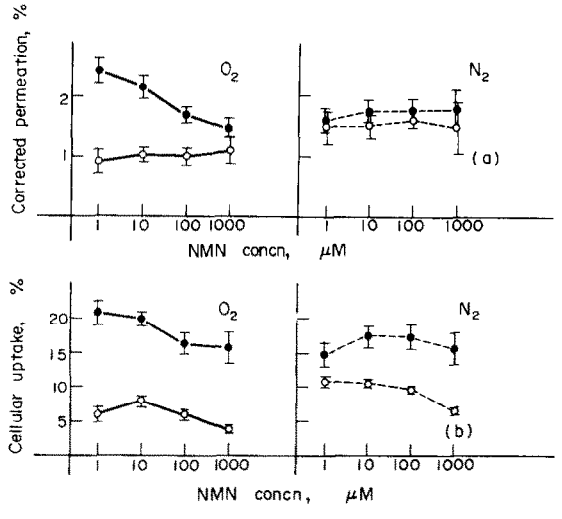

Fig. 3 Corr. permeation (a) and cellular uptake (b) of NMN from the blood side (●) and the lumen side (○) during aerobiosis (O_2) and anaerobiosis (N_2). Abscissa: concentration of NMN administered (logarithmical scale); ordinate: corr. permeation (a) and cellular uptake (b) within 45 min. Each point represents the mean ± S.E.N.

A permeation ratio of 1 during anaerobiosis was reached both by a decrease in corr. permeation from blood to lumen and an increase in corr. permeation from lumen to blood as compared to aerobic conditions. Which of these two effects contributed more to a permeation ratio of 1 varied among the individual compounds: with NMN and TEA both mechanisms were approximately equally effective whereas with NMScop it was primarily the corr. permeation from blood to lumen which was greatly decreased as compared to aerobic conditions, whilst the corr. permeation from lumen to blood was elevated only by a small amount.

The cellular uptake of the three monoquaternary ammonium compounds was still higher under anaerobic conditions from the blood side than from the lumen side (Figs. 2–4, lower right portions) but the difference in uptake from the two sides was reduced as compared to aerobiosis. This decrease was accomplished by a significant reduction in uptake from the blood side (with 50–400 μM TEA), by a significant increase in uptake from the lumen side (NMScop), or by both (low concentrations NMN). With NMScop the uptake from the blood side was unchanged; with TEA the luminal uptake remained practically unaltered during anaerobiosis in comparison to aerobiosis (the measured reduction was small and statistically insignificant). In contrast to the permeation the concentration dependence in uptake from the blood side was not totally abolished by anaerobiosis. Therefore, adsorption cannot be excluded as being involved.

Uphill transport

In the experiments under aerobic conditions described above it was demonstrated that the permeation from the blood to the lumen side is higher than that from the lumen to the blood side at suitable concentrations. This saturable, metabolically dependent mechanism could represent a secretory system

capable of creating a gradient between the blood and the lumen side. In order to show uphill transport the following experiments were carried out: 1 μM NMScop, a concentration at which the corr. permeation blood-to-lumen was twelve times that in the reverse direction (Fig. 2), was given simultaneously to both the lumen and the blood side of the mucosa under aerobic conditions and a concentration gradient developed between both sides which increased with time (Fig. 5). After 180 min the luminal concentration reached 122 $\pm$ 4 per cent of the initial value, whereas on the blood side the NMScop concentration had decreased to 74 $\pm$ 3 per cent. This distribution corresponds to a concentration ratio lumen/blood of 1.65.

The establishment of this gradient was linked to oxidative metabolism, since it was reduced practically to zero when the same experiments were carried out during anaerobiosis (Fig. 5). The remaining small difference was statistically insignificant ($P > 0.1$).

A similar, but somewhat smaller gradient was established with NMN. The concentration ratio lumen/blood after 180 min was 1.20. This phenomenon was least readily shown with TEA, which barely exceeded 100 per cent on the lumen side after 180 min, with 86 per cent on the blood side, yielding a concentration ratio lumen/blood of 1.15. But the luminal concentration was still rising at 180 min, so it is likely that higher values would be reached at a later time. This observation was also made with the other substrates tested, so the given concentration ratios do not reflect the true equilibria.

Inhibition of permeation and cellular uptake of NMN by NMScop

Another set of experiments was carried out to study whether monoquaternary ammonium compounds are transported by a common mechanism.

Ten μM ^{14}C-labelled NMN was administered alone and together with 10, 100, and 1000 μM of unlabelled NMScop on the blood or the lumen side of the jejunal preparation, which was supplied with O_2 and incubated for 45 min. NMScop was chosen to inhibit the transport of NMN because according to the evidence presented earlier, NMScop seems to possess a relatively high affinity for the transport mechanism. From Fig. 6 it is apparent that both the secretion of NMN and the uptake from the blood side were progressively inhibited by increasing concentrations of NMScop.

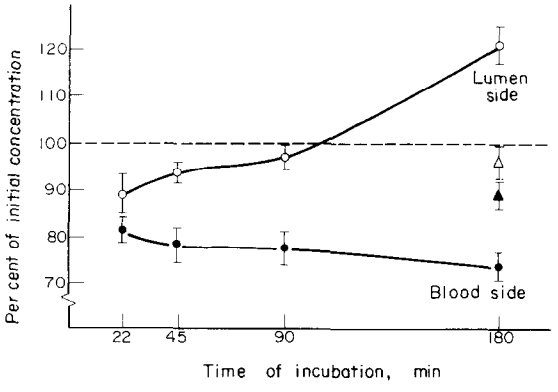

Fig. 5 Distribution of NMScop between the lumen side (○,△) and the blood side (●,▲) during aerobiosis (circles) and anaerobiosis (triangles) after simultaneous administration of 1 μM NMScop on both sides of isolated jejunal epithelium. Abscissa: time after NMScop administration; ordinate: concentration of NMScop in per cent of the concentration originally administered. Each point represents the mean $\pm$ S.E.M. of four experiments.

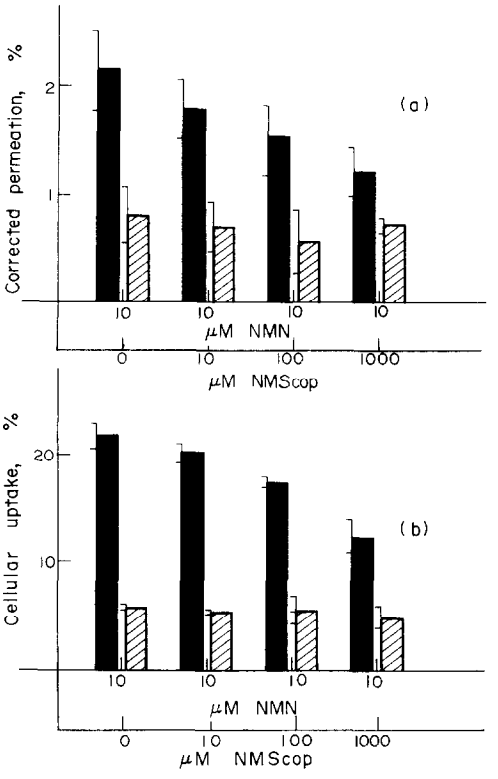

Fig. 6 Inhibition of the corr. permeation (a) and the cellular uptake (b) from the lumen side (light columns) and from the blood side (dark columns) of 10 μM NMN by 10, 100 and 1000 μM NMScop. All columns represent the mean $\pm$ S.E.M. Incubation: 45 min at 37°, aerobiosis.

The influence of K^+ on the intestinal transport of monoquaternary ammonium compounds

In order to test whether a secretory system for K^+ interferes with the demonstrated secretion of monoquaternary ammonium compounds, the permeation from blood to lumen of TEA was examined with varying K^+ concentrations. There was no significant difference in TEA permeation with 7, 14, or 28 mM K^+ in the incubation medium.

DISCUSSION

Intestinal secretion of monoquaternary ammonium compounds. The present results demonstrate the existence of a secretory system for monoquaternary ammonium compounds in the intestinal mucosa. This has been established by the following observations:

1. The permeation ratio blood-to-lumen/lumen-to-blood was far greater than 1 for all three compounds tested at concentrations below 500 μM. Certainly a permeation ratio greater than 1 has to be expected with positively charged compounds, since the lumen

side of the epithelium is negatively charged in comparison to the blood side. A potential difference of 2–4 mV has been measured on the preparation used in this study [4, 23] which agrees well with the 3–6 mV in guinea pig jejunum obtained by other investigators [27, 28]. But the corr. permeation ratios obtained in the present experiments—12 with 1 μM NMScop, 2.5 with 1 μM NMN, and 2 with 50 μM TEA—far exceed the values to be expected for passive distribution of charged species according to Ussing's flux ratio test [29]. With a potential difference of 3 mV the calculated flux ratio is only 1.12, whereas a potential difference of 66 mV would be necessary for a flux ratio of 12.

2. The transport system was concentration dependent and exhibited saturation kinetics; the permeation ratios approached 1 with high concentrations.

3. The establishment of permeation ratios greater than 1 and the concentration dependence of the permeation from blood to lumen were abolished by a decrease in oxidative energy.

4. In accordance with the preferential flux direction blood-to-lumen, the system was capable of uphill transport, creating a concentration gradient between the lumen and the blood side of the mucosa. The establishment of this gradient was also abolished by anaerobiosis.

Therefore, this secretory mechanism must be classified as an active transport system. The fact that one monoquaternary ammonium compound inhibited the transport of another in a concentration-dependent manner is consistent with a common transport system for these substances.

Since the demonstration of an active secretory system for cardiac glycosides [2–5], the intestinal transport of monoquaternary ammonium compounds represents a second system which secretes compounds of a chemically distinct group of substances into the intestinal lumen. But whereas an active transport system for cardiac glycosides has been additionally demonstrated only in the liver [30, 31], and, possibly, in the kidney [32, 33] that for quaternary ammonium compounds seems to be widely distributed throughout the body. Quaternary ammonium compounds are reported to be actively transported by the liver [34–37: reviews: 8, 38, 39], the kidney [40–43; reviews: 44, 45], the choroid plexus [46, 47], nervous tissue [48, 49], myocardial cells [50], and erythrocytes [51, 52].

Close similarities of the transport system for quaternary ammonium compounds seem to exist between the intestine and other tissues with respect to substrate affinity. In the present study NMScop was most readily transported. Compounds with a chemical structure similar to that of NMScop (a highly polar group at one end of the molecule and a lipophilic structure at the opposite end) are also most rapidly excreted in bile, for instance mepiperphenidol (Darstin®), benzomethamine, oxyphenonium or procaine amide ethobromide [37, 39]. In the kidney substances with an asymmetrical charge distribution like Darstin, other dialkyl-piperidinium compounds, and tetraalkylammonium derivatives with one longer alkyl substituent have a relatively high affinity for the organic base transport system, measured by inhibition studies [53–55]. Those quaternary ammonium compounds which lack the nonpolar component in their structure, for example TEA, are slowly excreted in bile [37] and possess a relatively low affinity for the renal transport system [53–55]. These results agree well with the present findings, in which TEA was also the substrate least transported by the active mechanism described. The lipoid solubilities of the tested compounds (chloroform–water partition coefficient: 0.06 for TEA, 0.06 for NMN, and 0.10 for NMScop) are consistent with the suggestion that a nonpolar side chain facilitates the interaction with binding sites at the cell wall [53, 54].

Furthermore the molecular weight may also contribute to the quantitative differences in transport. Quaternary ammonium compounds with relatively low molecular weights (< 200–300) tend to be poorly excreted in bile, but biliary excretion increases as the molecular weight of the compounds rises [56, 57]. Similarly in the present study NMScop, which has a molecular weight of 318, was far better transported than NMN and TEA with molecular weights of 137 and 130, respectively.

Differentiation of permeation into a saturable and a nonsaturable component. The permeations in the secretory direction decrease more or less rapidly with increasing concentrations (Figs. 2–4). This kind of curve is to be expected when a carrier system reaches its saturation. But even with high concentrations there is no tendency of the permeation from blood to lumen to approach zero, indicating the participation of a second, nonsaturable permeation mechanism. The latter is more clearly apparent from Fig. 7, in which the absolute amount of NMScop secreted per unit time and unit area is plotted versus the concentration, both for the total permeation (Fig. 7a) and the corr. permeation (Fig. 7b). In both cases the flux may be described as the sum of a saturable term displaying Michaelis–Menten kinetics plus a second nonsaturable term, since the flux tended to become proportional to the concentration offered as the latter was increased. Thus the flux from blood to lumen can be described by the equation

$$Y = \frac{V \cdot S}{K_m + S} + P \cdot S, \qquad (1)$$

where: Y = amount secreted/(time · area), V = maximum velocity of transepithelial carrier-mediated transport, K_m = half-saturation constant of transepithelial carrier-mediated transport, P = permeability coefficient (proportionality constant for nonsaturable process), S = substrate concentration offered.

V, K_m, and P were calculated by approximating the parameters of eq. (1) to the experimental data for the NMScop permeation from blood to lumen by an iteration procedure [58]. The values obtained are given in the legend to Fig. 7. The satisfactory fit of the calculated curves to the values measured is illustrated in Fig. 7.

The values obtained for V and K_m by using either the total or the corr. permeation for the approximation procedure were not significantly different from one another. Only the permeability coefficient was approximately four times higher, when the values for total permeation were used. This fact may be considered as additional evidence for the practicability

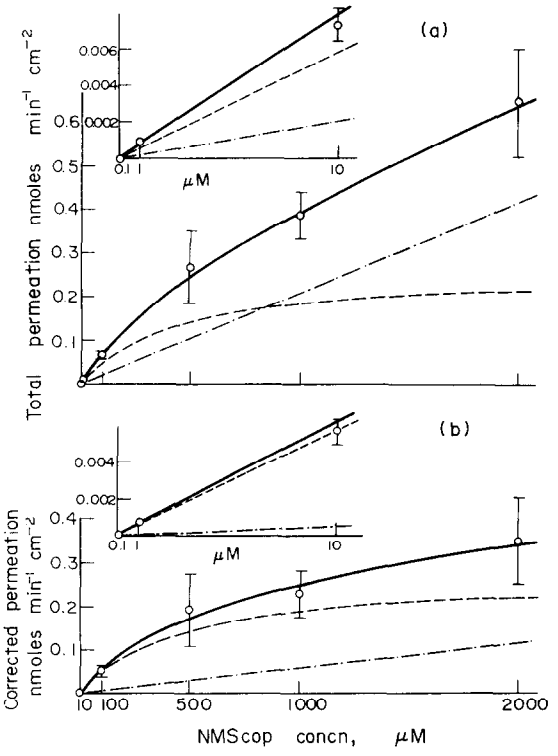

Fig. 7 Permeation from blood to lumen of NMScop in absolute terms as a function of the concentration administered. Abscissa: concentration administered (linear scale); ordinate: total (a) and corr. permeation (b) in nmoles $min^{-1} cm^{-2}$. Insets: permeations with low concentrations offered. Curves correspond to the equations

$$\frac{V \cdot S}{K_m + S} + P \cdot S(\text{——}),$$

$$\frac{V \cdot S}{K_m + S}(\text{– – –}),$$

and $$P \cdot S(\text{– · – · –}),$$

calculated by use of the constants obtained by approximating the parameters of eq. (1) to the experimental data. The constants used were:

V (nmoles $min^{-1} cm^{-2}$)	K_m (μM)	P (10^{-5} cm min^{-1})
(a) 0.268	445	20.94
(b) 0.277	467	5.84

of the correction method employed for the extracellular substrate permeation described above. However, the values obtained for the transport parameters are only approximations, since they are based on permeation rates which also include a non-steady-state period.

Cellular localization of the transport system. The proof of the intestinal secretion of monoquaternary ammonium compounds by a saturable, metabolically dependent transport system poses the question where is this mechanism located within the epithelial cell.

The existence of a transport mechanism in the basolateral membranes is suggested by the saturability of the uptake from the blood side of the epithelium (Figs. 2–4). If no further transport system is assumed to be involved in the transepithelial permeation, a decrease in cellular uptake from the blood side would be expected with inhibition of secretion. Yet with NMScop the cellular uptake from the blood side was unchanged in anaerobiosis, although the secretion is markedly decreased in comparison to aerobic conditions. These experimental findings may be explained by the assumption of a second transport system in the luminal membrane, secreting monoquaternary amines toward the lumen. If less substrate enters and leaves the cells in anaerobiosis, the cellular concentration may remain unchanged in comparison to aerobic conditions, although the secretion is inhibited. Thus, the cell model which fits all experimental data is that of two transport mechanisms in series, paralleled by diffusional pathways across both membranes. Analogous cell models have been derived previously from studies of the secretion of cardiac glycosides [4, 5] and the absorption of 3-*O*-methyl-D-glucose [59] by the isolated mucosa.

The existence of the luminal system is substantiated by the increase in cellular uptake from the lumen side in anaerobiosis in comparison to aerobiosis as observed with NMScop and NMN. A decrease in basal efflux cannot be responsible for this finding, because the basal efflux coefficient is unchanged under these conditions (Turnheim and Lauterbach, in preparation). The unaltered luminal uptake of TEA in anaerobiosis might be attributed to an especially low affinity to this pump, which is in good agreement with the demonstrated low secretory rate of TEA when placed on the blood side. The unsaturability of the luminal uptake may be explained by a low affinity of the substrates for this system, as would be expected from the preferential direction of transport. Therefore the concentrations studied in which the basal uptake exhibited saturation were probably less than the saturation range of the transport system on the outside of the luminal membrane.

Intestinal transfer of non-quaternary amines. In a number of organs a common transport system is shared by quaternary and non-quaternary amines, as shown by inhibition studies [41, 43, 52, 55, 60, 68]. It is quite conceivable, therefore, that non-quaternary amines in their cationic form are also substrates for the intestinal secretory system demonstrated for quaternary ammonium compounds. As a matter of fact, the tertiary amine atropine (pK_a 9.9) has been shown to be a potent inhibitor of NMScop transport in the isolated jejunal mucosa (Sund, Lauterbach, Turnheim; unpublished). Intestinal secretion may thus be responsible for the finding of Hogben *et al.* [69] that the distribution ratio intestinal lumen/plasma of organic bases was higher than that which had been expected on the assumption that only the unionized form transgresses the cell boundaries. The dose-dependence of the absorption of the tertiary amines quinine and quinidine which has been observed under certain conditions by Ragozzino and Malone [70] and which is very similar to that recorded for benzomethamine [21] may also be due to saturable intestinal secretion.

Therefore the demonstration of a secretory system for quaternary ammonium compounds not only

emphasizes the excretory function of the intestine but may also provide an explanation for the peculiarities in absorption kinetics of drugs, which thus far could not be fully explained by the non-ionic diffusion theory.

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