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THE STIMULATING AND INHIBITORY EFFECT OF MONOQUATERNARY AMMONIUM COMPOUNDS ON DECAMETHONIUM UPTAKE BY RAT KIDNEY CORTEX SLICES

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Abstract—Decamethonium is concentrated as the unchanged compound in rat kidney cortex slices incubated (oxygen–carbon dioxide atmosphere 95 : 5 v/v %) in Krebs–Ringer bicarbonate medium (37°, pH 7·4) containing [^{14}C]-decamethonium. Decamethonium uptake was a saturable and metabolically dependent process. When added to medium at relatively low concentrations carbamoylcholine, choline, neostigmine and tetraethylammonium stimulated 1 hr uptake of decamethonium (2×10^{-6} M), whereas these monoquaternary ammonium compounds at higher concentrations inhibited uptake. Initial decamethonium uptake (5 min) was increased when slices were preincubated (90 min) with a monoquaternary ammonium compound (without decamethonium) before transfer to a medium containing only decamethonium (2×10^{-6} M). This suggests that stimulation is related to the presence of monoquaternary ammonium compounds in the slices. No decamethonium efflux (30 min) occurred from slices preincubated (1 hr) with decamethonium (2×10^{-6} M), which means that stimulation cannot be attributed to inhibition of decamethonium efflux by monoquaternary ammonium compounds. The stimulation phenomenon can be interpreted as an example of substrate-facilitated carrier transport, which implies that efflux of monoquaternary ammonium compounds accelerates decamethonium influx. This interpretation is consistent with the conclusion from the experiments on mutual inhibition that the polymethylene–bisquaternary ammonium compound decamethonium and the above monoquaternary ammonium compounds share a common carrier mechanism.

MANY quaternary ammonium compounds are concentrated in renal slices by active transport processes in many respects similar to those involved in the tubular secretion of these substances by the intact kidney (Peters).[1]

We have recently shown that decamethonium, carbamoylcholine and tetraethylammonium are concentrated as the unchanged compounds in mouse kidney slices by saturable metabolically dependent processes.[2–4] Further studies suggested that the polymethylene–bisquaternary ammonium decamethonium and the monoquaternary ammonium compounds carbamoylcholine, choline, neostigmine and tetraethylammonium share a common carrier-mediated transport mechanism in mouse kidney slices.[5–7]

It has previously been reported that rat kidney cortex slices accumulate decamethonium, tetraethylammonium and choline.[8–10] We therefore thought it of interest to investigate whether decamethonium and the above four monoquaternary ammonium compounds also share a common transport mechanism in rat kidney cortex slices. Furthermore, experiments were performed to establish whether decamethonium uptake was a saturable and metabolically dependent process. The possibility

of metabolic transformation of decamethonium was investigated by paper chromatography (Christensen *et al.*).[11] For the sake of comparison with decamethonium, the uptake of hexamethonium was also studied.

MATERIALS AND METHODS

The following isotope-labeled compounds were used; [^{14}C]methyldecamethonium dibromide (Radiochemical Centre, Amersham) with a specific activity of 15 mc/mM, [^{14}C]methylhexamethonium dichloride (New England Nuclear Corp.) with a specific activity of 1·71 mc/mM and inulin–carboxyl-^{14}C (New England Nuclear Corp.) with a specific activity of 1·95 mc/g.

The following unlabeled compounds were used; decamethonium dibromide (syncurine®, Burroughs Wellcome & Co.), decamethonium diiodide (Koch–Light Laboratories Ltd.), carbamoylcholine chloride (Ph. Nord. 1963), neostigmine bromide (Ph. Nord. 1963), choline chloride (Merck), tetraethylammonium bromide (Bie & Berntsen, Denmark) and iodoacetic acid (Merck).

Experimental procedure. Kidney slices were prepared and used as previously described.[2] Albino rats of a single strain (Wistar) with a body weight of 150–200 g were decapitated and bled. A few experiments (Table 1) were performed with male

TABLE 1. THE UPTAKE (S/M RATIO AFTER 1 HR INCUBATION) OF [^{14}C]DECAMETHONIUM (2×10^{-6} M) AND [^{14}C]HEXAMETHONIUM (3×10^{-6} M) BY CORTICAL AND MEDULLARY SLICES OF RAT KIDNEY

Compound	Sex	S/M ratio		Difference (%)
		Cortical slices	Medullary slices	
Decamethonium	♂	5·2 ± 0·3	2·2 ± 0·1	−58 ± 4*
Decamethonium	♀	6·2 ± 0·2	2·7 ± 0·2	−56 ± 4*
Hexamethonium	♀	0·87 ± 0·04	0·73 ± 0·02	−15 ± 5†

Cortical and medullary slices were prepared as stated in text. Results are given as the mean of values from six experiments ± S.E.M.

* $P < 0·001$.

† $P < 0·05$.

animals, but otherwise only female animals were used. Unless otherwise stated, four slices (total wet wt. 150 mg) were cut from each kidney (two outer slices from each anterior and posterior surface) with a razor blade and placed in a test tube, which contained 20 ml Krebs–Ringer bicarbonate solution with glucose (11 mM/l.). This procedure made it possible to incubate kidney tissue from each animal (distributed in two test tubes) simultaneously under different experimental conditions (decamethonium alone or decamethonium plus another drug). Each animal thus served as its own control (method of paired comparison).[12] In some experiments (Table 1) each kidney was halved by a central section parallel to the anterior and posterior surface, and two outer slices (cortical; one from each anterior and posterior surface) as well as two inner slices (medullary, one from each central surface) were prepared. The method of paired comparison[12] was also used in the latter type of experiments

(Table 1), as kidney tissue from each animal was distributed in two test tubes (four cortical slices in one, and four medullary slices in the other).

Incubations were carried out by shaking (60 oscillations/min) the test tubes at 37° (pH 7·4). Drugs were always added to the media 10 min after the incubation had begun. Unless otherwise stated, the media were gassed with a mixture of oxygen–carbon dioxide (95:5, v/v %). When incubating under anaerobic conditions (Table 2),

TABLE 2. THE UPTAKE (S/M RATIO AFTER 1 HR INCUBATION) OF [^{14}C]DECAMETHONIUM (10^{-4}, 5×10^{-4} AND 10^{-3} M) BY RAT KIDNEY CORTEX SLICES

Decamethonium conc. (M)	S/M ratio	
	Oxygen–carbon dioxide atmosphere	Nitrogen–carbon dioxide atmosphere
10^{-4}	3·9 ± 0·3	1·24 ± 0·06
5×10^{-4}	2·5 ± 0·1	1·07 ± 0·07
10^{-3}	2·2 ± 0·1	1·05 ± 0·03

Slices were incubated in an atmosphere of oxygen–carbon dioxide (95:5 v/v %) as well as in an atmosphere of nitrogen–carbon dioxide (95:5 v/v %). Results are given as the mean of values from six experiments ± S.E.M.

the media were gassed with a mixture of nitrogen–carbon dioxide (95:5, v/v %). An amount of 0·13–0·54 μc [^{14}C]decamethonium was added to each test tube together with varying amounts of unlabelled decamethonium. When used (Table 1) [^{14}C]-hexamethonium was added to the medium in an amount of 0·1 μc. Unless otherwise stated, other drugs were added to the media just before [^{14}C]decamethonium. In the experiments recorded in Table 7 slices were incubated for 90 min (in the absence of [^{14}C]decamethonium) either with a monoquaternary ammonium compound (pre-loaded), or without (control) before being transferred to a final incubation medium, which contained [^{14}C]decamethonium only.

At the end of the incubation period the slices were separated from the media by filtration on PVC-covered fibre glass nets placed upon cotton. Slices were transferred to another medium by submerging of nets plus slices. Otherwise the slices were weighed (wet wt.) immediately after the separation procedure.

Measurement of radioactivity. Samples of tissue and medium were prepared for radioactivity measurements in a Packard Tri-Carb liquid scintillation spectrometer (model 3375) as previously described.[2]

The results were expressed as the slice-to-medium (S/M) concentration ratio of ^{14}C, which was calculated as the counting rate per gram slice (post incubation wet wt.)/counting rate per millilitre medium.

The difference between S/M ratio in control experiment (decamethonium alone) and experiment with decamethonium plus another drug (a monoquaternary ammonium compound or iodoacetate) was expressed as per cent of control value. The difference between S/M ratio in cortical and medullary slices (Table 1) was expressed as per cent of the S/M ratio value found in cortical slices. The significance of all differences was estimated by Student's *t*-test.[12]

The water content (expressed as per cent of total wet wt.) was determined in freshly

prepared slices (one kidney) as well as in slices incubated for 1 hr (the other kidney) by drying the tissue at 105° for 24 hr. The water content increased from 77·8 ± 0·6 before incubation to 81·4 ± 0·5 after incubation (Mean ± S.E.M. of values from six experiments). The percentage increase in water content differed significantly from zero (4·6 ± 1·3, $P < 0·05$).[12]

Preparation of chromatograms. Following incubation (1 hr) with [^{14}C]decamethonium (2×10^{-6} M) protein-free kidney extracts were prepared as described,[2] and chromatographed on paper by the method previously described.[11] Two different systems were used as mobile phase; (a) *n*-butanol, ethanol, glacial acetic acid, water (8:2:1:3). (b) pyridine, *n*-butanol, water (3:2:3).

RESULTS

Deca and hexamethonium uptake by cortical and medullary slices

Table 1 shows the uptake of [^{14}C]decamethonium (2×10^{-6} M) and [^{14}C]hexamethonium (3×10^{-6} M) by cortical and medullary slices (preparation: see Methods). The uptake was expressed as the slice-to-medium (S/M) concentration ratio after incubation for 1 hr.

It is seen that both cortical and medullary slices take up decamethonium against a concentration gradient, but that the decamethonium concentration in cortical slices is more than twice as high as that in medullary slices. There is no sex difference with regard to decamethonium uptake. There is a considerable difference between the two methonium compounds deca–hexamethonium regarding their renal uptake. The concentration of decamethonium in cortical slices is thus 5–6 times higher than that of the medium, whereas the concentration of hexamethonium in cortical slices does not exceed that of the medium. The concentration of hexamethonium in cortical slices is only slightly higher than that in medullary slices.

Decamethonium uptake under aerobic and anaerobic conditions with varying concentrations of decamethonium in the medium

The uptake of [^{14}C]decamethonium (10^{-4}, 5×10^{-4} and 10^{-3} M) was measured in an atmosphere of oxygen–carbon dioxide as well as in an atmosphere of nitrogen–carbon dioxide. The uptake was expressed as the slice-to-medium (S/M) concentration ratio after incubation for 1 hr.

The results of these experiments (Table 2) show that S/M ratio under aerobic conditions decreases with increasing concentrations of decamethonium in the medium. S/M ratio is thus around 4 with 10^{-4} M decamethonium in medium, but only around 2 with 10-fold higher concentration of decamethonium in the medium. Under anaerobic conditions S/M ratio is around 1, and does not decrease when raising the external decamethonium concentration.

Effect of iodoacetate on decamethonium uptake. Table 3 shows the effect of iodoacetate on the uptake of [^{14}C]decamethonium (2×10^{-6} M). The uptake was expressed as the slice-to-medium (S/M) concentration ratio after incubation for 1 hr. It is seen that decamethonium uptake is significantly reduced to one-third of the control value in the presence of 10^{-3} M iodoacetate.

TABLE 3. EFFECT OF IODOACETATE (10^{-3} M) ON THE UPTAKE (S/M RATIO AFTER 1 HR INCUBATION) OF [^{14}C]DECAMETHONIUM (2×10^{-6} M) BY RAT KIDNEY CORTEX SLICES

S/M ratio		
Control	Iodoacetate added	Difference (%)
4·7 ± 0·2	1·47 ± 0·03	−68 ± 1*

Slices were incubated either with or without (control) iodoacetate as stated in text. Results are given as the mean of values from six experiments ± S.E.M.
* $P < 0.001$.

Paperchromatographic results. Protein-free extracts from kidney slices, which had been incubated (1 hr) in an atmosphere of oxygen–carbon dioxide with [^{14}C]decamethonium (2×10^{-6} M), were subjected to paperchromatography using the two systems described in the method section. The radioactivity of the chromatograms could not be distinguished from that of authentic [^{14}C]decamethonium.

Effect of carbamoylcholine on decamethonium uptake. Table 4 shows the effect of carbamoylcholine on the uptake of [^{14}C]decamethonium (2×10^{-6} M). The uptake was expressed as slice-to-medium (S/M) concentration ratio after incubation for 1 hr.

TABLE 4. EFFECT OF CARBAMOYLCHOLINE ON THE UPTAKE (S/M RATIO AFTER 1 HR INCUBATION) OF [^{14}C]DECAMETHONIUM (2×10^{-6} M) BY RAT KIDNEY CORTEX SLICES

		S/M ratio		
No. of exp.	Carbamoylcholine conc. (M)	Control	Carbamoylcholine added	Difference (%)
6	10^{-5}	4·7 ± 0·3	5·1 ± 0·2	+11 ± 7
6	10^{-4}	5·4 ± 0·3	6·6 ± 0·5	+22 ± 5*
9	3×10^{-4}	5·4 ± 0·1	7·7 ± 0·5	+45 ± 10†
6	10^{-3}	4·4 ± 0·2	7·2 ± 0·3	+63 ± 10†
6	3×10^{-3}	4·2 ± 0·3	7·5 ± 0·5	+77 ± 4‡
6	5×10^{-3}	5·0 ± 0·3	8·3 ± 0·5	+67 ± 4‡
9	10^{-2}	5·1 ± 0·2	6·9 ± 0·5	+35 ± 10*
6	$1{\cdot}8 \times 10^{-2}$	5·0 ± 0·3	5·9 ± 0·4	+21 ± 13
6	$3{\cdot}4 \times 10^{-2}$	4·2 ± 0·2	4·2 ± 0·2	0 ± 5
8	5×10^{-2}	5·0 ± 0·1	3·8 ± 0·2	−24 ± 3‡
9	8×10^{-2}	4·7 ± 0·3	3·2 ± 0·3	−32 ± 5‡

Slices were incubated either with or without (control) carbamoylcholine as stated in text. Results are given as mean values ± S.E.M.
* $P < 0.01$.
† $P < 0.005$.
‡ $P < 0.001$.

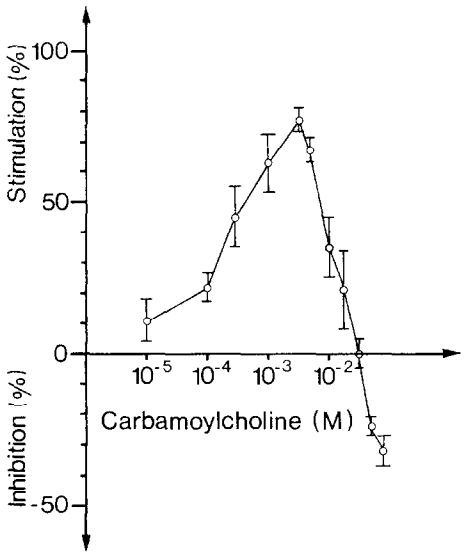

FIG. 1. Percentage stimulation and inhibition (mean values from Table 4) of decamethonium (2×10^{-6} M), uptake by rat kidney cortex slices as a function of log carbamoylcholine concentration in medium. Vertical bars indicate S.E.M.

Carbamoylcholine at a concentration of 10^{-5} M had no statistically significant effect on decamethonium uptake. A significant and increasing stimulation of decamethonium uptake was seen, when carbamoylcholine concentration in medium was raised from 10^{-4} to 3×10^{-3} M. The uptake was stimulated to the highest extent (80 per cent) in the presence of 3×10^{-3} M carbamoylcholine. However, stimulation was seen to decrease, when carbamoylcholine concentration was raised from 3×10^{-3} to $3{\cdot}4 \times 10^{-2}$ M. The uptake was significantly increased in the presence of 5×10^{-3} M (70 per cent) and 10^{-2} M (40 per cent) carbamoylcholine, whereas neither $1{\cdot}8 \times 10^{-2}$, nor $3{\cdot}4 \times 10^{-2}$ M carbamoylcholine had any significant effect on uptake. A significant and increasing inhibition of decamethonium uptake was seen, when carbamoylcholine concentration was raised from $3{\cdot}4 \times 10^{-2}$ to 8×10^{-2} M. The uptake was thus reduced by one-fourth in the presence of 5×10^{-2} M carbamoylcholine and by one-third in the presence of 8×10^{-2} M carbamoylcholine.

In Fig. 1 the mean percentage differences (stimulation and inhibition) from Table 4 have been plotted against log carbachol concentration.

Figure 1 shows that the effect of carbamoylcholine on 1 hr uptake of decamethonium (2×10^{-6} M) is log dose dependent. Both increase and decrease in percentage stimulation of decamethonium uptake seem to be linear functions of log carbamoylcholine concentration. Furthermore, the increase in percentage inhibition of uptake seems to be a linear function of log carbamoylcholine concentration with a slope very similar to that of the descending part of the stimulation curve.

Effect of carbamoylcholine on decamethonium uptake with varying concentrations of decamethonium in the medium

Table 5 shows the effect of carbamoylcholine (3×10^{-3} M) on the uptake of [^{14}C]decamethonium (10^{-4} and 10^{-3} M). The uptake was expressed as slice-to-

medium (S/M) concentration ratio after incubation for 1 hr. The presence of carbamoylcholine is seen to increase the uptake by two-thirds with 10^{-4} M decamethonium in medium, but only by one-sixth when the decamethonium concentration in medium is 10-fold higher.

TABLE 5. EFFECT OF CARBAMOYLCHOLINE (3×10^{-3} M) ON THE UPTAKE (S/M RATIO AFTER 1 HR INCUBATION) OF [^{14}C]DECAMETHONIUM (10^{-4} AND 10^{-3} M) BY RAT KIDNEY CORTEX SLICES

Decamethonium conc. (M)	S/M ratio		Difference (%)
	Control	Carbamoylcholine added	
10^{-4}	3·3 ± 0·2	5·4 ± 0·2	+66 ± 12*
10^{-3}	2·0 ± 0·1	2·3 ± 0·1	+17 ± 5†

Slices were incubated either with or without (control) carbamoylcholine as stated in text. Results are given as the mean of values from six experiments ± S.E.M.
* $P < 0.005$.
† $P < 0.05$.

Effect of choline, neostigmine and tetraethylammonium on decamethonium uptake

Table 6 shows the effect of choline, neostigmine and tetraethylammonium on the uptake of [^{14}C]decamethonium (2×10^{-6} M). The uptake was expressed as slice-to-medium (S/M) concentration ratio after incubation for 1 hr.

TABLE 6. EFFECT OF CHOLINE, NEOSTIGMINE AND TETRAETHYLAMMONIUM ON THE UPTAKE (S/M RATIO AFTER 1 HR INCUBATION) OF [^{14}C]DECAMETHONIUM (2×10^{-6} M) BY RAT KIDNEY CORTEX SLICES

No. of exp.	Drug	Conc. of drug (M)	S/M ratio		Difference (%)
			Control	Drug added	
6	Choline	10^{-3}	5·3 ± 0·1	9·2 ± 0·4	+77 ± 10*
9	Choline	3×10^{-3}	4·8 ± 0·2	8·9 ± 0·4	+89 ± 11*
9	Choline	5×10^{-2}	6·0 ± 0·2	4·2 ± 0·1	−30 ± 3*
6	Neostigmine	10^{-4}	4·8 ± 0·2	6·7 ± 0·3	+40 ± 3*
6	Neostigmine	10^{-3}	4·9 ± 0·4	5·3 ± 0·4	+11 ± 13
6	Neostigmine	10^{-2}	5·2 ± 0·2	2·4 ± 0·1	−53 ± 1*
7	Tetraethylammonium	10^{-4}	4·8 ± 0·4	9·4 ± 0·6	+98 ± 17†
7	Tetraethylammonium	10^{-3}	4·9 ± 0·3	5·5 ± 0·5	+14 ± 10
7	Tetraethylammonium	10^{-2}	4·6 ± 0·3	2·4 ± 0·1	−47 ± 3*

Slices were incubated either with or without (control) drug as stated in text. Results are given as mean values ± S.E.M.
* $P < 0.001$.
† $P < 0.005$.

Decamethonium uptake is seen to be significantly increased in the presence of 10^{-3} and 3×10^{-3} M choline, 10^{-4} M neostigmine and 10^{-4} M tetraethylammonium.

The presence of either 10^{-3} M neostigmine or 10^{-3} M tetraethylammonium had no significant effect on uptake. The above monoquaternary ammonium compounds inhibited decamethonium uptake, when they were present in medium at relatively high concentrations. Neostigmine and tetraethylammonium were more potent inhibitors than choline, as the former two agents at a concentration of 10^{-2} M halved decamethonium uptake, whereas the uptake was only reduced by one-third in the presence of 5×10^{-2} M choline.

Initial decamethonium uptake by slices preloaded with carbamoylcholine, choline, neostigmine or tetraethylammonium

Table 7 shows the initial [^{14}C]decamethonium uptake by slices incubated for 90 min (preloaded) with a monoquaternary ammonium compound before transfer to a final medium, which only contained [^{14}C]decamethonium (2×10^{-6} M). The uptake was expressed as slice-to-medium (S/M) concentration ratio after incubation for 5 min. It is seen that preloading with carbamoylcholine (3×10^{-3} M), choline (3×10^{-3} M), neostigmine (2×10^{-4} M) and tetraethylammonium (2×10^{-4} M) significantly increases initial decamethonium uptake.

TABLE 7. INITIAL UPTAKE (S/M RATIO AFTER 5 MIN INCUBATION) OF [^{14}C]DECAMETHONIUM (2×10^{-6} M) BY RAT KIDNEY CORTEX SLICES PRELOADED WITH CARBAMOYLCHOLINE, CHOLINE, NEOSTIGMINE AND TETRAETHYLAMMONIUM

No. of exp.	Drug	S/M ratio		Difference (%)
		Control	Preloaded with drug	
9	Carbamoylcholine (3×10^{-3} M)	0·95 ± 0·04	1·22 ± 0·03	+30 ± 5*
9	Choline (3×10^{-3} M)	1·00 ± 0·03	1·22 ± 0·03	+23 ± 5†
8	Neostigmine (2×10^{-4} M)	0·92 ± 0·03	1·19 ± 0·04	+29 ± 3*
11	Tetraethylammonium (2×10^{-4} M)	0·93 ± 0·03	1·15 ± 0·04	+25 ± 6†

Slices were preincubated either with (preloaded) or without (control) drug for 90 min as stated in text. Results are given as mean values ± S.E.M.

* $P < 0·001$.

† $P < 0·005$.

Efflux of decamethonium. Slices were incubated (1 hr) with 2×10^{-6} M [^{14}C]-decamethonium, and then transferred to another medium (20 ml) containing 2×10^{-6} M unlabelled decamethonium. Following incubation for 30 min in the latter medium the [^{14}C] S/M ratio was found to be 3·8 ± 0·3 (the mean ± S.E.M. of values from six experiments), using the [^{14}C] concentration in the first medium as a reference. This is practically the same S/M ratio value as that found after incubation for 1 hr with 2×10^{-6} M [^{14}C]decamethonium (Tables 4 and 6), when it is presumed that [^{14}C]decamethonium has been washed out of the extracellular space during the second incubation period.

The size of the extracellular space of the slices was estimated by determining the slice-to-medium concentration ratio (1 hr of incubation) of [^{14}C]inulin (10^{-6} M), which was 0·40 ± 0·01 (mean value ± S.E.M. from six experiments).

DISCUSSION

The present results show that decamethonium is taken up in rat kidney cortex slices against an apparent concentration gradient, whereas this is not the case with hexamethonium. The same observation was made by Mc Isaac,[8] who also investigated the uptake of these two methonium compounds in rat kidney cortex slices. There is a particularly high accumulation of decamethonium in the cortical areas of the kidney (Table 1), which suggests that the transport system for decamethonium has its main localization in the tubule cells of the cortex. Decamethonium uptake shows saturation under aerobic conditions, as S/M ratio decreases with increasing concentration of decamethonium in medium (Table 2). Under anaerobic conditions however, the uptake seems to be directly proportional to the external decamethonium concentration with a practically constant S/M ratio value around 1 (Table 2). Furthermore, decamethonium uptake is a metabolically dependent process, as it is depressed under anaerobic conditions (Table 2) and in the presence of the metabolic inhibitor iodoacetate (Table 3). Our paperchromatographic results indicate that decamethonium does not undergo any metabolic transformation in the tissue.

The monoquaternary ammonium compounds carbamoylcholine, choline, neostigmine and tetraethylammonium have two effects on 1 hr uptake of decamethonium (2×10^{-6} M); at relatively low concentrations these four compounds stimulate decamethonium uptake and at higher concentrations they inhibit it (Tables 4 and 6). The percentage stimulation of 1 hr decamethonium uptake in the presence of carbamoylcholine decreases with increasing decamethonium concentration in medium (Table 5). The increased initial decamethonium uptake by slices preloaded with carbamoylcholine, choline, neostigmine or tetraethylammonium (Table 7) suggests that the stimulation is related to the presence of these monoquaternary ammonium compounds in the slices. An explanation for the increase in initial net uptake (influx–efflux) of decamethonium might be that the above monoquaternary ammonium compounds, when they are present in the slices, compete with decamethonium for a common efflux mechanism. However, this assumption is not valid, as no decamethonium efflux was shown to occur from slices preloaded with decamethonium (2×10^{-6} M).

A number of papers have appeared in recent years reporting accelerated flux phenomena in association with transport of hexoses and amino acids. For instance, Levine, Oxender and Stein,[13] who studied the efflux of [^{3}H]glucose from human erythrocytes into a medium containing varying concentrations of unlabelled glucose, found an increase in the rate of efflux of [^{3}H]glucose as the external glucose level was increased. Furthermore, the rate of [^{3}H]glucose exit into a galactose medium was higher than that into a galactose-free medium. These results were interpreted as examples of substrate-facilitated carrier transport or accelerative exchange diffusion, which means that the rate of movement across the membrane of the substrate-carrier complex is supposed to be greater than that of the free carrier. The flux of a substrate in one direction (influx of unlabelled glucose or galactose) should thus accelerate the flux of the same or another substrate in the opposite direction (efflux of [^{3}H]glucose) by increasing the rate of return of the carrier (as a substrate-carrier complex) from external to internal side or vice versa. Heinz and Walsh,[14] who studied the uptake of [^{14}C]glycine by Ehrlich mouse ascites carcinoma cells, found that preloading the cells with unlabelled glycine accelerated influx of [^{14}C]glycine. A similar effect on [^{14}C]-

glycine influx was obtained by preloading the cells with *N*-methylglycine, whereas this amino acid if present in external medium depressed influx of [^{14}C]glycine.

The stimulating effect of carbamoylcholine, choline, neostigmine and tetraethylammonium on decamethonium uptake is most likely to be interpreted as an example of accelerative exchange diffusion or substrate-facilitated carrier transport, which should mean that efflux of these monoquaternary ammonium compounds accelerates decamethonium influx. This interpretation is consistent with the conclusion from the experiments on mutual inhibition that these monoquaternary ammonium compounds and the polymethylene–bisquaternary ammonium compound decamethonium share a common carrier mechanism in the rat kidney. It should be mentioned that the above monoquaternary ammonium compounds were also shown to stimulate and inhibit decamethonium uptake by mouse kidney slices.[5–7]

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REFERENCES

1. L. PETERS, *Pharmac. Rev.* **12,** 1 (1960).
2. J. HOLM, *Acta Pharmac. Tox.* **28,** 192 (1970).
3. J. HOLM, *Acta Pharmac. Tox.* **28,** suppl. 1, 51 (1970).
4. J. HOLM, *Acta Pharmac. Tox.* **29,** suppl. 4, 21 (1971).
5. J. HOLM, *Biochem. Pharmac.* **20,** 2983 (1971).
6. J. HOLM, *Acta Pharmac. Tox.* **30,** 81 (1971).
7. J. HOLM, *Acta Pharmac. Tox.* **30,** 89 (1971).
8. R. J. MCISAAC, *J. Pharmac. exp. Ther.* **168,** 6 (1969).
9. C. P. SUNG and R. M. JOHNSTONE, *Can. J. Biochem.* **43,** 111(965).
10. C. P. SUNG and R. M. JOHNSTONE, *Biochim. biophys. Acta.* **173,** 548 (1969).
11. C. BROEN CHRISTENSEN and J. HOLM, *Acta Pharmac. Tox.* **27,** 17 (1969).
12. A. BRADFORD HILL, *Principles of Medical Statistics, Lancet,* 149 (1966).
13. M. LEVINE, D. L. OXENDER and W. D. STEIN, *Biochim. biophys. Acta* **109,** 151 (1965).
14. E. HEINZ and P. WALSH, *J. biol. Chem.* **233,** 1488 (1958).