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THE EFFECTS OF MERCAPTOMERIN ON THE WATER AND CATION EXCHANGES IN SLICES OF RAT TISSUE

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In a previous paper (Maizels & Remington, 1958) the penetration of sulphate into slices of kidney cortex incubated in a sulphate medium without and with mercaptomerin was considered. The nature of the Na and K changes in these experiments was obscured by the fact that slices were transferred from a leaching medium containing 150 mM-NaCl and no K, to an incubation medium containing (m-equiv/l.) Na 190, K 6 and sulphate 183. Hence, not only was the external base concentration altered at the start of incubation, but in addition sulphate slowly diffused into the tissue throughout the period of observation. For this reason a number of observations are recorded in the present investigation, where cortex slices were incubated and leached in simple chloride media, and changes in Na, K and water measured in the presence and absence of mercurial diuretics. Mudge (1951) and Kleinzeller & Cort (1957) have already investigated this problem, but their observations were made at 25° C. In the present work observations were made at 37° C, and it was found that the results varied somewhat with the temperature of incubation and with the mercurial diuretic used. It was not considered that the findings *in vitro* had any close bearing on the action of the mercurial diuretics *in vivo*.

METHODS

Cortex slices were leached in a simple NaCl medium, usually 150 mM, but sometimes, following Kleinzeller & Cort (1957), 154 mM. With the vessel containing the slices standing on a tray of ice the temperature of the medium was 8–10° C, whereas when immersed in ice-cold water the temperature was about 3° C. After leaching, slices were incubated in Robinson's (1956) NaCl medium containing (mM) NaCl 130, KCl 5, $CaCl_2$ 2·5, $MgSO_4$ 1, sodium phosphate at pH 7·4 8 and glucose 5·6. Frequently 5 m-mole α-oxoglutarate was added per litre of medium, and though when this was omitted cation movements were less vigorous, they were still of considerable magnitude (Table 1).

Methods and standard errors of Na, K and water estimations are the same as in the previous paper (Maizels & Remington, 1958). The lithium medium was prepared as follows: 2 g human serum albumin was dialysed against water and then made up to about 90 ml. with 155 mM LiCl. 0·5 ml. 250 mM $CaCl_2$, 0·5 ml. 100 mM $MgCl_2$ and 1 ml. 400 mM H_3PO_4 were added and the pH

brought to 7·4 with a saturated solution of Li_2CO_3, the medium being shaken at 37° C to assist escape of CO_2. Finally the volume was made up to 100 ml. with the LiCl solution. Phosphate in the LiCl medium was reduced to minimize the risk of precipitating lithium phosphate: albumin served the same purpose and also increased the buffering power of the medium. The latter contained about 160 m-equiv Li, a few m-equiv Na and about 1 m-equiv K/l., the amount of the two last constituents depending on the duration of dialysis of the albumin.

After incubation in the LiCl medium, slices were dried and ashed, but owing to the insolubility of the lithium salts (presumably phosphate or pyrophosphate) the residue was extracted as follows: 0·2 ml. H_2SO_4 (5%, v/v) was added, the water driven off at 105° C, and the residual traces of acid containing the various salts in solution were appropriately diluted with water. Li was estimated with a flame photometer using a suitable light filter. The standard error was ±9·9 m-equiv/kg dry wt for 24 duplicate or triplicate observations.

RESULTS

Effects of incubating slices of renal cortex in NaCl media

Mercaptomerin. If cortex slices of rat kidney are leached for 15–30 min in cool NaCl medium, and then incubated at 37° C in a similar medium containing K and glucose but no mercaptomerin, loss of Na and gain of K occur against the concentration gradients, loss of Na exceeding gain of K with a consequent

TABLE 1. Effects of mercurial diuretics, BAL and iodoacetate on the Na, K and water contents of slices of renal cortex leached for 20–30 min in NaCl solution at 8° C, and then incubated in a nutrient medium (mM) NaCl 130, KCl 5, $CaCl_2$ 2·5, $MgSO_4$ 1, sodium phosphate at pH 7·4 8, glucose 5·6

Expt.	Agent added (m-mole/l. medium)	Time (min at 37° C)	Water (g/kg dry wt. tissue)	Tissue cation (m-equiv/kg dry wt. tissue)			O_2 used (l./kg dry wt. tissue/hr)
				Na	K	Na + K	
1*a*	None	0	3550	476	162	638	—
b	None	10	2680	261	252	513	—
c	Mercaptomerin 1	10	2720	269	248	517	—
d	Esidrone 1	10	2970	361	189	550	—
e	None	60	2630	257	255	512	—
f	Mercaptomerin 1	60	4430	656	110	766	—
g	Esidrone 0·25	60	3500	476	178	654	—
h	Esidrone 0·5	60	4020	583	135	718	—
i	Esidrone 1	60	4250	627	111	738	—
2*a*	None	0	3260	430	160	590	—
b	None	60	2640	239	280	519	—
c	BAL 1	60	2710	260	262	522	—
d	Mercaptomerin 0·25	60	3630	486	190	676	—
e	BAL 1 + mercaptomerin 0·25	60	2920	305	253	558	—
f	Mercaptomerin 0·25 + BAL	60	3260	390	229	619	—
3*a*	None	0	3460	466	154	620	—
b	None	60	3140	368	230	598	16·0
c	Mercaptomerin 1	60	4130	605	139	744	9·8
d	Iodoacetate 0·025	60	3340	477	143	620	9·1
e	Iodoacetate 0·04	60	3680	537	136	673	7·9

5 m-mole α-oxoglutarate added to 1 l. medium in expts. 1 and 2. BAL + mercaptomerin = BAL added 3 min before mercaptomerin; mercaptomerin + BAL = mercaptomerin added 3 min before BAL. Additions were made during incubation only, not during the preliminary leaching.

Standard errors: Na + 11·2; K ± 6·8 and Na + K ± 11·0 m-equiv/kg dry wt. irrespective of the total amounts of Na, K and Na + K present.

decrease in total base per cell and in cell water (Table 1 and Figs. 1 and 3*a*). It may be presumed that incubation has reversed the changes that have occurred during the preliminary leaching, the movements of cations against concentration gradients being 'active' and requiring energy (active cation transport). If mercaptomerin is added to the incubation medium to give a final concentration of 1mM, loss of Na from the tissue remains almost unaffected for the first 10 min at 37° C, but after 15 or 20 min the content Na

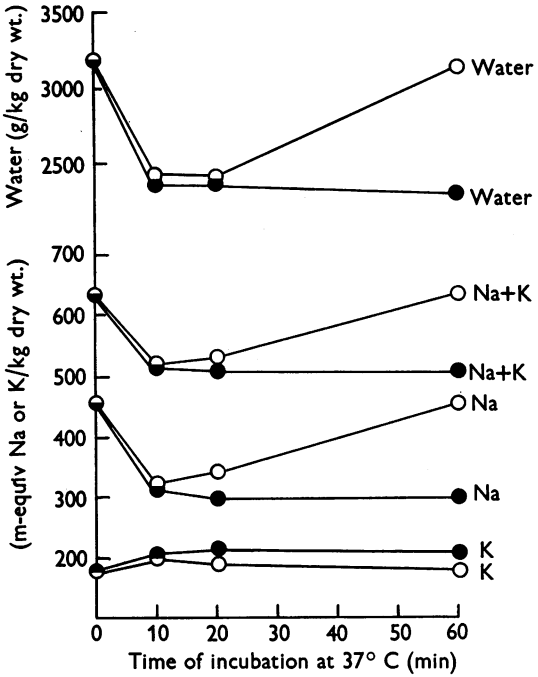

Fig. 1. Water, Na and K contents of cortex slices leached in a mercaptomerin-free NaCl solution for 30 min and then incubated at 37° C in nutrient media without or with 1 mM mercaptomerin. ●, no mercaptomerin; ○, mercaptomerin present. External concentrations, (m-equiv/l. medium): Na 150, K 5 (p. 289).

though less than the '0 min' value, is greater than that of the corresponding mercaptomerin-free control. After 60 min incubation with mercaptomerin the Na content has risen again and may exceed the initial value by 0-100 m-equiv/kg dry wt. It will be noted that even in those experiments where in the presence of mercaptomerin gain of Na is marked, the absolute decrease in K is relatively small (Table 1, Expt. 1 and Figs. 1 and 3*a*), the Na + K changes averaging $+81 \pm 19$ m-equiv/kg dry wt. in fourteen experiments. It follows that the swelling of cortex slices in the presence of mercaptomerin is largely determined by the increase in cell Na.

With regard to the ratio of the concentrations of Na + K in the water of

tissues and medium (R), this may be expected to vary with the concentration of non-penetrating anion within the cells, and hence to vary inversely with the amount of cell water. For the sake of simplicity R has been omitted from the table, but in ten experiments with parallel data available the results were analysed, each individual result itself being the mean of observations in duplicate or triplicate (corresponding to 6 or 9 slices). The findings were as follows: (*a*) Slices leached in NaCl media at about 10° C, mean water content 3520 g/kg dry wt., R (mean and S.D.) $1{\cdot}18 \pm 0{\cdot}028$; (*b*) slices leached and incubated for 1 hr in mercaptomerin-free nutrient media, mean water content 2820 g/kg dry wt., R $1{\cdot}27 \pm 0{\cdot}029$; (*c*) slices leached and incubated for 1 hr in a nutrient medium containing 1 mM mercaptomerin, mean water content 3830 g/kg dry wt., R $1{\cdot}23 \pm 0{\cdot}027$. *a* and *c* show that although the water content of the latter is higher, R in the case of *c* is also higher than in *a*. It is possible that raising the temperature to 37° C increases the ionization of non-penetrating anion within the cell. If so, this factor will also operate in the case of *b*. In view of the fact that the water content of *c* is about 30% greater than in *b*, it is surprising that R in the former is only 0·04 less than in *b*, the unpoisoned preparation. This might arise if the concentration of fixed anion within the cells increased in the presence of mercaptomerin. Values for R in *a* and *b* are qualitatively similar to those of Whittam & Davies (1953).

Esidrone (Ciba) is the sodium salt of pyridinedicarboxy-β-mercuri-ω-hydroxypropylamidetheophylline. The changes in cortex slices leached in cool NaCl solutions for 20 min and then incubated in a nutrient medium containing Esidrone are shown in Table 1 (Expt. 1). After 10 min incubation the slices have lost water and Na and gained, K, though the changes are not as marked as they are on incubation with mercaptomerin or in the controls. After 1 hr at 37° C the slices incubated with Esidrone have swollen again, but now water, Na and K contents are all similar to the corresponding values for slices incubated for 1 hr with mercaptomerin. It will be seen later, however, that slices simply leached for $2\frac{1}{2}$ hr with Esidrone and *not* incubated swell much more than when similarly leached with mercaptomerin.

The results of leaching kidney cortex slices at 3° C without and with mercurial diuretics, and the effects of subsequent incubation

In previous experiments slices were leached in cool NaCl media free from mercurial diuretics and then incubated without or with these agents. In the present series slices were leached without or with mercaptomerin or Esidrone, and then incubated similarly in nutrient media. The experiments were undertaken in consequence of investigations carried out by Kleinzeller & Cort (1957), and differed from theirs in that rats were used instead of rabbits. Kleinzeller & Cort's results on leaching with Esidrone were confirmed, but the findings with mercaptomerin differed from those with Esidrone.

Mercaptomerin. The water content of slices leached for 2½ hr at 3° C in the presence of mercaptomerin is only slightly greater than that of slices leached in a simple buffered NaCl solution (Fig. 2). So too, on subsequent incubation for 10 min, the mercaptomerin-leached slices, whether in a simple nutrient medium at 37° C or in one reinforced by a further addition of mercaptomerin, show as much shrinkage as slices leached and incubated in unpoisoned media, fall of Na and gain of K being comparable in all three. However, between 10 and 60 min at 37° C the water and Na content of slices previously leached with mercaptomerin rises and K falls, the effects being marked when mercaptomerin is present in both the leaching and the incubation media, and quite slight if the mercaptomerin is present only during leaching and not during incubation as well. The latter effect is shown in Fig. 2.

Esidrone. Unlike mercaptomerin, Esidrone added to leaching media induces considerable tissue swelling after 2½ hr at 3° C (Fig. 2) and the effects of Esidrone are far greater than those elicited by much higher concentrations of mercaptomerin. In three experiments with unpoisoned media and with media containing 1 mM-mercaptomerin or Esidrone, water contents were (g/kg dry wt.): (*a*) 3760, 3840, 5040; (*b*) 3250, 3320, 4650; (*c*) 3470, 3500, 4720. In another experiment the water content of slices leached with 1 mM-mercaptomerin was 3800 g/kg dry wt. and with media containing respectively 1, 0·5 and 0·25 mM Esidrone, the values were 5340, 5190, 4080.

When slices previously leached for 2½ hr with Esidrone are incubated in an Esidrone-free medium, shrinkage still occurs, but after 10 min at 37° C the water content is still much greater than in the controls leached and incubated in the absence of Esidrone (3830 compared with 2930—see Fig. 2). If, however, slices are leached and incubated with Esidrone, no decrease in water occurs after 10 min at 37° C, the respective figures being 5230 and 5250 g/kg dry wt., though Na falls slightly (from 897 to 821 m-equiv/kg dry wt. in one experiment) and K shows a small absolute increase (from 22 to 79 m-equiv).

After 60 min incubation it is found that as long as slices have been leached in media containing Esidrone, water and Na are high and K is low, whether Esidrone be present in the incubation medium or not (Fig. 2). As already noted this contrasts with the findings when cortex slices are briefly leached in Esidrone-free media and then incubated for 60 min with Esidrone, for in these circumstances, though shrinkage is inhibited, increase in water is only moderate and is not greater than with mercaptomerin (Table 1, Expt. 1).

It is clear that there are considerable differences between the effects of the various mercurial diuretics on cortex slices. After prolonged leaching in the cold, Kleinzeller & Cort (1957) have shown that Esidrone, mercury perchloride and *p*-chloromercuribenzoate all cause cortex slices to swell, and this is true also of mersalyl. Mercaptomerin, on the other hand, causes little swelling in the cold. Again when slices have been leached with Esidrone,

washed, and incubated in Esidrone-free media, restoration of normal composition is incomplete while in the case of slices similarly treated with mercaptomerin little adverse effect is apparent (Fig. 2). On the other hand, both agents

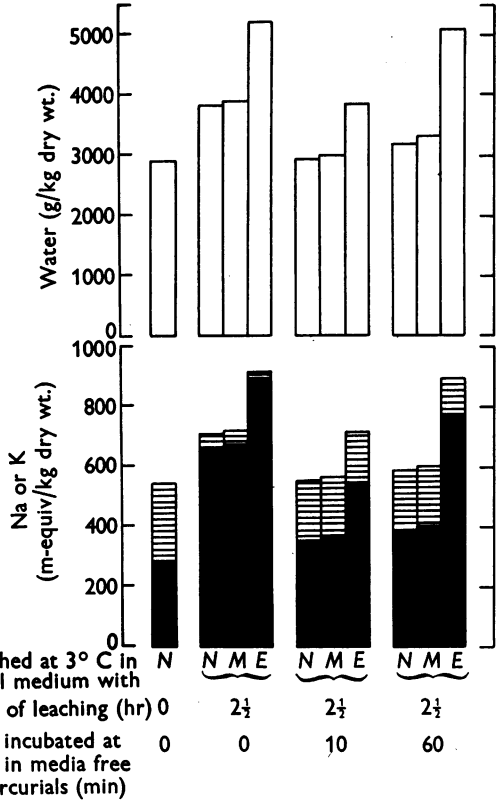

Fig. 2. Water, Na and K contents of cortex slices leached at 3° C for 2½ hr in NaCl solution containing either no mercurial or else 1 mM mercaptomerin or Esidrone (*N*, *M* or *E* above); samples then incubated in a nutrient medium containing no mercurial diuretic. The single column represents original untreated slices. ■, Na; ▤, K; □, water.

prevent maintenance of normal tissue composition at 37° C. The findings suggest that both Esidrone and mercaptomerin combine strongly with cortex tissue at 37° C, but that at low temperatures the attachment of mercaptomerin is slight.

Effects of incubating cortex slices in lithium chloride media without and with mercurial diuretics

These experiments were undertaken in an attempt to determine whether the mercurial diuretics act mainly by increasing the permeability of cortex cells or by inhibiting active cation movements against the concentration

gradients. The rationale is discussed later, but in the meantime the results of leaching cortex slices in NaCl media free from mercurial diuretics, and incubating in a nutrient medium containing LiCl (165 m-equiv/l) without or with mercaptomerin may be considered. The findings in Fig. 3 are typical and may be summarized as follows: (1) If leached slices are dipped in LiCl solution and withdrawn immediately, there is already a considerable gain of Li, largely in

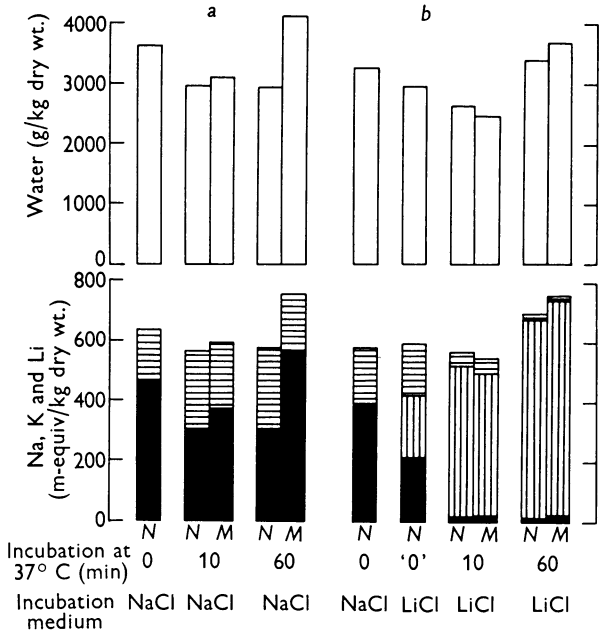

Fig. 3. The effects on the water and cation contents of cortex slices incubated in NaCl (*a*) and LiCl (*b*) nutrient media without mercaptomerin (*N*) and with mercaptomerin (*M*). Incubation preceded by leaching at 10° C for 30 min in mercaptomerin-free NaCl solution. Cation composition of nutrient media; NaCl medium, Na 150 and K 5 m-equiv/l.; LiCl medium, Li 165 m-equiv/l. ■, Na; ▤ K; ▥, Li; □, water. Note: *a* and *b* are from separate experiments and are not entirely comparable.

exchange for tissue Na. It may be surmised that this rapid initial entry of Li is largely at the expense of Na in the extracellular space. When the temperature of the LiCl medium is 4° C, the gain at '0 min' is about 15% of that gained in an hour at 37° C; at 12° C the gain is 25%, and at 37° C the immediate gain is about 30%. (2) Between '0' and 10 min the tissue shrinks because loss of Na+K exceeds gain of Li. This may arise because active efflux of Na exceeds active influx of K plus passive influx of Li, or because Na and K moving outwards with their concentration gradients move more rapidly than Li flowing inwards. These phenomena are also seen 10 min after incubation with mercaptomerin, though here tissue Na is a little higher in the unpoisoned systems, while K is about the same in both. (3) After 60 min

unpoisoned and poisoned slices have again swollen considerably, though the Na content of the former is still less than that of the latter, and the Na concentration in tissue water is also less than the external concentration, indicating that active transport still persists. In the case of poisoned slices, on the other hand, tissue Na concentration is slightly greater than the external concentration. The K concentration of slices is always greater than the external concentration. Thus in Fig. 3, the Na concentrations of slices incubated for 60 min in LiCl media without and with mercaptomerin were respectively 4·4 and 6·5 m-equiv/l. tissue water, while the external concentration was 6·2 m-equiv/l. (data not shown in figure); the corresponding concentrations of tissue K were 6·8 and 5·7 m-equiv/l. tissue water with an external K concentration of 1·06 m-equiv. The persistence of a relatively high tissue K even in the presence of mercaptomerin suggests that tissue K is not completely exchangeable (cf. Whittam & Davies, 1954).

It has also been shown that both in the absence and presence of mercaptomerin tissue slices have shrunk after 10 min in LiCl media at 37° C, and have swollen again after 60 min. There is rather more swelling with mercaptomerin than in unpoisoned systems, the excess corresponding on the average to 204 g water/kg dry wt. (6 expts.).

Effects of BAL on slices of renal cortex of rats incubated with mercaptomerin

It is generally thought that the mercurial diuretics act on SH-groups, because their diuretic action is opposed by BAL. Thus Borghraef & Pitts (1956) found that the simultaneous injection of 'five times a molar equivalent of 10% BAL in oil' prevented the diuresis of Chlormerodrin in rats. These authors do not imply that such an excess is essential, and indeed Farah & Maresh (1948) had previously shown that 0·5 mole BAL/mole mersalyl sufficed to prevent diuresis in anaesthetized dogs.

In the present experiments, with far higher concentrations of mercaptomerin and BAL than are used *in vivo*, it was necessary to consider possible effects of BAL itself. The following result is typical: leached slices contained 3480 g water/kg dry wt.; on incubating for 60 min at 37° C in media containing 4, 2, 1 and 0 mM BAL, the respective water contents were 3550, 3050, 2810 and 2870 g/kg dry wt. It was therefore thought undesirable to use BAL in concentrations greater than about 1 m-mole/l. medium.

Having ascertained a suitable concentration of BAL, the effect of BAL on inhibition by mercaptomerin was examined. Leached slices were incubated for 60 min with mercaptomerin, BAL being added either 3 min before or 3 min after mercaptomerin. In one experiment 0·25 mM mercaptomerin and 1 mM BAL were present in the medium (Table 1), and in another 0·35 mM mercaptomerin and 1·05 mM BAL. It was found that BAL added after mercaptomerin gave rather little protection against the inhibitory action of the poison, while

BAL added just before gave partial but definite protection. It is perhaps surprising that though the inhibitory effects of mercaptomerin have not begun to be apparent within 10 min incubation (see Table 1, Expt. 1 and Figs. 1 and 2), the previous addition of BAL affords only incomplete protection against ultimate inhibition by mercaptomerin.

In view of these findings it was not expected that BAL added 60 min after incubation with mercaptomerin would lead to any restoration of function, and in fact no such restoration occurred. Slices were incubated for 60 min in a medium containing 0·25 mM mercaptomerin, and then leached and re-incubated in mercaptomerin-free nutrient media containing 1·05 mM BAL. Fall in Na and water and rise in K failed to occur, the findings corresponding to those with slices similarly treated with mercaptomerin alone. Unpoisoned slices, on the other hand, incubated, leached and re-incubated showed a typical fall in water and Na contents with rise of K. So too, slices incubated in medium containing 0·25 mM mercaptomerin, and then leached and re-incubated in poison-free media containing 2 mM glutathione, failed to show any significant recovery. These results were disappointing because they showed that mercaptomerin, in an amount sufficient to produce definite alteration in the composition of cortex slices on incubation at 37° C, induced irreversible changes in the tissue.

One other point is of interest. BAL affords some protection against the swelling induced in cortex slices when leached in the cold with Esidrone (without subsequent incubation). Thus unpoisoned controls leached in NaCl solution at 3° C had the following water contents (g/kg dry wt.) after '0', 60 and 150 min, respectively: 3180, 3220, and 3380; with 1 mM Esidrone the figures were 3180, 4030 and 4720, while after 150 min at 3° C with 1 mM Esidrone and 1 mM BAL the water content was 3740.

Respiration and cation transfer by slices of renal cortex incubated in media without and with mercurial diuretics

When in the following experiments mercurial diuretics were used, they were only present during incubation and not during the preliminary leaching.

The respiration in the present series of experiments varied between 14 and 17 l. O_2/kg dry wt./hr at 37° C. Robinson's (1956) figures correspond to about 20 l. at 38° C; a temperature coefficient of 3 per 10° C would largely account for this difference. In the presence of mercaptomerin (1 m-mole/l. medium) respiration was inhibited by about 30% at $\frac{1}{4}$–$\frac{1}{2}$ hr, and by about 45% at $\frac{3}{4}$–1 hr. The 0–$\frac{1}{4}$ hr period was occupied by equilibration in the Warburg flasks, but it is likely that the inhibition during this time was less; if so, this might account in part for the maintenance of a relatively normal distribution of cations during the earlier part of incubation.

It was thought of interest to compare the effects of mercaptomerin on the

Na and K contents of slices with that of a second inhibitor, because Robinson (1956) had shown that when a comparable degree of inhibition is effected by cyanide, tissue swelling is less than with mercaptomerin, the respective water contents being about 3900 compared with 4700 g/kg dry wt. In the present experiments iodoacetate was preferred to cyanide, because the pH does not fall during incubation, though its use is limited to systems where glucose is the chief substrate and where α-oxoglutarate is absent. A typical experiment (Table 1, No. 3) shows that iodoacetate inhibits the respiration of cortex slices (presumably by its more selective action on glycolysis) and that cation transfer against concentration gradients is less active than in its absence. When the effects of iodoacetate and mercaptomerin are compared, it is seen that with similar inhibitions of respiration, differences in K contents (m-equiv/kg dry wt.) are small, but the Na content and hence the Na + K content and also the water content are much higher in the presence of mercaptomerin than with iodoacetate. Thus it is probable that mercaptomerin has a twofold effect on cation movements, one due to an inhibitory action on respiration, and the other exerted on some other mechanism concerned with the movements of cations.

Three other experiments were done with Esidrone, in which inhibition of respiration averaged 42% at 37° C. One observation with meralluride (U.S.P., another mercurial diuretic) gave 45% inhibition at 37° C. It was thought that the inhibitory action of all these substances was exerted against respiration rather than glycolysis, because when succinate without glucose was added to leached cortex slices, inhibition of respiration was not decreased, the results being 48% with Esidrone and 52% with mercaptomerin (1 mM).

Comparison of the effects of mercaptomerin on slices of rat liver and kidney

Fig. 4*a* shows that mercaptomerin has a definite inhibitory effect on the output of Na and uptake of K when leached liver slices are incubated, while Fig. 4*b* shows that even after 60 min incubation with mercaptomerin, when the Na content may be less than the initial value, it is still greater than 30 min incubation with mercaptomerin. The figure further shows that the shrinkage of tissue occurring between '0' and 30 min at 37° C even in the presence of mercaptomerin is greater than the corresponding decrease in kidney slices. In six experiments with parallel observations decrease in liver water averaged 600 g/kg dry wt. (3400–2800), whereas for kidney cortex the fall was 450 g/kg dry wt. (3800–3350). Between 30 and 60 min water in liver slices now increases, the average for six experiments being 290 g/kg dry wt. (2800–3090), while the average increase for cortex slices was 720 (3350–4070). Thus the inhibitory effects of mercaptomerin on loss of water during incubation is definitely less in the case of liver slices than with cortex slices.

With regard to cation changes, liver slices incubated with 1 mM-mercaptomerin usually lose more Na and Na + K/g dry wt. at 0–30 min than do kidney

slices, while between 30 and 60 min liver slices always gain much less Na and Na + K (Fig. 4*b*). For the whole 60 min period of incubation gain of Na and Na + K by liver slices is less than the gain by kidney slices, and in the case of the former Na and Na + K may sometimes be less than the initial value even after incubation for 60 min with 1 mM mercaptomerin. Thus the effects of mercaptomerin on slices of renal cortex are more marked than they are on liver slices.

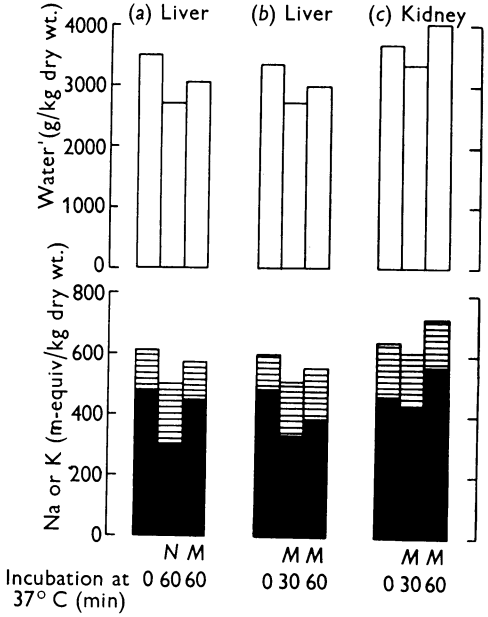

Fig. 4. Water, Na and K contents of liver and kidney slices leached at 10° C for 30 min in mercaptomerin-free NaCl solution (first column of each group), and then incubated at 37° C in a NaCl nutrient medium without mercaptomerin (*N*) and with 1 mM mercaptomerin (*M*). Note mercaptomerin was present in *both* incubated samples of (*b*) and (*c*). Cation composition of medium: Na 150 and K 5 m-equiv/l. ■, Na; ▤, K.

DISCUSSION

In this section the phrase 'active cation transport' is used to indicate cation movements against gradients of concentration (and potential). The slice, however, is considered as a whole, and individual variations in the amount and direction of transport in the several parts of the renal tubule are ignored. Since after leaching the Na concentration in the slice as a whole is still less than the external concentration, while the K concentration is greater, fall of Na and rise of K during incubation are regarded as evidence of active cation transport. Changes in the reverse direction are considered to be passive.

When kidney slices are placed in cool glucose-free media, metabolism is depressed and cations move with the concentration gradients so that Na,

Na + K and water rise, while K falls. On incubation with a similar medium containing 5 mM-K and 5·6 mM glucose these changes are reversed. In the presence of mercaptomerin there is a latent period of about 10 min, when Na and K continue to move against the concentration gradients. At this stage the tissue seems to be virtually unaffected by the poison and shrinks. Then between 10 and 60 min the tissue swells again with a marked rise in Na content and a relatively small fall in K content (Table 1 and Figs. 1, 2 and 3*a*). It has been shown that the late swelling is associated with changes in the cell which cannot be reversed by BAL or glutathione. The delayed action of mercaptomerin may be due to gradual combination with cell structures, or to the temporary availability of sources of energy which can be utilized in the presence of mercaptomerin, but which cannot be replaced (cf. mouse ascites tumour cells: Maizels, Remington & Truscoe, 1958).

It has been noted that after 60 min incubation with mercaptomerin cortex slices swell, the gain of Na being considerable, while loss of K is relatively slight. These findings probably arise from the fact that while Na tends to enter the tissue cells and K to leave in accordance with the concentration gradients, entry of Na is assisted and escape of K hindered by the Donnan asymmetry resulting from the presence of non-penetrating anion within the cells.

Causes of the cation changes induced in cortex slices by the mercurial diuretics

Kleinzeller & Cort (1957) have shown that when rabbit cortex slices are leached in cold NaCl solution containing Esidrone (also mercury perchloride or *p*-chloromercuribenzoate), increase in water and Na is much greater than on leaching in simple NaCl solution (see our Fig. 2). It is then argued that the increased swelling with Esidrone at 0° C must be due solely to permeability effects, since at this temperature metabolism and active cation movements (against concentration gradients) will be abolished. This conclusion, however, may well be incorrect, for in poison-free leaching media there is a very rapid initial increase in slice water of about 500 g/kg dry wt. (from 2700 to 3200) with a further increase of only about 200 g during the subsequent $2\frac{1}{2}$ hr at 3° C. So too, Whittam & Davies (1953) find that when kidney slices are placed in cold hypotonic media, swelling of the tissue and decrease in the sum of Na and K are complete in about 10 min. Since kidney slices are moderately permeable at low temperatures, it is difficult to see why the Donnan asymmetry does not determine continued swelling, unless this is counteracted in some degree by active movement, in which case part at least of the effects of Esidrone might be exerted against such active movements. Kleinzeller & Cort (1957) proceed to show that if cortex slices are leached in media containing ^{24}Na, gain of tracer is greater in the presence of Esidrone than in its absence, while on subsequent incubation at 25° C in media free from both tracer and Esidrone, the efflux of ^{24}Na is greater from those slices originally leached with Esidrone. These

authors suggest that the increased efflux from Esidrone-leached slices excludes any deleterious effect on active Na movements, and that therefore the effects of the mercurial diuretics must be exerted against passive movements. This view, however, involves oversimplification. Thus after leaching in a medium containing ^{24}Na and transfering to tracer-free medium, ^{24}Na escapes not only from the cells but also from the extracellular space. ^{24}Na concentration of the latter roughly equals that of the original leaching medium and exceeds that of the cells: the extracellular space also equilibrates more quickly with the tracer-free medium than will the cells. Further, in Kleinzeller & Cort's communication, as in the present paper, values are referred to dry weight, and although the extracellular space constitutes a fairly constant proportion of the wet weight of slices, whether the tissue be swollen or not (Robinson, 1950), extracellular fluid referred to dry weight of tissue will increase as the tissue swells. Hence in Esidrone-leached slices extracellular ^{24}Na (referred to dry weight) will be contributed in considerably greater amount than is the case with the less swollen controls, and this may lead to an apparent increase in efflux when this is calculated on the basis of dry weight. Kleinzeller & Cort's data do not permit calculation of ^{24}Na efflux from the actual tissue cells, which may perhaps be no greater when Esidrone-leached slices are incubated than is the case with the unpoisoned control. In addition to this, the initial concentration of ^{24}Na (counts/l. tissue water) was about 30% higher in their Esidrone preparations, while the content of non-penetrating anion was less than in the controls. Both these factors will facilitate a higher Na efflux in the case of slices treated with Esidrone, and the higher observed efflux of ^{24}Na from the swollen slices so treated might, nevertheless, be associated with decreased activity in the processes which energize Na transport. So far therefore, there is no evidence either in favour or against Kleinzeller & Cort's views that the action of the mercurial diuretics is due to increasing permeability of the cortex cells, though experiments to be discussed suggest that changes in cell permeability are probably a minor factor in the action of mercaptomerin on the composition of cortex slices.

If slices are kept at 37° C for 60 min in a nutrient medium containing LiCl instead of NaCl, the composition of the slices is practically the same whether mercaptomerin be present or not. If it be accepted that Li movements are purely passive, this indicates that any action of mercaptomerin on passive penetration is quite subsidiary. In support of the assumption that Li movements are passive, it may be noted that the Li composition of slices in LiCl media at 10 min is 190 m-equiv/l. tissue water compared with an external concentration of 165 m-equiv/l., while at 60 min the tissue concentration has only risen by 2%, though water and Li/kg dry wt. have risen by about 30% (Fig. 3). This shows that the concentration of Li in the water entering the tissue between 10 and 60 min is roughly constant at a figure which is slightly

in excess of the external concentration, and suggests that the inflow is governed by the Donnan asymmetry. If then the inflow of Li is indeed passive and if also the main effect of mercaptomerin is to increase the passive fluxes of cations, then mercaptomerin added to LiCl media should increase the rate of penetration of Li into tissue slices, the additional swelling induced in LiCl media being of the same order as that seen with NaCl. If, however, one considers slices in NaCl and LiCl media without and with mercaptomerin, it is found that after 10 min loss of cation exceeds gain, and in all cases the slices shrink, the water content being between 2500 and 2900 g/kg dry wt. After a further 50 min slices in NaCl medium without mercaptomerin still maintain this water content, but in the other three samples (NaCl with mercaptomerin, and LiCl without and with mercaptomerin) the slices have all swollen, the water contents being between 3500 and 3700 g/kg dry wt. (Fig. 3). These findings are consistent with the view that the mercurial diuretics act mainly on active transport, for with active transport presumed to be absent in all three samples there is no reason why any additional specific inhibitory action by mercaptomerin should be manifest. On the other hand, the contrary view that the mercurial diuretics act solely on permeability might explain the gain of water by slices in NaCl media containing mercaptomerin, but in the case of LiCl media, Li is common to unpoisoned and poisoned samples, and the presence of mercaptomerin in the latter should still increase the rate of penetration of Li and induce greater tissue swellings in this sample. In other words, the effect of mercaptomerin in LiCl media should be to some extent additive, and should be clearly evident, even though the water content of slices in LiCl media without mercaptomerin is relatively high when compared with the water content of slices in simple NaCl media. Experimentally it was found that the water contents of slices incubated for 60 min in NaCl media without and with mercaptomerin were about 2600 and 3600 g/kg dry wt., while the contents of slices similarly incubated in LiCl were 3650 and 3850 (mean of 6 expts.) Hence the findings suggest that mercaptomerin slightly increases permeability, but exerts its main effects against the active movements of Na and K.

Respiration and the mercurial diuretics

Robinson (1956) states that mercaptomerin depresses respiration, and in our experiments this inhibition amounted to about 40%; Esidrone and meralluride have similar effects. Absence of inhibition of respiration by Esidrone (Kleinzeller & Cort, 1957) and by meralluride (Mudge, 1951) in rabbit cortex slices at 25° C may be due to a high temperature coefficient of inhibition. If the latter were about 3 between 27 and 37° C, effects on respiration might well escape detection at 25° C. It has been shown that the inhibition of respiration is not the chief factor in the increase in water and Na and decrease in K of cortex slices treated with the mercurial diuretics.

Action of mercaptomerin on liver slices

Kleinzeller & Cort (1957) report that Esidrone is without effect on slices of rabbit liver. This may be due in part to the fact that their observations were made at 0° C. If rat liver slices are leached in the cold and then incubated with mercaptomerin at 37° C, loss of water and Na and gain of K are smaller than in the controls (Fig. 4). It may be noted that after the injection of chlormerodrin (3-chloromercuri-2-methoxypropylurea) in rats, the mercury content of the kidney *in vivo* may be more than 100 times greater than in the liver (Borghraef & Pitts, 1956). It is likely, however, that the content of mercurial diuretic in liver slices *in vitro* not in competition with kidney for the diuretic agent may be comparatively high. Nevertheless, liver is less affected by the mercurial diuretics than cortex slices (Fig. 4), either because accumulation in the liver slice is less, or because of relative insusceptibility of the liver cells.

Effective concentrations of the mercurial diuretics acting in vivo and in vitro

It may be wondered what bearing observations on kidney cortex slices *in vitro* have on the action of the mercurial diuretics on the intact organ *in vivo*. Kleinzeller & Cort (1957), adapting the data of Borghraef & Pitts (1956), suggest that the human kidney after treatment with Esidrone is likely to contain about 5×10^{-4} moles of mercurial/l. tissue water, which is about one third of the amount usually present in their media and a little in excess of the minimum amount of mercurial diuretic which must be present in a medium to elicit a response *in vitro*. They conclude that the Esidrone content of their slices is of the same order as that required to produce a clinical effect *in vivo*. This idea rests on a misconception, for Kleinzeller & Cort's calculations merely show that the therapeutic concentration in the actual kidney tissue *in vivo* is similar to that in the external medium used *in vitro*. In fact, Borghraef & Pitts' data show that *in vivo*, with an amount of chlormerodrin which corresponds to ten times the therapeutic dose, plasma chlormerodrin is $2{\cdot}2 \times 10^{-5}$ M, while the kidney content is $2{\cdot}9 \times 10^{-3}$ mole/kg wet wt. Larger doses *in vivo* increase the plasma content, but decrease the kidney uptake and are definitely toxic. If, as seems likely, the medium *in vitro* may be compared to the plasma *in vivo*, then slices *in vitro* are exposed to 50 times the maximum amount of mercurial diuretic that can be tolerated *in vivo*, and to more than 250 times the therapeutic plasma concentration. It is true that *in vitro* an effect is clearly detectable after slices have been kept at 37° C for 1 hr in a medium containing $1{\cdot}25 \times 10^{-4}$ M mercaptomerin, but $0{\cdot}62 \times 10^{-4}$ M is ineffective though the latter is three times the maximum tolerated plasma content. How the tissue concentrations vary with the very high external concentrations used

in vitro is unknown, but they are probably a great deal higher than those found *in vivo*. It is worthy of note that effects both *in vitro* and *in vivo* may be elicited after a similar interval of time. Thus Fawaz & Fawaz (1954) have found that maximum diuresis occurs 2–3 hr after the intravenous injection of mersalyl, but an effect is well marked within an hour. Thus so far as can be judged from the different types of effect elicited at 37° C, it appears that the action of the mercurials *in vitro* differs from the action *in vivo* in requiring much larger amounts of these agents, and in the firm combination of the mercurials with the tissue which persists even after washing or after treatment with BAL. For these reasons it is considered that findings *in vitro* have no bearing at all on the physiological action of the mercurial diuretics *in vivo*.

SUMMARY

1. Most of the mercurial diuretics cause slices of the renal cortex of rats to swell considerably on leaching at 3° C. In the case of Esidrone this property seems to be associated with the agent becoming firmly fixed to the tissue even at low temperatures. Mercaptomerin does not combine firmly in these circumstances and causes little swelling in the cold, though it becomes firmly fixed to tissues at 37° C.

2. Slices leached in the cold without mercurial diuretics and then incubated with mercaptomerin respond like unpoisoned controls between '0' and 10 min, fall in the contents of water and Na and rise in the content of K being similar. After 10 or 15 min there is a gradual increase in the contents of water and Na, with a fall in the content of K. Once these changes have occurred, they cannot be reversed by BAL.

3. BAL added 3 min after the addition of mercaptomerin at 37° C gives partial protection against the action of mercaptomerin, and if it is added 3 min before the poison protection is marked but is not complete.

4. If leached slices are incubated in nutrient media containing LiCl instead of NaCl and KCl, swelling occurs which is almost as great in the absence of mercaptomerin as in its presence. If it be assumed that Li transfer is purely passive, it follows that mercaptomerin acts chiefly on active cation transport and has little effect on passive transfer.

5. The mercurial diuretics in a concentration of 1 m-mole/l. medium depress the respiration of cortex slices by about 40 % at 37° C, but this is probably only a minor factor in the rise in the contents of tissue water and Na and fall in the content of K induced by the mercurial diuretics.

6. The effects of mercaptomerin on liver slices is similar to that on renal cortex, but is much less marked.

7. The lowest concentration of mercurial diuretic which elicits a definite effect *in vitro* is many times greater than the maximum tolerated plasma

concentration in rats *in vivo*, and it is doubtful if experiments with cortex slices *in vitro* have any relevance to diuretic action *in vivo*.

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