SUMMARY

1. A method is described for the accurate quantitative analysis of ketone bodies from small samples (0.1 ml.) of blood and urine.

2. The method is based on the conversion of all ketone bodies into acetone, on the reaction of acetone with 2:4-dinitrophenylhydrazine and on subsequent colorimetric determination of acetone-2:4-dinitrophenylhydrazone in carbon tetrachloride.

3. A combined reflux and distillation apparatus has been designed which ensures reproducibility in the estimation of β -hydroxybutyric acid and allows the separate and direct estimation of acetone plus acetoacetic acid and of β -hydroxybutyric acid from one sample.

4. At a ketonaemic level of 40 mg./100 ml., the standard deviation of an individual reading is less than ± 0.2 mg./100 ml.

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The Mechanism of Action of Mercurial Preparations on Transport Processes and the Role of Thiol Groups in the Cell Membrane of Renal Tubular Cells*

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The powerful diuretic action of mercurial preparations and investigation of the mechanism of their action *in vivo* have led to the generally accepted view that these substances directly or indirectly interfere with the reabsorption of sodium chloride and water by the tubular cells of the kidney (Schwartz & Wallace, 1951). It is also agreed that mercurial preparations exert their effect by inhibiting SH groups of some essential cellular enzymes (see, for example, Barron & Singer, 1945; Barron, 1951), but no clear-cut evidence is so far available concerning the localization of their action and detailed biochemical mechanism. Mudge (1951) observed that mercuric chloride (and also meraluride, an organic mercurial) at appropriate concentrations *in vitro*, inhibits the uptake of K^+ ion and loss of water by slices of kidney cortex which have previously been leached in isotonic sodium chloride at 0°, without, however, affecting the oxygen uptake of the tissue. A statement to the same effect has been made by Robinson (1954).

These results appeared to suggest therefore that mercurials interfere with the active transport of electrolytes in kidney-cortex slices, some authors maintaining that the primary effect is on Cl^- ion transport (Schwartz & Wallace, 1951; Axelrod & Pitts, 1952).

In this paper evidence is given for the view that mercurials interfere with the permeability of the membrane of tubular cells by increasing passive influx of Na^+ ion, Cl^- ion and water into the cells, without interfering with the active extrusion of Na^+ ion. Furthermore, evidence will be presented suggesting that the state of SH groups of proteins of the cellular membrane is a controlling factor in permeability. No evidence was found suggesting a direct effect of mercurials on Cl^- ion transport *per se*.

METHODS

The experimental procedure used has already been described in some detail (Cort & Kleinzeller, 1956) and will only be summarized here. Unless otherwise stated, slices of kidney cortex of normal rabbits (the uppermost slice being rejected) were leached in 0.154M-NaCl at 0° for 2.5 hr., and samples of slices (usually 100-200 mg. wet wt.) were then

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incubated at 25° in 3 ml. of phosphate-saline containing a mercurial preparation or other additions, and in most experiments also substrate (0.005 M α -oxoglutarate). Slices incubated in the same medium without mercurials (or other additions) served as controls.

The contents of water, Na⁺ and K⁺ ions were estimated in the slices previously described (Cort & Kleinzeller, 1956). Where the content of Cl⁻ ion was also estimated, the dry tissue was crushed, extracted with 0.2 N-H₂SO₄ for about 5 days, and Na⁺, K⁺ and Cl⁻ ions were estimated in the extract, the last by amperometric titration, a calomel-dropping-Hg electrode system, a 5% KNO₃ bridge and 0.02 N-HgNO₃ being used for microtitration (Kolthoff & Miller, 1941; Kubie, in preparation). This method is accurate to a total Cl⁻ ion sample of about 2 μ -equiv. in 1 ml. of solution. Wherever a significantly lower amount was found, it has been designated as 0.

The results are expressed in m-equiv. of the appropriate ion/100 g. of dry solids. Samples of tissue were analysed at the following intervals: initial value (*I*), after leaching (*T*₀), and at various intervals after incubation, the subscript of *T* indicating minutes of incubation. The same procedure was adopted where other tissues were used.

In experiments with ²⁴Na, the procedure was similar to that described earlier (Cort & Kleinzeller, 1956). Tissue slices were leached in isotonic NaCl with or without ²⁴Na present. The difference between the values of Na⁺ ion at *I* and *T*₀ was used to estimate the relative passive penetration of Na⁺ ion into the cells. Samples of slices, leached in labelled NaCl, were then quickly washed in non-isotopic NaCl, and transferred to vessels containing 2 ml. of phosphate-saline with or without mercurial, containing 0.005 M α -oxoglutarate as substrate. After incubation for 10 min. at 25° the slices were removed, blotted and weighed, and dried at 110° over P₂O₅ for 5 hr.; the dry solids were weighed and digested for 15 min. at 100° with 1 ml. of conc. HNO₃. The Na⁺ and K⁺ ion content in these digests was then estimated. Portions of the filtered medium were used for measuring radioactivity. Measurements were made with a low-voltage liquid counter of approximately 4 π geometry. From these measurements the relative inward and outward fluxes of ²⁴Na (*M*_i and *M*_o) were estimated. At the same time, relative *M*_{i,Na} was estimated directly by leaching the slices in non-labelled NaCl, incubating them in phosphate-saline labelled with ²⁴Na, and measuring the specific activity of ²⁴Na in the tissue. The results are expressed as relative fluxes (*M*_i and *M*_o) in 10⁷ counts/sec./100 g. of tissue solids.

Oxygen uptake of tissue slices was measured at 37° by the Warburg technique, 3 ml. of phosphate-saline, or a specific medium as described in the text, being used. The results are expressed as *Q*_{O₂}.

In some experiments the non-protein and protein N in the medium after incubation of the slices were estimated as follows: the medium was centrifuged, and 0.5 ml. of 10% trichloroacetic acid was added to a 2 ml. portion. After standing overnight, the samples were centrifuged, and 2 ml. of the supernatant was digested with the Cu-Se catalyst described by Sober, Mayer & Gottfried (1944). The sediment was washed once with 2 ml. of 5% trichloroacetic acid, centrifuged, then dissolved in 0.5 ml. of N-KOH and digested. Ammonia was then estimated by the micro-diffusion technique (Conway, 1947). The results are expressed in μ g. of N/10 mg. of dry solids.

All values are the mean of two individual analyses, which generally did not differ by more than 3–5%. All experiments were repeatedly confirmed; representative results only are presented.

Most of the experiments described were carried out with either HgCl₂ (4–5 $\times$ 10⁻⁴ M, final concn.) or 1.45 $\times$ 10⁻³ M (final concn.) Esidrone (i.e. theophyllino-mercuri-monooxypropylamido sodiumquinolinate, Ciba, Basel, Switzerland); in some of the experiments 2.8 $\times$ 10⁻³ M (final concn.) Novurit (i.e. sodium *N*-(γ -hydroxymercuri- β -methoxy)-propylcamphoramate, SPOFA, Prague, Czechoslovakia), or *p*-chloromercuribenzoate (10⁻⁴ M) was used with essentially the same results.

Reduced glutathione and *p*-quinone were commercial preparations; α -oxoglutaric acid was obtained from Dr J. Kocourek, dehydroascorbic acid was prepared by Dr J. Liebster.

RESULTS

Effect of mercurials added during incubation on the transport of electrolytes and water. Mudge (1951) observed in experiments of 30 min. duration that mercuric chloride at a concentration of 5 $\times$ 10⁻⁴ M (and also at higher concentrations), and also meralluride 10⁻³ M, inhibited the transport of water and K⁺ ion, without commensurably affecting the O₂ uptake. Similar results concerning water were obtained by Robinson (1954) with another mercurial. If, however, the effect of Esidrone (or Novurit) is examined at shorter time intervals, it is found that during the first 10 min. of incubation the slices lose water and Na⁺ ion at a rate only slightly lower than the controls without mercurial added,

Fig. 1. Time curve of effect of Esidrone added during incubation on transport of Na⁺ ion and water. Slices were leached for 2.5 hr. at 0° in 0.154 M-NaCl and then incubated at 25° in 3 ml. of phosphate-saline containing 0.005 M α -oxoglutarate, without or with 1.45 $\times$ 10⁻³ M-Esidrone. —, Controls; ---, Esidrone. The top set of curves represent water (ml./100 g. of dry solids), the lower Na⁺ (m-equiv./100 g. of dry solids).

Table 1. *Effect of incubation with Esidrone on the transport of water, Na⁺, K⁺ and Cl⁻ ions in kidney-cortex slices*

Slices were leached for 2.5 hr. at 0° in 0.154 M-NaCl and then incubated at 25° in 3 ml. of phosphate-saline containing 0.005 M- α -oxoglutarate and Esidrone. All values are in ml. or m-equiv./100 g. of dry solids.

Mercurial	Time interval (min.)	Water (ml.)	Na ⁺	K ⁺ (m-equiv.)	Cl ⁻
Nil	Initial	287	25.7	30.7	17.9
Nil	0	377	53.6	14.7	54.4
Nil	10	317	34.5	18.2	32.8
Esidrone (1.45 $\times$ 10 ⁻³ M)	10	319	37.4	17.6	35.3
Nil	30	302	33.2	25.4	30.4
Esidrone (1.45 $\times$ 10 ⁻³ M)	30	330	43.7	14.7	39.9

Table 2. *Effect of incubation with mercurials on the loss of nitrogenous substances by kidney-cortex slices*

Slices were leached for 2.4 hr. at 0° in 0.154 M-NaCl and then incubated in 3 ml. of medium A₂ (Robinson, 1949) containing 0.005 M- α -oxoglutarate. NPN, Non-protein N; PN, protein N. All values in μ g./10 mg. of dry solids in the medium after incubation.

Mercurial	Time interval (min.)	Expt. 1		Expt. 2		Expt. 3	
		NPN (μ g.)	PN (μ g.)	NPN (μ g.)	PN (μ g.)	NPN (μ g.)	PN (μ g.)
Nil	10	33.8	21.3	11.6	18.5	7.7	13.7
HgCl ₂ (5 $\times$ 10 ⁻⁴ M)	10	25.6	25.4	10.2	19.2	8.6	14.5
Esidrone (1.45 $\times$ 10 ⁻³ M)	10	—	—	—	20.4	7.0	14.9
Nil	30	29.5	20.2	12.0	22.3	16.0	23.2
HgCl ₂ (5 $\times$ 10 ⁻⁴ M)	30	36.6	64.5	19.7	44.2	18.8	39.6
Esidrone (1.45 $\times$ 10 ⁻³ M)	30	—	—	—	28.6	36.6	26.5

Fig. 2. Effect of HgCl₂ added during incubation on transport of cations and water. Conditions were as described for Fig. 1. —, Without HgCl₂; ---, 3.7 $\times$ 10⁻⁴ M-HgCl₂; - · - ·, 5 $\times$ 10⁻⁴ M-HgCl₂. Top set of curves: water; middle set: Na⁺ ion; bottom set: K⁺ ion.

but take up water and Na⁺ ion again during further incubation (Fig. 1); the movement of K⁺ ion is reciprocal to that of Na⁺ ion. The water, Na⁺ and K⁺ ion contents of the slices after 30 min. incubation differ little from, or may even be higher than, the T₀ values.

No appreciable inhibitory effect of Esidrone (at the concentration used) on the O₂ uptake of the tissue was found (Q_{O₂} values: controls, -15.2 without substrate, -21.4 with substrate; with added mercurial, -12.1 without substrate, -22.0 with substrate).

Although the time curve for the effect of mercuric chloride followed the same trend, the effect was less clear. Fig. 2 shows the effect of two concentrations of mercuric chloride (3.7 and 5 $\times$ 10⁻⁴ M) on the transport of water, Na⁺ and K⁺ ions.

It has been suggested (Schwartz & Wallace, 1951; Axelrod & Pitts, 1952) that mercurials may specifically affect the transport of Cl⁻ ion. As shown in Table 1, the values for Cl⁻ ion appear to follow Na⁺ ion, and the changes of Cl⁻ ion are of the same order as Na⁺ ion changes (see also Leaf, 1956). This result does not support a specific effect of mercurial on Cl⁻ ion transport, but does not exclude it. A simultaneous movement of α -oxoglutarate anion may also have occurred under these conditions (see Whittam & Davies, 1953).

Effect of mercurials added during incubation on the loss of nitrogenous substances by slices of kidney cortex. It is known that kidney-cortex slices, when incubated in phosphate-saline, lose some protein nitrogen into the medium; such loss can be reduced in a slightly modified medium (see Robinson, 1949; Aebi, 1952). It will be seen from Table 2 that incubation of the slices in medium A₂ (Robinson, 1949), containing 0.005 M- α -oxoglutarate as substrate, and mercurial brings about a marked loss of protein

nitrogen between 10 and 30 min. incubation, as compared with controls without added mercurials. The increased breakdown of tissue protein in the presence of mercurials may contribute to the renewed uptake of electrolyte and water by the tissue after 10 min. incubation, as shown above.

Effect of mercurials added during leaching on the transport of cations and water by slices of kidney cortex. The results reported above suggested that mercurials affected a process other than active transport of cations (and water?) by tubular cells, since no effect was observed in the first 10 min. of incubation. Further evidence was provided by the following experiment: if slices of kidney cortex are leached in isotonic NaCl containing 1.45×10^{-3} M-Esdrone (the leaching temperature of 0° inhibiting active transport), the tissue takes up considerably more Na^+ ion and water, and loses more K^+ ion, than controls leached in NaCl alone. If the slices leached

in NaCl and Esdrone are then incubated in phosphate-saline with or without substrate (the slices being quickly washed in NaCl before transferring them into vessels for incubation in order to remove adhering fluid containing mercurial, there being a residual component bound to tissue proteins), the rate of Na^+ ion and water loss, and the rate of K^+ ion uptake, do not differ from that of controls leached in NaCl, or may even be higher; however, a new steady state of the levels of Na^+ ion, water and K^+ ion in the tissue is established, the Na^+ ion and water content being higher, the K^+ ion content lower, than in the controls (Fig. 3). The same result was obtained with leaching in NaCl containing, for example, 4×10^{-4} M-mercuric chloride.

Since mercurials are firmly bound by the proteins of kidney cortex (Borghgraef & Pitts, 1956; Greif, Sullivan, Jacobs & Pitts, 1956; Leuschner, 1956),

Fig. 3. Effect of Esdrone added during leaching on transport of cations and water. Open points, controls; filled points, 1.45×10^{-3} M-Esdrone. For other conditions see text. Top set of curves: water; middle set: Na^+ ion; bottom set: K^+ ion.

Fig. 4. Fluxes of ^{24}Na and the effect of Esdrone thereon. —, Controls; ---, Esdrone (1.45×10^{-3} M) added during leaching; - - - - Esdrone added during incubation. The period of $T-T_0$ represents the passive influx of ^{24}Na (M_i); T_0-T_{10} represents the active extrusion of ^{24}Na (M_o). The columns represent passive influx of ^{24}Na added to separate groups of slices during incubation. Filled-in column, control; cross-hatched column, with Esdrone added during incubation. For details of conditions see text.

Table 3. *Effect of various mercurial preparations on the transport of cations and water in kidney-cortex slices during leaching*

Slices were leached at 0° for 2.5 hr. in 0.154 M-NaCl with or without a mercurial present. All values are in ml. or m-equiv./100 g. of dry solids.

Mercurial	Initial values			After leaching in NaCl			After leaching in NaCl with mercurial		
	Water (ml.)	Na^+ (m-equiv.)	K^+	Water (ml.)	Na^+ (m-equiv.)	K^+	Water (ml.)	Na^+ (m-equiv.)	K^+
HgCl_2 (4×10^{-4} M)	276	19.4	23.7	400	47.0	13.1	478	53.0	12.0
Esdrone (1.45×10^{-3} M)	317	24.5	30.4	438	52.2	11.3	512	66.0	8.3
p-Chloromercuribenzoate (10^{-4} M)	311	16.9	24.6	423	48.9	7.9	515	63.9	7.1

Table 4. *Effect of leaching in isosmolar sodium sulphate and sodium chloride on the transport of cations and water in kidney-cortex slices*

Slices of kidney cortex were leached at 0° for 2.5 hr. in 0.154M-NaCl (0.308 osmolar), 0.1145M-NaCl (0.229 osmolar) and 0.0764M-Na₂SO₄ (0.229 osmolar). All values are in ml. or m-equiv./100 g. of dry solids.

Time	Leaching medium	Water (ml.)	Na ⁺ (m-equiv.)	K ⁺ (m-equiv.)
Initial	—	298	20.7	22.6
T ₀	0.154M-NaCl	390	51.7	9.7
T ₀	0.1145M-NaCl	432	51.6	12.1
T ₀	0.0764M-Na ₂ SO ₄	368	48.8	8.2

this result is regarded as evidence that mercurials affect primarily the permeability of the tubular cell membrane for Na⁺ ion and water.

The increased uptake of Na⁺ ion and water by slices of kidney cortex was also observed with another mercurial preparation, *p*-chloromercuribenzoate, as shown in Table 3. The effect described appears therefore to be characteristic of mercuric compounds, inorganic and organic.

Effect of Esidrone on the fluxes of ²⁴Na. The experimental results reported above were confirmed in four experiments with ²⁴Na. The results of a representative experiment (Fig. 4) show that in the course of the experiment (lasting 10 min.) the addition of 1.45 × 10⁻³M-Esidrone to the medium did not affect the active transport of Na⁺ ion out of cells (*M_o*), while considerably increasing the passive inflow (*M_i*). Addition of Esidrone during leaching in NaCl at 0° greatly increased *M_i*, while the rate of extrusion of ²⁴Na from cells during 10 min. incubation at 25° was even higher than that of controls. This last observation suggests that the active transport of Na⁺ ion out of cells is also a function of the concentration gradient across the cell membrane. Such a conclusion has also been reached in experiments in which the concentration gradient of Na⁺ ion has been changed (Cort & Kleinzeller, 1957).

Effect of mercurials added during leaching on the transport of Cl⁻ ion. Since Schwartz & Wallace (1951) and Axelrod & Pitts (1952) have expressed the view, on the strength of experiments *in vivo*, that mercurials primarily affect the transport of Cl⁻ ion in the kidney, and since Koefoed-Johnsen & Ussing (1956) have reported experiments suggesting that another heavy metal (copper) decreases the permeability for Cl⁻ ion of the frog skin, further experiments were directed to elucidate the primary biochemical action of mercurials. The effect of mercurials on the transport of Cl⁻ ion was studied under conditions where the concentration gradient of Cl⁻ ion between tissue and medium was changed, without varying the concentration gradient of sodium. In these experiments SO₄²⁻ ion replaced Cl⁻ ion as the anion. The Na₂SO₄ solution used (0.0764M) is hypotonic as compared with 0.154M-NaCl solution, the Na⁺ ion concentration being the

same in both. Since the osmolarity of the medium is a determinant of water and cation transport during leaching (Robinson, 1950), as a preliminary to the study of the effect of mercurials the effect of the hypotonic Na₂SO₄ solution on these transport processes was examined. The results are presented in Table 4, in which changes of water and cation contents during leaching in 0.154M-NaCl are compared with changes on leaching in 0.0764M-Na₂SO₄ and 0.1145M-NaCl, the latter solution being isosmolar with the Na₂SO₄ solution.

It will be seen that the uptake of water and Na⁺ ion during leaching is less in the Na₂SO₄ solution than in the 0.154M-NaCl solution. Such a result would be expected in a hypotonic solution containing an anion to which the cell membrane is far less permeable than Cl⁻ ion. The uptake of water due to reduced osmolarity may be assessed as follows: slices leached in 0.1145M-NaCl took up an additional 42 ml. of water/100 g. of dry solids, as compared with slices leached in 0.154M-NaCl. This value has to be compared with the uptake of 70 ml. of water in slices leached in Na₂SO₄.

The following procedure was used to examine the effect of mercurial on the transport of Cl⁻ ion: kidney-cortex slices were leached at 0° in either 0.154M-NaCl or 0.0764M-Na₂SO₄, with or without 1.45 × 10⁻³M-Esidrone. After leaching the slices were incubated for 10 min. at 25° either in the usual phosphate-saline or in a modified medium in which all Cl⁻ ion was replaced by SO₄²⁻ ion; this medium is also somewhat hypotonic (245 m-osmolar as compared with 305 m-osmolar of the standard medium). The results of one such experiment are shown in Table 5.

It will be seen that Cl⁻ ion leaves cells practically completely on leaching in Na₂SO₄ solution, while Na⁺ ion increases. The continued increase in cell Na⁺ ion must be balanced by another anion. Our own results do not permit an answer to this question, but it may be suggested that protein degradation in the cold (Conway, Geoghegan & McCormack, 1955) might provide the additional anion equivalence.

In accordance with the low permeability of the cell membrane for SO₄²⁻ ion no appreciable effect of Esidrone on the uptake of water and Na⁺ ion was

Table 5. *Effect of Esidrone, added during leaching, on transport of Cl⁻ ion in kidney-cortex slices*

Slices were leached for 2.5 hr. at 0° in either 0.154M-NaCl or 0.077M-Na₂SO₄, with or without 1.45 × 10⁻³M-Esidrone. Slices were then incubated for 10 min. at 25° either in 3 ml. of phosphate-saline of usual composition (Cl⁻ ion medium), or in 3 ml. of a phosphate-saline in which Cl⁻ ion was replaced by SO₄²⁻ ion (SO₄²⁻ ion medium); 0.005M-α-oxoglutarate was present as substrate. All values are in ml. or m-equiv./100 g. of dry solids.

Leaching medium	Initial				T ₀				Cl ⁻ ion medium T ₁₀				SO ₄ ²⁻ ion medium T ₁₀			
	Water (ml.)	Na ⁺ (m-equiv.)	K ⁺ (m-equiv.)	Cl ⁻ (m-equiv.)	Water (ml.)	Na ⁺ (m-equiv.)	K ⁺ (m-equiv.)	Cl ⁻ (m-equiv.)	Water (ml.)	Na ⁺ (m-equiv.)	K ⁺ (m-equiv.)	Cl ⁻ (m-equiv.)	Water (ml.)	Na ⁺ (m-equiv.)	K ⁺ (m-equiv.)	Cl ⁻ (m-equiv.)
—	302	32.6	30.1	19.8	—	—	—	—	—	—	—	—	—	—	—	—
NaCl	—	—	—	—	412	58.8	13.6	46.3	325	37.1	22.2	29.9	346	34.5	21.6	4.6
NaCl + Esidrone	—	—	—	—	475	78.4	10.3	59.6	393	48.4	17.8	42.9	391	51.8	14.0	4.0
Na ₂ SO ₄	—	—	—	—	376	54.3	9.5	0	332	48.8	21.2	23.9	364	41.8	19.7	0
Na ₂ SO ₄ + Esidrone	—	—	—	—	365	52.3	8.4	0	357	41.8	23.6	21.8	359	38.0	22.6	0

Table 6. *Transport of cations and water in kidney slices leached in the presence of Esidrone and incubated in phosphate-saline with and without K⁺ ion*

Slices were leached at 0° for 2.5 hr. in 0.154M-NaCl without and with 1.45 × 10⁻³M-Esidrone. Slices were then incubated at 25° in 3 ml. of phosphate-saline (6.25 m-equiv. of K⁺ ion/l.) or phosphate-saline in which K⁺ ion was replaced by an equivalent amount of Na⁺ ion; 0.005M-α-oxoglutarate was present as substrate. All values are in ml. or m-equiv./100 g. of dry solids.

Leaching medium	K ⁺ ion concn. in incubation (m-equiv./l.)	Initial				T ₀				T ₁₀				T ₃₀			
		Water (ml.)	Na ⁺ (m-equiv.)	K ⁺ (m-equiv.)	Water (ml.)	Na ⁺ (m-equiv.)	K ⁺ (m-equiv.)	Water (ml.)	Na ⁺ (m-equiv.)	Water (ml.)	Na ⁺ (m-equiv.)	K ⁺ (m-equiv.)	Water (ml.)	Na ⁺ (m-equiv.)	Water (ml.)	Na ⁺ (m-equiv.)	K ⁺ (m-equiv.)
NaCl	6.25	313	23.3	21.9	391	45.0	11.1	309	31.9	309	31.9	19.0	294	29.8	294	29.8	23.4
NaCl + Esidrone	6.25	—	—	—	590	70.6	10.7	417	56.3	417	56.3	16.1	369	47.3	369	47.3	16.2
NaCl	0	—	—	—	—	—	—	323	41.7	323	41.7	10.6	285	42.1	285	42.1	10.5
NaCl + Esidrone	0	—	—	—	—	—	—	442	65.2	442	65.2	8.4	391	59.2	391	59.2	8.3

observed on leaching in 0.0764M- Na_2SO_4 . This result also suggests that mercurials do not affect the extracellular space of the slices and that mercurials during leaching act directly on tubular cells.

Slices leaching in Na_2SO_4 and then incubated in the standard phosphate-saline lose water and re-accumulate K^+ ion at approximately the same rate as those leached in NaCl, the extrusion of Na^+ ion being considerably slower. However, slices leached in Na_2SO_4 containing Esidrone extrude Na^+ ion and re-accumulate K^+ ion, while their content of water does not change. This is the third instance where a dissociation between the transport of water and Na^+ or K^+ ion has been observed (see Cort & Kleinzeller, 1956). Concerning the movement of Cl^- ion, it must be pointed out that Cl^- ion is lost on leaching in Na_2SO_4 and re-accumulates at the same rate on incubation in standard phosphate-saline, whether or not the slices were leached in Esidrone.

Slices leaching in either NaCl or Na_2SO_4 , with or without Esidrone, and incubated in the SO_4^{2-} ion medium, showed the following behaviour: slices leached in NaCl with or without Esidrone lost water and Na^+ ion, and re-accumulated K^+ ion in the same manner as in the standard phosphate-saline. At the same time a loss of Cl^- ion occurred in accordance with its concentration gradient. Slices leached in Na_2SO_4 with or without Esidrone extruded Na^+ ion and re-accumulated K^+ ion without appreciable change in water content. It should be mentioned that the rate of O_2 uptake is the same whether phosphate-saline or the SO_4^{2-} ion medium is used (Q_{O_2} in standard phosphate-saline: -25.2 ; Q_{O_2} in the SO_4^{2-} ion medium: -25.3). It will be noted that brain-cortex slices also do not show an impaired metabolic activity when incubated in a Cl^- ion-free medium (Thomas & McIlwain, 1956). It

thus appears that in kidney or brain cortex such metabolic activities as oxidation of substrates do not require Cl^- ion.

The results reported above are difficult to reconcile with the suggestion that mercurials affect a specific transport process for Cl^- ion, especially that part of the experiment where slices were leached and incubated in the appropriate SO_4^{2-} ion media.

Effect of Esidrone added during leaching on the transport of K^+ ion. It might be concluded from the work of Mudge (1951, 1953) that mercurials acted primarily on the transport of the K^+ ion. If that were the case, slices leached in NaCl and mercurial might behave differently from controls when incubated in a medium not containing K^+ ion. It has been shown previously (Cort & Kleinzeller, 1956) that slices leached in NaCl and then incubated in a modified phosphate-saline without K^+ ion lose water, the cation content remaining constant. Slices leached in NaCl and Esidrone behave in the same way (Table 6). The loss of water during incubation does not appear to be dependent on the intracellular concentration of K^+ ion within the limits of about 5 m-equiv./100 g. of dry solids (lowest values for K^+ ion in slices leached in NaCl and Esidrone) and about 30 m-equiv./100 g. of dry solids (normal slices).

Effect of mercuric chloride added during leaching on the transport of cations and water in other tissues. In view of the drastic effect of mercurials on renal function it was considered of interest to examine the effect of mercuric chloride during leaching on the transport of cations and water in various tissues. The results are summarized in Table 7. It will be seen that leaching in NaCl containing 5×10^{-4} M-mercuric chloride increases the Na^+ ion and water content in brain and heart, as in kidney, but does

Table 7. *Effect of 5×10^{-4} M-mercuric chloride on the transport of cations and water during leaching of various tissues from the guinea pig*

Slices of tissue were leaching at 0° for 2.5 hr. in 0.154M-NaCl with and without 5×10^{-4} M- HgCl_2 . All values are in ml. or m-equiv./100 g. of dry solids. Standard error is listed for those values where five experiments were performed. The figures otherwise presented are averages. The number of experiments is given in parenthesis.

Tissue	Initial values			After leaching in NaCl			After leaching in NaCl + HgCl_2		
	Water (ml.)	Na^+ (m-equiv.)	K^+	Water (ml.)	Na^+ (m-equiv.)	K^+	Water (ml.)	Na^+ (m-equiv.)	K^+
Kidney (2)	354	21.3	29.6	431	42.4	13.1	544	63.5	9.2
Liver (2)	245	10.4	29.0	524	76.5	6.6	496	73.8	5.6
Brain cortex (5)	501 ± 14	36.7 ± 3.0	31.8 ± 3.6	887 ± 34	105.6 ± 5.7	6.9 ± 0.9	954 ± 17	116.9 ± 7.3	3.2 ± 0.5
Diaphragm (2)	302	19.8	28.8	570	71.2	6.9	558	75.8	4.1
Somatic muscle (gracilis) (5)	368.5 ± 15	16.6 ± 1.3	38.2 ± 1.7	577 ± 11	79.8 ± 3.9	8.3 ± 0.8	588 ± 27	74.8 ± 4.3	4.9 ± 0.9
Myocardium (5)	368 ± 2	14.3 ± 0.7	22.3 ± 0.6	477 ± 8	66.1 ± 1.6	5.9 ± 0.3	523.5 ± 18	72.5 ± 2.1	3.3 ± 0.4

Table 8. *Effect of various concentrations of p-quinone during leaching on the transport of cations and water in kidney-cortex slices*

Slices were leached at 0° for 2.5 hr. in 0.154M-NaCl with or without quinone. All values are in ml. or m-equiv./100 g. of dry solids.

p-Quinone concn. (M)	Time	Water (ml.)	Na ⁺ (m-equiv.)	K ⁺ (m-equiv.)
—	Initial	291	21.2	21.4
—	T ₀	397	47.3	12.1
5 × 10 ⁻⁴	T ₀	442	50.2	11.4
10 ⁻³	T ₀	435	53.0	11.7
2 × 10 ⁻³	T ₀	477	57.4	6.5

not affect the Na⁺ ion and water content in liver or striated muscle (gracilis and diaphragm). However, in all tissues examined mercuric chloride brought about a decrease of the K⁺ ion content.

Effect of p-quinone on the transport of cations and water during leaching. Since p-quinone reacts with SH groups (see Barron, 1951), its effect on the transport of cations and water in kidney-cortex slices during leaching was examined. As shown in Table 8, p-quinone in concentrations 5 × 10⁻⁴–2 × 10⁻³M brings about a considerable increase of Na⁺ ion and water content of the slices, as compared with slices leached in NaCl alone. Further experiments, not reported in detail here, showed that the effect of p-quinone during leaching is partly reversible on incubation in a similar manner to the effect of mercurials.

DISCUSSION

Effect of mercurials on electrolyte transport in kidney-cortex cells. The results reported above indicate that mercurials (organic and inorganic) increase the passive movement of Na⁺ ion, Cl⁻ ion and water into renal tubular cells, leaving unaffected active transport of Na⁺ ion out of the cells. It may also be argued that the changes depicted in Figs. 1 and 2 could be the result of a relatively slow penetration of mercurials into the cells. However, two facts militate against such an interpretation of the results. First, the results obtained on leaching slices in sodium chloride containing Esidrone (Fig. 3) or mercuric chloride: since it is known that mercurials are firmly bound by proteins of kidney tissue and cannot be removed, e.g. by dialysis (Greif *et al.* 1956), this experiment appears to exclude the possibility of mercurials affecting active-transport processes. Also our experiments with ²⁴Na in the medium did not show any change of sodium efflux as compared with controls. Secondly, it should be noted that a similar, if somewhat more rapid, effect on the movement of Na⁺ ion and water during incubation was obtained with nearly equivalent amounts of either mercuric chloride or Esidrone, in spite of considerable differences of molecular weight (equivalence being calculated on

the basis of mercury free to react with SH groups; such calculation appears to be justified in view of the fact that in experiments both *in vivo* and *in vitro* it has been reported that the C–Hg bond in organic mercurials is not broken by the metabolic activity of the whole organism (Weiner & Müller, 1955) or tissue homogenate (Leuschner, 1956). The uptake of Na⁺ ion, Cl⁻ ion and water by the slices after the first 10 min. of incubation appears therefore to be the result of an irreversible damage to the cells by the high concentrations of mercurials present, accompanied by a release of protein nitrogen into the medium, rather than the result of inhibition of active transport. In all experiments it was found that mercurials added during leaching brought about a decrease in cell K⁺ ion, whether or not Na⁺ ion and water content was affected. This phenomenon was not studied further, but it would appear from the results of Mudge (1953) that mercurials may have affected the amount of non-exchangeable K⁺ ion in the tissue.

The view that mercurials primarily affect the transport of Cl⁻ ion (Schwartz & Wallace, 1951; Axelrod & Pitts, 1952) is based essentially on observation of the effect of mercurials on excretion of Cl⁻ ion and chloride balance in whole animals and humans. This conclusion must be taken as indirect since the experimental approach precluded any information about cellular changes in the kidney. Not only have our own results failed to confirm this view, but it is clear from the experiments in which SO₄²⁻ ion replaced Cl⁻ ion in the medium, that in fact Cl⁻ ion and Na⁺ ion can move in opposite directions across the cell membrane at a time when there is a clear effect of mercurials on Na⁺ ion penetration. These results also provide further evidence for the view (Whittam, 1956) that in kidney-cortex cells Cl⁻ ion is passively transported. The only clear effect on electrolytes that we could demonstrate for mercurials was on the diffusion resistance of the tubular-cell membrane for Na⁺ ion.

Considerable evidence has been accumulated showing that mercurials affect metabolic processes of the cells by combining with SH groups of cell enzymes. Since it has been here shown that mercurials primarily decrease the resistance of the

cell membrane towards passive movements of Na^+ ion and water, it may be concluded that such SH groups of the protein component of the cellular membrane are of importance in the regulation of cell permeability. In view of the fact that only kidney-cortex tissue and, to a lesser extent, myocardium and brain cortex appear to be affected by mercurials, it would follow that these tissues may contain in their cell membranes a protein component capable of combining specifically with mercurial preparations. It should be mentioned that in myocardium radioactive mercury is fixed on the cellular membrane, as shown by autoradiography (Koshtoyants, personal communication).

Mercurials are most often used clinically in congestive cardiac failure, but aside from kidney and brain, the myocardium is the only other significant tissue (including somatic muscle) whose transport activity is specifically depressed by mercurials.

Mechanism of diuretic action of mercurials. Recently, Borghgraef & Pitts (1956) provided evidence that the main site of action of mercurial diuretics is the cells of the renal cortex, since 80 % of the mercurial injected was localized in this tissue. How do the concentrations used in our experiments compare with those required for a physiological or clinical effect in the intact animal? Since diuresis is produced clinically by the intravenous administration of 2 ml. (0.216 m-mole) of Esidrone, and since both average human kidneys are 600–700 g., of which about two-thirds is cortex, one may roughly calculate that the concentration of mercurial/l. of cortical-tissue water was of the order of $5 \times 10^{-4}\text{M}$. This figure is about one-third of the concentration used by us, but it must be pointed out that the effect of mercurial on tissue slices can be observed with far lower concentrations, but that for purposes of greater constancy and statistical significance we wished to measure a maximal, but not too toxic, effect. Mudge (1951) found a clear effect of mercuric chloride on kidney-cortex slices at a concentration as low as $3 \times 10^{-4}\text{M}$. The overwhelming amount of mercurial in kidney was found in the soluble fraction of the disintegrated tubular cells (Greif *et al.* 1956). In view of the very high activity of mitochondria as compared with whole cells in the active transport of cations (see Davies, 1954), and the relatively small proportion of mercury bound by the mitochondrial fraction (Greif *et al.* 1956), these results would also be consistent with the view that mercurials affected a process other than the active extrusion of Na^+ ion. By histochemical methods no changes of the morphology of tubular-cell mitochondria have been found after administration of mercurial (Lojda, personal communication). The main mechanism of action of mercurials *in vivo* appears therefore to be a change in the permeability of the cell membrane

of tubular cells, thus increasing the passive movement of Na^+ ion, Cl^- ion and water into cells, and resulting in a reduction of total reabsorbed Na^+ ion, Cl^- ion and water.

It has been previously suggested (Cort & Kleinzeller, 1956) that the active extrusion of Na^+ ion from tubular cells is primarily localized at or near the basal membrane of the tubular cells. Since this membrane is in most direct contact with the blood vessels through which mercurials are transported in the kidney and into which the flow of Na^+ ion and water from tubular urine is directed, it would appear to be in accord with available evidence to suggest that this is also the place where mercurials exert their effect, by chemical reaction with SH groups of the membrane proteins. Since mercurials and mercury in blood are bound to albumin (Edsall, 1954), only an insignificant fraction of serum mercury would be filtered.

Role of SH groups of membrane proteins in cellular permeability. The results obtained with *p*-quinone provide further evidence, in addition to that obtained with mercurials, that SH groups of proteins of the cell membrane play a role in the permeability of tubular cells for cations and Cl^- ion. So far, no conclusive results have been obtained with dehydroascorbic acid as an acceptor of hydrogen from the SH groups, in conditions under which *p*-quinone was effective, although it is known to oxidize SH groups of, for example, glutathione, in a non-enzymic reaction (Szent-Györgyi, 1928). An explanation for this may be sought in the lower redox potential of the dehydroascorbic-ascorbic acid system as compared with the *p*-quinone-quinol system. No consistent results have been obtained with reduced glutathione (10^{-3}M) added during leaching, although in most of the experiments the Na^+ ion content in the slices was slightly lower, the K^+ ion content somewhat higher, than in the controls without glutathione.

Davson (1943) and LeFevre (1948) provided evidence suggesting that SH groups in the membrane of erythrocytes also played a role in the permeability of these cells. It would appear therefore that the state of SH groups in membrane proteins (which is a reflexion of the redox potential at the cell surface) influences cell permeability and is thus of more general importance for cell metabolism. Such conclusion is in full agreement with that of Barron (1951).

SUMMARY

1. The effect of mercuric chloride and organic mercurials [Esidrone (CIBA), Novurit (SPOFA), *p*-chloromercuribenzoate] and *p*-quinone on the transport of Na^+ , K^+ and Cl^- ions and water by slices of rabbit kidney cortex was investigated.

2. Addition of 1.45×10^{-3} M-Esidrone, or 2.8×10^{-3} M-Novurit, affected only slightly the decrease of Na^+ ion and water, and increase of K^+ ion, during 10 min. incubation at 25° . Further incubation in the presence of these mercurials brought about a new uptake of Na^+ ion and water, and a loss of K^+ ion. The same response was found with $4-5 \times 10^{-4}$ M-mercuric chloride. Chloride ion followed Na^+ ion changes.

3. Incubation of slices of kidney cortex for 30 min. in the presence of mercuric chloride or Esidrone at the above concentration brought about a loss of protein nitrogen into the medium, as compared with controls without mercurials.

4. Slices of kidney cortex, leached at 0° in 0.154 M-sodium chloride containing 5×10^{-4} M-mercuric chloride or 1.45×10^{-3} M-Esidrone or 10^{-4} M-*p*-chloromercuribenzoate, took up considerably more Na^+ ion and water than controls leached in sodium chloride alone. On further incubation at 25° , the decrease of Na^+ ion and water, and the uptake of K^+ ion, proceeded at the same rate as in controls. However, a new steady state was established in slices leached in the presence of mercurials, in that Na^+ ion and water came into equilibrium at higher levels, K^+ ion at lower levels, than in controls.

5. Measurements of fluxes of ^{24}Na showed that 1.45×10^{-3} M-Esidrone increased the passive influx of Na^+ ion, leaving the active extrusion of Na^+ ion unaffected.

6. The transport of Cl^- ion followed its own concentration gradient irrespective of whether mercurials were present or not.

7. Slices of brain cortex and myocardium took up more Na^+ ion and water on leaching at 0° in isotonic sodium chloride containing 5×10^{-4} M-mercuric chloride than controls leached without added mercuric chloride, while striated muscle (gracilis and diaphragm) and liver were not affected.

8. *p*-Quinone (5×10^{-4} – 2×10^{-3} M) added during leaching in 0.154 M-sodium chloride at 0° brought about an increased uptake of Na^+ ion and water. The effect of *p*-quinone was reversible on incubation at 25° .

9. The mechanism of diuretic action of mercurials is discussed. It is suggested that mercurials change the permeability of the basal membrane of renal tubular cells, thus increasing the passive movement of Na^+ ion, Cl^- ion and water back into cells, resulting in a decreased reabsorption of salt and water.

10. It is suggested that the state of SH groups of proteins in the membrane of renal tubular cells is a determinant of cell permeability to Na^+ ion and perhaps water.

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