depletion and that the usually prescribed doses might result in accumulation of toxic levels of the unconjugated drug.

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Transport and binding of decamethonium in mouse kidney slices

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MANY quaternary ammonium compounds are concentrated in renal slices by specialized transport processes, in many respects similar to those involved in the tubular secretion of these substances by the intact kidney.[1] The polymethylene-bisquaternary ammonium compounds, hexamethonium (C_6) and decamethonium (C_{10}) are concentrated in kidney cortex slices in various species: C_6 and C_{10} in chickens, C_6 in cats and C_{10} in rats and mice.[2–4] It was a characteristic result in these experiments, that slice-to-medium concentration ratio of C_6 or C_{10} continued to increase linearly over a period of time without reaching a steady state level even after an incubation of several hours.[2–4] The continued accumulation of C_6 and C_{10} with time indicates that efflux of these two methonium compounds from renal slices is very slow, as compared to influx. Recent results from our laboratory, showing that no C_{10} efflux occurs from mouse and rat kidney slices preloaded with C_{10}, confirm this suggestion.[5,6] McIsaac[3] has suggested that methonium compounds are bound to a specific carrier, which is capable of transporting quaternary ammonium compounds into renal tubule cells. The continued accumulation of methonium compounds, should, according to McIsaac,[3] be due to a strong binding of these substances to some intracellular structure. If the latter hypothesis is correct, a steady state ratio (influx equals efflux) between the concentration of methonium compound in renal slices and in external medium would not be obtained until equilibrium has been established between bound and unbound (freely diffusible) methonium compound in the tissue. The above-mentioned studies were only performed with relatively low (10^{-5} M or less) C_6 or C_{10} concentrations in external media.[2–6] In the present study C_{10} uptake by mouse kidney slices was investigated with a high C_{10} concentration in

external medium, as equilibrium between unbound and bound C_{10} under these conditions would be expected to occur within a relatively short period of time.

Male albino mice of a single strain (NMRI) with a body weight of 28–32 g were decapitated and bled. Kidney slices were prepared and used as previously described.[4,5] Slices (total wet wt, 150 mg) were incubated at 37°, pH 7·4, aeration: 95% O_2 and 5% CO_2, in 20 ml Krebs–Ringer bicarbonate solution with glucose (11 mM/l.). [^{14}C]Methyldecamethonium dibromide (Radiochemical Centre, Amersham, England) with a sp. act. of 15 mCi/mM was added to the incubation medium (0·3 μCi) together with varying amounts of unlabelled decamethonium diiodide (Koch–Light Laboratories Ltd., England). In the experiments recorded in Fig. 1 each test tube contained only kidney slices from the same animal (both kidneys). Each of the remainder of the experiments was carried out as a paired

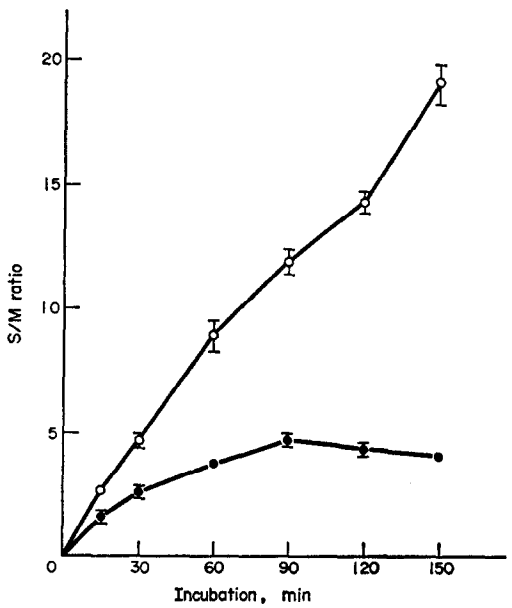

FIG. 1. [^{14}C]Decamethonium uptake by mouse kidney slices as a function of the duration of incubation (min). The [^{14}C]-slice-to-medium concentration ratio (S/M ratio) was measured with 5×10^{-5} M (○) or 5×10^{-4} M (●) decamethonium in the medium. Each circle represents the mean value of six experiments with S. E. M. (vertical bars for values higher than $\pm$ 0·1).

comparison with kidney tissue from two animals. Slices from two kidneys, one kidney from each animal, were thus placed in each test tube, this procedure making it possible to incubate tissue from each animal (distributed in two test tubes) simultaneously under different experimental conditions (control or noncontrol conditions). Each animal thus served as its own control; method of paired comparison.[7] At the end of the incubation period the slices were separated from the media by filtration on PVC-covered fibre glass nets placed on cotton. Slices were transferred to another medium by submerging the nets plus slices. Otherwise, the slices were weighed (wet weightt) immediately after the separation procedure. Samples of tissue and medium were prepared for radioactivity measurements in a Packard Tri-Carb liquid scintillation spectrometer (model 3375) as previously described.[4,5] The results were expressed as the slice-to-medium (S/M) concentration ratio of [^{14}C], which was calculated as:

$$\frac{\text{Counting rate per gram slice (post incubation wet wt)}}{\text{Counting rate per millilitre medium.}}$$

Paperchromatographic studies have previously shown that [^{14}C]C_{10} does not undergo any metabolic transformation in mouse kidney slices.[4] The difference between S/M ratio in control experiment and noncontrol experiment was expressed as per cent of control value. The significance of these differences was estimated by the Student's *t*-test.[7]

Time course of decamethonium uptake. Figure 1 shows that C_{10} slice-to-medium (S/M) concentration ratio has already reached a steady state value around 4 after incubation for 60–90 min with 5×10^{-4} M C_{10} in external medium. However, with a 10-fold lower C_{10} concentration (5×10^{-5} M) S/M ratio increases almost linearly with time during 150 min without reaching a steady state.

Efflux of decamethonium. Slices from each animal were distributed in two test tubes and incubated for 1 hr with [^{14}C]C_{10} (5×10^{-4} M). At the end of the incubation period tissue in one test tube was separated from medium and prepared for analysis (control), whereas slices in the other test tube were transferred to another medium (20 ml) containing 5×10^{-4} M unlabelled C_{10}. Following incubation for 15 min in the latter medium, the [^{14}C]C_{10} S/M ratio was found to be $2{\cdot}3 \pm 0{\cdot}1$ (the mean ± S.E.M. of values from six experiments) using the [^{14}C]C_{10} concentration in the first medium as a reference (no [^{14}C]-activity could be measured in the second incubation medium, as the bulk of the medium was more than 100-times larger than that of the slices). This S/M ratio value differs significantly (31 ± 5 per cent, $P < 0{\cdot}005$) from that found in control slices, namely $3{\cdot}4 \pm 0{\cdot}2$. When correction had been made for the wash-out of [^{14}C]C_{10} from extracellular space (inulin space in mouse kidney slices was 0·4),[5] it can be roughly estimated that one-fourth of the [^{14}C]C_{10} amount accumulated in the tissue was released during the second incubation period. No C_{10} efflux was shown to occur from mouse kidney slices preloaded with a low C_{10} concentration in medium.[5] This suggests that C_{10} when present in the slices at a low concentration does not exist as a freely diffusible ion. As shown in Fig. 1, a steady state can be obtained with a high C_{10} concentration in a medium. At a steady state efflux equals influx, which should mean that C_{10} when present in the slices at a high concentration is at least to some extent releasable and freely diffusible. The present data showing a considerable C_{10} efflux from slices preloaded with a high C_{10} concentration in medium confirms this suggestion.

Exchange between decamethonium in slices and decamethonium in medium. The exchange between [^{14}C]C_{10} accumulated in mouse kidney slices and [^{14}C]C_{10} in external medium was investigated (Table 1). In B experiment slices were preincubated with [^{14}C]-labelled C_{10}, whereas slices in A experiment (control experiments) were preincubated with unlabelled C_{10} at the same concentration. With

TABLE 1. EXCHANGE BETWEEN [^{14}C]DECAMETHONIUM ACCUMULATED IN MOUSE KIDNEY SLICES AND [^{14}C]DECAMETHONIUM IN EXTERNAL MEDIUM

[^{14}C]-Slice-to-medium concentration ratio		
A	B	
Preincubated (1 hr) with 2×10^{-6} M decamethonium	Preincubated (1 hr) with 2×10^{-6} M [^{14}C]decamethonium	Difference (%)
$3{\cdot}9 \pm 0{\cdot}1$	$7{\cdot}5 \pm 0{\cdot}4$	90 ± 9*

Slices were preincubated (1 hr) either with 2×10^{-6} M decamethonium (A) or with 2×10^{-6} M [^{14}C]decamethonium, (B) before transfer to a medium containing 5×10^{-4} M [^{14}C]decamethonium. [^{14}C]-slice-to-medium concentration ratio was measured after incubation for 90 min in the latter medium. Results are given as the mean of values from six experiments ± S. E. M.

* $P < 0{\cdot}001$.

this exception the two types of experiments were performed in the same way (cf. Table 1). However, [^{14}C] S/M ratio in B experiment was almost twice as high as in A experiment. This means that in B experiment half the final [^{14}C]-activity in the slices derives from [^{14}C]C_{10}, which has remained in the tissue from the preincubation period. The [^{14}C]C_{10} amount from the preincubation period can only represent a C_{10} concentration of 7×10^{-6} M ($3{\cdot}6 \times 2 \times 10^{-6}$ M) as calculated from the difference between [^{14}C] S/M ratios in A and B experiments (7·5–3·9) and the [^{14}C]C_{10} concentration in the preincubation medium (2×10^{-6} M). In other words, the amount of [^{14}C]C_{10} deriving from the preincubation period represents half part of the final [^{14}C]-activity in the tissue, but less than half per cent of the final C_{10} amount. The final C_{10} concentration in the slices was 2×10^{-3} M ($3{\cdot}9 \times 5 \times 10^{-4}$ M) as calculated from the S/M ratio in A experiment (3·9) and the C_{10} concentration in the final medium (5×10^{-4} M). The data in Table 1 thus shows that the exchange between [^{14}C]C_{10} accumulated in the slices during the preincubation period (low C_{10} concentration; high degree of [^{14}C]-labelling) and [^{14}C]C_{10} in the second medium (high C_{10} concentration; low degree of [^{14}C]-labelling) has not been completed even after 90 min incubation. This extremely slow rate of exchange between

internal and external C_{10} is consistent with the assumption that C_{10} is strongly, "irreversibly", bound to some intracellular structure.

Effect of carbamoylcholine on the rate of uptake and steady state concentration ratio of decamethonium. The quaternary ammonium compounds C_{10} (polymethylene-bisquaternary) and carbamoylcholine (monoquaternary), which are concentrated in mouse kidney slices by a saturable metabolically dependent transport mechanism,[4,8] can mutually inhibit each others' transport.[5,8] When added to an external medium at a relatively low concentration, carbamoylcholine was also able to stimulate C_{10} uptake.[5] Initial C_{10} uptake was increased in slices preloaded with carbamoylcholine, which suggests that stimulation was related to the presence of carbamoylcholine in the slices.[5] Stimulation of C_{10} (net) uptake could not be attributed to a competitive inhibition of C_{10} efflux by internal carbamoylcholine, as no C_{10} efflux was shown to occur from slices preloaded with C_{10}. C_{10} Uptake thus represents a true influx, which is accelerated when carbamoylcholine is present in the tissue. A number of papers have appeared in recent years reporting accelerated flux phenomena in association with transport of hexoses and amino acids, Levine, Oxender and Stein,[9] 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,[10] 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 on decamethonium uptake is most likely to be interpreted as an example of accelerative exchange diffusion, which should mean that carbamoylcholine efflux accelerates decamethonium influx. This interpretation is consistent with the conclusion from the experiments on mutual inhibition that decamethonium and carbamoylcholine share a common carrier-mediated transport mechanism in mouse kidney slices.[5,8] According to our interpretation of the stimulation phenomenon carbamoylcholine should only be expected to increase the rate of C_{10} uptake, but not to increase the final steady state ratio between C_{10} concentration in tissue and medium. However, the above stimulation experiments were only performed with a relatively low concentration (2×10^{-6} M) of C_{10} in external medium,[5] and C_{10} concentration ratio therefore continued to increase linearly with time without reaching a steady state level even after incubation for several hours.[4] We thus found it of interest to investigate the effect of carbamoylcholine (10^{-3} M) on C_{10} uptake with 5×10^{-4} M C_{10} in medium, as a steady state can be reached with this high concentration of C_{10} (Fig. 1). Table 2 shows that carbamoylcholine (carbamoylcholine chloride, Ph. Nord. 1963) significantly increases the 15 min S/M ratio value, which is placed on the ascending part of the uptake curve (Fig. 1), whereas carbamoylcholine does not increase 90 min S/M ratio, which is placed on the steady state level of the curve (Fig. 1). On the contrary, there is a slight but statistically significant inhibition of the steady state ratio in the presence of carbamoylcholine.

TABLE 2. EFFECT OF CARBAMOYLCHOLINE (10^{-3} M) ON THE UPTAKE (S/M RATIO) OF [^{14}C]DECAMETHONIUM (5×10^{-4} M) BY SLICES OF MOUSE KIDNEY

No. exp.	Incubation period (min)	S/M ratio		Difference (%)
		Control	Carbamoylcholine added	
7	15	1·6 ± 0·1	2·1 ± 0·1	+33 ± 8*
6	90	4·4 ± 0·2	3·8 ± 0·1	−14 ± 3*

Slices were incubated either with or without (control) carbamoylcholine as stated in text. Carbamoylcholine was added to medium 30 min before [^{14}C]decamethonium. Mean values ± S. E. M. are given.

* $P < 0{\cdot}01$.

Conclusion. The present data and previous findings[3–6,8] are consistent with the assumption (1) that C_{10} is transported into renal cells by a carrier mechanism capable of transporting quaternary ammonium compounds (for instance carbamoylcholine); and (2) that C_{10} once inside the cell is strongly bound to some intracellular structure.

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Effect of suramin (Bayer 205) on hepatic microsomal cytochromes P-450, b_5, and demethylation activity in rats

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SURAMIN is commonly used for protecting an individual against trypanosomiosis (sleeping sickness) because of its powerful trypanocidal action.[1] It is the symmetrical 3″-urea of the sodium salt of 1-(3-benzamido-4-methylbenzamido)-napthalene-4,6,8-trisulphonic acid which was first synthesized in 1922.[2] The precise mode of action of suramin on trypanosomes is not yet clear. It is possible that the suramin combines with the plasma protein, which may then gradually be released in the blood stream and pass into trypanosomes in amounts sufficient to inactivate essential enzymes of the trypanosomes.[3] However, a prolonged latent period of contact of suramin and trypanosomes before the trypanocidal effect is manifested, has been reported by the earlier workers.[4] In addition to plasma protein, various body tissues in animals, such as kidneys, spleen, liver, heart, brain and adrenals have also been found to bind suramin for a considerable period after administration.[5] It seems probable that the long retention of suramin in the plasma and tissues without being metabolized[5] accounts for the very satisfactory prophylactic value of this drug as far as sleeping sickness is concerned. However, a knowledge which would be useful is whether suramin retention in the body can impair detoxication mechanisms. If suramin retention decreases the efficiency of detoxication mechanisms, then it may be supposed that toxic manifestations might occur in suramin treated persons.

The present work was undertaken to investigate the effect of suramin on the NADPH-dependent oxidative demethylation of various drugs, e.g. ethylmorphine and the concentrations of cytochrome b_5 and cytochrome P-450 which are probably involved in liver microsomal drug-hydroxylating reactions.[6] This study is merited because suramin is retained in the liver, where it might interact with the drug-metabolizing enzymes.

Weanling Sprague–Dawley rats were used throughout and maintained on a stock pellet diet (Diet 41B) and water *ad lib.* The rats were divided into four groups: group A was given 0·95% saline and served as controls, groups B, C and D were treated with suramin (20 mg/kg), phenobarbital sodium (100 mg/kg) and suramin plus phenobarbital, respectively 24 hr before the animals were