

ACCUMULATION OF SULFATE LABELLED WITH S^{35} BY RAT TISSUE IN VITRO*

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INTRODUCTION

During the course of studies on the water and electrolyte exchange of rat renal tissue *in vitro*, it has been observed that, under specifically defined conditions, sulfate ion labelled with S^{35} may be strikingly concentrated within the tissue as compared with its concentration in the medium in which the tissue is incubated. This observation was followed up in some detail because of the chance that it might offer an approach to the hitherto little studied mechanisms of renal sulfate transport. In addition, information about vertebrate sulfate metabolism is scanty, and it seemed worthwhile to explore the possibility of gaining further information in this general field.

Methods

Briefly, the method used in the present experiments was to compare the concentration of S^{35} -labelled sulfate in tissue fragments and in the various defined media in which they were incubated for stated periods of time.

Preparation and Incubation of Tissue Samples.—Tissue fragments were obtained from kidneys and other organs of rats sacrificed by exsanguination after rapid general anesthesia with ether. Kidney tissue samples were prepared as thin slices (about 0.35 mm. in thickness) and divided, by means of a razor blade, into cortical and medullary regions (1, 2). In most experiments, only the cortical fragments were used. Occasionally, however, comparisons were made of $S^{35}O_4$ distribution in two roughly defined regions of the medulla as well—a more peripheral region, contiguous with the cortex, and a more central portion consisting largely of the renal pelvis and large collecting ducts. Similarly, in some comparative experiments, liver slices were prepared and, further, unsliced strips of colon, diaphragm, and aorta were tested occasionally. Such tissue samples were transferred as rapidly as possible to Warburg

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flasks containing 1.5 or 2.0 ml. of incubation medium, and, in general, were incubated in a Warburg apparatus for periods of $\frac{1}{2}$ to $1\frac{1}{2}$ hours at 37°C . with O_2 atmosphere. The media in which the tissues were immersed varied considerably in composition, although in every case the total solute concentration was maintained as close as possible to being equivalent to 0.15 M NaCl (300 m.osm/liter). In many experiments, Krebs-Ringer-phosphate (3) or a similar solution buffered with $\text{K}_2\text{HPO}_4\text{-HCl}$ instead of $\text{Na}_2\text{HPO}_4\text{-HCl}$ was used. In other tests, incubation media were made of simpler mixtures of isotonic solutions, including 0.15 M NaCl , 0.15 M KCl , and 0.3 M sucrose . Occasionally, glucose or sodium acetate was utilized as substrate, and sometimes NaCN was incorporated into the medium or added from the side-arm of the Warburg flask. The use of the various solutions mentioned will be given in more detail below. Radiosulfate-containing sulfate ion was added to the medium in the form of a carrier-free solution of H_2SO_4 . This was obtained from the Atomic Energy Research Establishment, Harwell, England (initial activity: 45.7 mc./ml.). The original solution was diluted by a factor of the order of 2×10^4 in the preparation of the final incubation media.

Measurement of Tissue Water, Na, K, and S^{35}O_4 Contents.—Control (non-incubated) slices and slices which had been incubated were, in general, divided into aliquots for weight and ion determinations, and for measurement of radiosulfate content. The aliquots taken for weight determinations were blotted quickly and placed in tared aluminum foil cups. They were weighed rapidly on a torsion balance, then dried for 2 hours between 100 and 110°C ., and reweighed, so that it was possible to define wet and dry weights and water content for every tissue sample. Subsequently, many of the samples were ashed for several (12 to 36) hours at 500°C . prior to solution in 0.01 to 0.1 N HCl for flame photometric analysis (Beckman flame photometer) of Na and K contents. The activities of tissue samples and aliquots of the media were estimated as follows: Tissue samples were extracted with relatively large volumes of water (at least 2 ml. water per 50 mg. of tissue) for 24 hours at room temperature, whereas media were diluted with distilled water in order to reach a final solute concentration approximating that of the tissue extracts. In general, duplicate samples of diluted media or tissue extracts were transferred in $100 \mu\text{l}$. aliquots to aluminum foil counting cups backed with lens paper discs and containing $25 \mu\text{l}$. aliquots of 10 per cent dextrose solution. The samples were dried, and the activity measured by means of a mica window (thickness 0.013 mm ., corresponding to 3.7 mg./cm^2) counter and scaling circuit.

Calculations Relating to Distribution of S^{35}O_4 in Tissues.—On the basis of the measured radiosulfate content, and wet and dry weights of the tissue, calculations were made of the relative distribution of S^{35}O_4 in the tissue samples as compared with the medium. More specifically, let

S_t = total amount of S^{35} in the tissue sample,

(S_m) = concentration of S^{35} in the external medium,

W_t = measured mass of tissue water;

then

$S_t/(S_m)$ = the virtual volume of water which would have to exist within the tissue in order that the concentration of S^{35} in the tissue water be equivalent to the concentration in the medium,

and

$[S_t/(S_m) \times 1/W_t] \times 100 =$ the virtual volume of tissue water expressed as per cent of the measured tissue water. This value will be designated, in the discussion to follow, as the *tissue relative sulfate volume*.

It may be noted that a calculated tissue relative sulfate volume of 100 might indicate that $S^{35}O_4$ was uniformly distributed throughout the tissue water, or that it was excluded from some cells and, in others, concentrated as compared with its level in the medium. Values of this function significantly above 100 (see discussion of errors, below) may be taken as unequivocal evidence that $S^{35}O_4$ is concentrated in at least some cells within the tissue. In the discussion which follows, values significantly above 100 are designated as indicative of "sulfate accumulation" without, however, any necessary implication of the mechanisms (exchange, actual uptake, etc.) of this concentration. Nor is the term "sulfate accumulation" used with explicit supposition that the S^{35} measured as within the tissue is in the chemically defined form of sulfate ion, SO_4^{2-} .

Sources of Error in the Procedure Used for Calculating Relative $S^{35}O_4$ Volumes of Tissues in Vitro.—The experimental error associated with the counting of individual $S^{35}O_4$ samples was estimated as of the order of ± 3 per cent. The simple method of extraction for obtaining radiosulfate in a form in which self-absorption by the samples could be disregarded was tested by placing small known volumes of $S^{35}O_4$ -containing solutions adjacent to measured amounts of kidney tissue. After allowing 40 to 150 minutes for the $S^{35}O_4$ to be partially or completely distributed in the tissue water, an attempt was made to test the recovery of sulfate. It was found that the recovery varied from 95.5 to 108.3 per cent (20 tests, average, 100.9 per cent) of the theoretical. In the procedure outlined in the preceding paragraphs, it is obvious that error is inherent because of the contamination of the tissue slices with adsorbed incubation medium. Furthermore, the tissue surface is irregular, and small gross deposits of medium may remain within such irregularities even after blotting. A certain proportion of the cells near the cut surface of the tissue slice must be severely damaged and, perhaps, opened for rapid penetration of the components of the medium. To evaluate the magnitude of the error resulting from these sources, tests were made in which samples of tissue were immersed in isotope-containing media, withdrawn as quickly as possible, blotted, and extracted according to the procedure used for incubated tissues. Virtual volumes of radiosulfate distribution, calculated as percentages of the total tissue water, ranged, for the various tissues used, from 8 to 34 per cent for kidney cortex to occasional values as high as 45 per cent for the highly irregular fragments of colon. Additional errors in the measurement, not directly connected with the calculation of $S^{35}O_4$ distribution, are not discussed here in detail because, in essence, they are the same as errors in previous studies on renal tissues *in vitro* (2).

RESULTS

Even the earliest preliminary experiments disclosed that the distribution of radiosulfate in renal cortical strips was such as to suggest the rapid penetration of the ion into tissue cells, and, indeed, an actual concentration of the substance in the tissue as compared with its concentration in the medium. Subsequent, more detailed studies showed that the ratio (apparent radio-

sulfate concentration in tissue water):(radiosulfate concentration in medium water) could rise to values as high as 15 to 17 during $\frac{1}{2}$ hour's incubation under definable conditions. Thus, during incubation at 37°C . in artificial media containing relatively high concentrations of K^+ (e.g., 0.04 M) and low concentrations of Na^+ , a marked concentration gradient of radiosulfate in the tissue as compared with the medium could develop (see, for instance, Fig. 1).

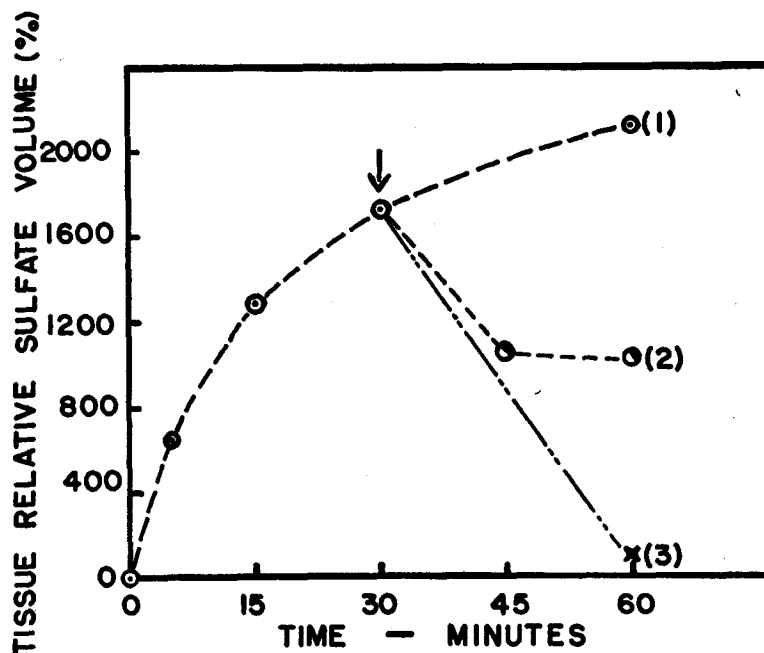


Fig. 1. Changes in renal tissue relative sulfate volume. Tissue incubated $\frac{1}{2}$ hour at 37°C . with oxygen atmosphere in medium 0.04 M KCl + 0.22 M sucrose. From the arrow, conditions as follows: Curve 1, incubation at 37°C . continued for additional $\frac{1}{2}$ hour. Curve 2, tissue in Warburg flask allowed to stand quiescently and cool to 23°C . Curve 3, NaCN added from side-arm of Warburg flask to give final concentration of CN^- in incubation medium of 0.02 M.

Such a concentration ratio failed to develop if NaCN (tested in a final concentration in the medium of 0.002 to 0.05 M) was present in the medium, and was abolished if CN^- was added during the course of incubation (Fig. 1). Likewise, other ways of interference with respiratory conditions reduced sulfate accumulation. Thus, Fig. 1 shows, too, the results of a representative experiment in which tissue slices which had been incubated in a radiosulfate-containing medium were cooled from 37°C . to $20\text{--}23^{\circ}\text{C}$. and allowed to remain quiescent for $\frac{1}{2}$ hour, with resultant appreciable decline in the tissue/medium

sulfate ratio. It may be noted that the addition of glucose (0.03 M) and sodium acetate (0.01 M) as exogenous substrates was without significant effect on the phenomenon of accumulation (Table I).

TABLE I
Effect of Substrates (Glucose and Sodium Acetate) on Tissue Relative Sulfate Volume

Substrate present	No. of experiments	Composition of medium	Tissue relative sulfate volume
None	4	KRPO ₄ *	219 ± 24
0.03 M glucose	4	"	180 ± 37
None	5	0.04 M KCl, 0.22 M sucrose	1410 ± 207
0.03 M glucose	4	0.04 " " , 0.19 " "	1314 ± 210
None	5	0.04 M KCl, 0.20 M sucrose, 0.01 M NaCl	1291 ± 380
0.01 M sodium acetate	5	0.04 M KCl, 0.20 M sucrose	1145 ± 90

* Krebs-Ringer-phosphate, pH 7.4.

TABLE II
Results of 8 Experiments in Which Renal Cortex Was Incubated for 1/2 to 1 1/2 Hours (37°C. O₂ Atmosphere) in Media Containing High Concentrations of Carrier SO₄⁻

Each figure represents the amount of exchangeable sulfur, calculated as percentage of the tissue dry weight, which would have to be present in the tissue in order to account for the observed SO₄ uptake on the basis of exchange alone. Data given are results of individual tests.

Duration of incubation	Calculated tissue sulfur for concentrations of carrier sulfate in the medium of		
	125-155 μM	530 μM	1030 μM
<i>hrs.</i>			
1/2	1.38 1.66 2.19 2.92	5.51 7.44	8.65 26.9
1	3.08 4.01	6.45 6.68	6.58 12.8
1 1/2	3.62 4.25	10.9 11.8	6.22 11.2

That the increased concentration of radiosulfate in the tissue as compared with the concentration in the medium represented an actual uptake of labelled sulfate, rather than a mere exchange of ions between the medium and sulfate or other S-containing entities initially present within the tissue, was demonstrated by results of experiments summarized in Table II. Kidney cortex was incubated in media containing relatively high concentrations of sodium

sulfate. To cite some representative data, the tissue relative sulfate volume ranged from 420 to 1310 per cent of the measured tissue water after $\frac{1}{2}$ hour's incubation in media which were 0.5 to 1.0 mM with respect to Na_2SO_4 . It may be seen in Table II that, if these values were attributable to exchange alone, it would be necessary to postulate that 6.45 to 12.8 per cent of the tissue dry weight was exchangeable sulfur. Such a conclusion appears absurd, and the

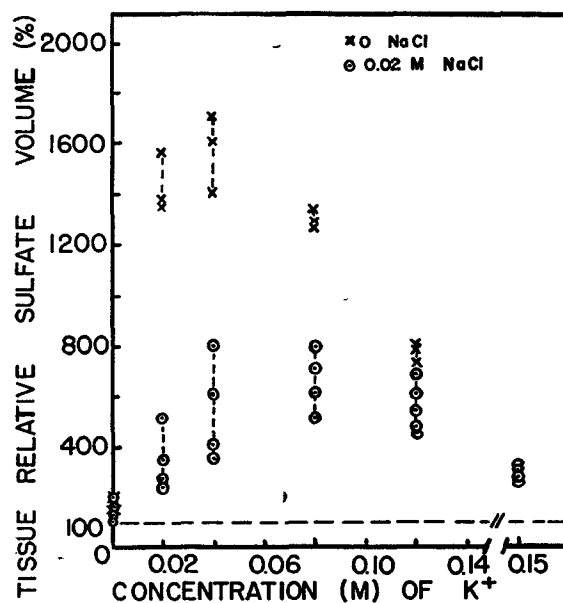


FIG. 2. Effect of varying concentrations of potassium ion in the incubation medium on radiosulfate uptake by kidney cortical slices. Incubation for $\frac{1}{2}$ hour, 37°C ., oxygen atmosphere. Most incubation media contained NaCl at concentrations of 0 or 0.025 M, and KCl and sucrose were present in concentrations varying to give a total solute concentration of 300 m.osm/liter. In one series of tests, incubation was in 0.15 M KCl only.

alternative hypothesis, that there is a real net uptake of sulfate by kidney tissue *in vitro*, is thus supported.

The quantitative dependence of sulfate accumulation on the presence and concentrations of the cations Na^+ and K^+ in the incubation medium has been mentioned above. Figs. 2 and 3 represent the results of experiments in which tissues were incubated in mixtures of 0.15 M NaCl , 0.15 M KCl , and/or 0.30 M sucrose. When NaCl concentration was held fixed (*e.g.*, at 0.02 M) and KCl allowed to vary, it was found that sulfate accumulation increased up to a concentration of 0.04 to 0.08 M KCl in the medium, and thereafter declined (Fig.

2). On the other hand, if KCl was fixed at its optimal concentration while NaCl varied, the sulfate accumulation decreased with rising NaCl concentrations (Fig. 3). Moreover, the presence of the cations calcium and magnesium depressed sulfate accumulation in media of which KCl and sucrose were the other constituents. In experiments in which comparative tests of the effects of variations in the concentrations of these ions were made, it was found that

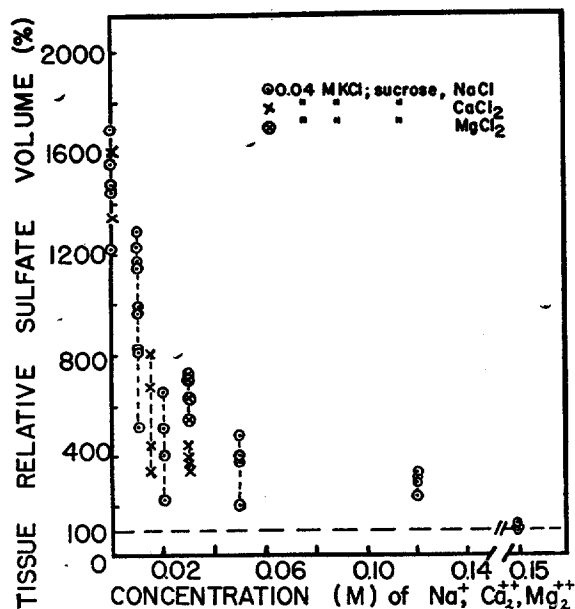


FIG. 3. Effect of varying concentrations of sodium ion in incubation medium on radiosulfate uptake by kidney cortical slices. Incubation for $\frac{1}{2}$ hour, oxygen atmosphere, 37°C . Most incubation media contained KCl at 0.04 M, and NaCl and sucrose were present in varying concentrations to give a total solute concentration of 300 m.osM/liter. In one series, incubation was in 0.15 M NaCl only. Also, data are included from experiments in which NaCl was absent from the medium, KCl was at a concentration of 0.04 M, and either CaCl_2 or MgCl_2 was present in concentrations of 0.001 to 0.03 M.

their effects were approximately similar to those of similar molarities of NaCl (Fig. 3). Choline ion resembled sodium in this respect, also. Thus, in modified Krebs-Ringer-phosphate solutions in which choline chloride was substituted for sodium chloride, and sodium phosphate was replaced by potassium phosphate, accumulation occurred and was slightly but consistently greater than in ordinary (Na^+ -containing) Krebs-Ringer-phosphate.

Kidney cortical tissue shows marked alterations in its content of sodium and potassium when incubated in various artificial media *in vitro* (4, and

others). It was found in the present study that sulfate accumulation showed a fairly consistent inverse variation with respect to tissue sodium concentration. Thus, as shown in Fig. 4, the data scatter about a curve approximating a hyperbola. On the other hand, the relationship between sulfate accumulation and tissue potassium content showed an entirely different pattern (Fig. 5). When the external medium was free of exogenous potassium, tissue potassium content was low and sulfate accumulation slight. At external concentrations of 0.02 to 0.04 M K^+ , the potassium content of tissues incubated for

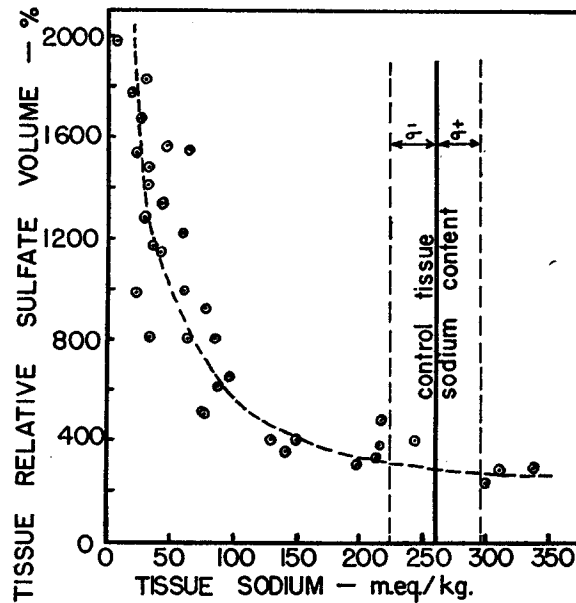


FIG. 4. Relationship between tissue relative sulfate volume and tissue sodium content. Results of 26 experiments on kidney cortical slices. Medium potassium concentration maintained at 0.04 M, sodium content varied from 0 to 0.11 M.

$\frac{1}{2}$ hour was observed to approximate the control (preincubation) level, and sulfate accumulation was maximal at the upper of these two values. Finally, as external KCl concentration rose towards the maximal ("isosmotic") value, 0.15 M, tissue sulfate declined with rising tissue potassium content.

As yet, a systematic series of experiments to characterize the pH dependence of sulfate accumulation has not been undertaken. It has been observed, however, that the uptake of sulfate in Krebs-Ringer-phosphate solution, buffered at pH 7.4, was essentially the same as in unbuffered mixtures of electrolytes containing Na^+ and K^+ concentrations equivalent to the concentrations in Krebs-Ringer-phosphate. In the unbuffered solutions, it may be supposed that

pH variations may be appreciably greater than in Krebs-Ringer-phosphate, and the results would suggest that close constancy of the pH of the external medium is not a critical factor affecting renal sulfate accumulation *in vitro*. A similar conclusion was reached by Cross and Taggart (5) with respect to PAH accumulation in kidney slices.

In the present series of experiments, some attempts were made to determine whether the phenomenon of sulfate accumulation was peculiar to the cortex of the rat's kidney, or could occur with other tissues as well. The results, sum-

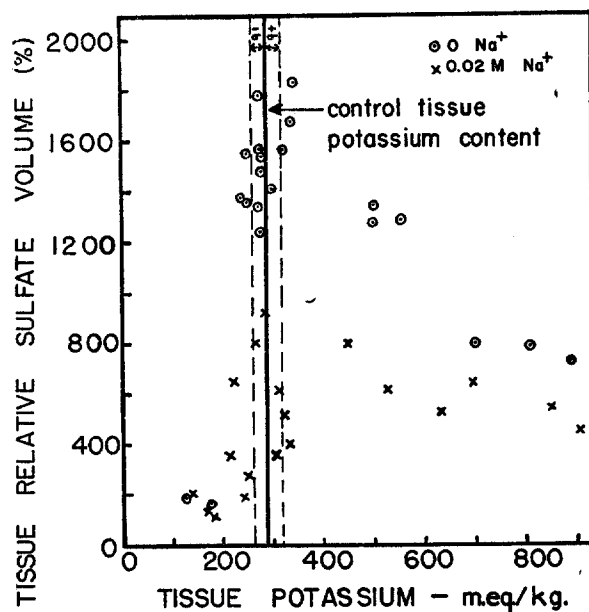


FIG. 5. Relationship between tissue relative sulfate volume and tissue potassium content. Results of 26 experiments on kidney cortical slices. Medium sodium concentration maintained at 0 or 0.02 M, potassium content varied from 0 to 0.15 M.

marized in Table III, indicate that the more peripheral portions of the renal medulla showed sulfate accumulation which might be even greater than the degree of uptake by kidney cortex under comparable conditions. Liver, also, showed some accumulation. S^{35} -containing sulfate was found in strips of colon, diaphragm, and aorta also, but calculated volumes of sulfate distribution in these tissues were not strikingly large as compared with the measured volumes of tissue water. These findings are cited here partly as evidence for a certain specificity in the observed process of significant sulfate uptake by kidney cortex and medulla. Finally, it may be worthwhile to note that no indication of specific uptake was ever obtained in tests on kidney tissue using S^{35} -labelled

thiourea and C¹⁴-labelled sucrose.¹ Although complete absence of uptake of these substances might be predicted on the basis of their *in vivo* behavior, the results of the experiments serve as an additional check on the validity of the method whereby sulfate accumulation was demonstrated.

TABLE III
Summary of Studies of Sulfate Uptake by Various Tissues

Tissue	Tissue relative sulfate volume		Tissue immersed, removed immediately from radi sulfate-containing solution
	Tissue incubated $\frac{1}{2}$ hr., 37°C., in		
	0.04 M KCl 0.11 M NaCl	0.04 M KCl 0.22 M sucrose	
Kidney Cortex	273 \pm 66 (6)*	1498 \pm 38 (4)*	21 \pm 10 (6)*, ‡
Kidney Medulla, peripheral zone	358 \pm 43 (6)	589 \pm 50 (4)	44 \pm 27 (5)
Kidney Medulla, central zone	187 \pm 23 (6)	—	—
Liver	160 \pm 17 (4)	316 \pm 91 (4)	17 \pm 11 (7)
Colon	95 \pm 38 (4)	193 \pm 40 (4)	14 \pm 9 (7)
Diaphragm	92 \pm 20 (4)	156 \pm 22 (4)	12 \pm 7 (7)
Aorta	111 \pm 35 (4)	160 \pm 28 (4)	—

* Figures in parentheses represent numbers of experiments from which means were derived.

‡ Kidney cortex and peripheral zone of medulla.

DISCUSSION

The observation that S³⁵-labelled sulfate not only penetrated into rat tissues in artificial media *in vitro*, but also could be actively accumulated by liver and kidney slices may seem somewhat surprising in view of the commonly held opinion that sulfate ion is approximately limited in its distribution to the extracellular fluids (6, 7). According to prediction from the Donnan effect, sulfate, as a divalent ion, might be expected to show a relative distribution between cells and extracellular water approximating the square of the distribution ratio of chloride or other monovalent ion. This might appear to be, or be interpreted as, a significant restraint to sulfate in penetrating cells with a negatively charged interior. That sulfate ion can enter into the intracellular phase of erythrocytes has been established, however (8, 9). In accord with the early observations of Borsook *et al.* (10), Walser and his coworkers (11) interpreted the time-concentration curves of radi sulfate following intravenous injection into man and dog as indicative in part of a gradual penetration (with or without metabolism) of sulfate into the cells. Moreover, the

¹ These labelled compounds were obtained from the Atomic Energy Research Establishment, Harwell, England.

kidneys of man and dog have been shown to have stable secretory (reabsorptive) mechanisms for sulfate (12-14), so that, in these species at least, it must be postulated that movement of sulfate ion into renal cells occurs *in vivo* as well as under the *in vitro* conditions described herein.

The interest of the observations presented in this paper consists partly in the fact that they may offer a further means of approach to the general understanding of sulfate metabolism. Radiosulfate has been observed in various organs and tissues after administration to developing or adult animals, or incorporation into the medium in tissue culture experiments (10, 14-16, and others). This has been attributed primarily to the incorporation of S^{35} into mucopolysaccharides or mucoproteins (*e.g.*, in cartilage, bones, tendons, gastrointestinal mucosa) (15, 17-21), while sulfolipids (19) and ethereal sulfates (22) represent additional sites of $S^{35}O_4$ incorporation. The type of accumulation described in the present paper, however, appears to differ from that of the *in vivo* and *in vitro* exchange or incorporation discussed by other workers. In the experiments described herein, the sulfate combination appears to be far more labile, and the uptake proportionately much greater, than in observations which have been described in the literature. The $S^{35}O_4$ which is extracted from kidney tissues after accumulation has been found to be in a form which is water-soluble and unprecipitable with trichloroacetic acid.² Although the S^{35} -containing substance(s) in such extracts cannot be identified, on the basis of the evidence so far available, with the compound(s) present in actively accumulating kidney tissue, it must be supposed that the latter differ appreciably from compounds localized in tissues by other workers even after the common procedures involving extensive treatment with water solutions. Furthermore, there is a difference in the observed relative uptake of $S^{35}O_4$ by various tissues in the present case as compared with the findings of other workers. For instance, Boström and Odeblad (19) noted S^{35} uptake into rat liver and kidney, but this was designated as occurring to a degree which was of the same order of magnitude as the incorporation by intestine and muscle, and much less than that by cartilage and aorta. Layton (15) found in *in vitro* studies on chick tissues that kidney cortex took up much less sulfate than tibial condyle and aortic arch, although more than skeletal muscle.

Although the phenomenon of *in vitro* sulfate accumulation has been studied most extensively with renal cortex, it may also occur in the case of slices from the peripheral region of the renal medulla and liver. At the present time, insufficient data are available for determining to what degree the accumulation

² Preliminary experiments have been carried out to determine whether all of the S^{35} extracted from accumulating tissues is precipitable with Ba^{++} . So far, the results have been equivocal. This is largely because it has not yet been possible to establish, in control studies, conditions for complete precipitation of $S^{35}O_4$ without the existence of factors such as high temperature and low pH which may be expected to favor the splitting of labile sulfate complexes.

in vitro may parallel events *in vivo*. Thus, the mechanism whereby the rat kidney handles sulfate is unknown, although, as noted above, sulfate reabsorption occurs in man and dog (11, 12). On the other hand, certain of the features of *in vitro* sulfate accumulation parallel those of the uptake of phenol red and *p*-aminohippurate in tissue slices from kidneys of fish, amphibia, and mammals. Most striking of these resemblances is the uniform potassium dependence of the three types of phenomenon mentioned. As in the case of sulfate, neither *p*-aminohippurate nor phenol red can be taken up from K^+ -free media (5, 23, 24, 26). The optimal concentration of K^+ in the medium is approximately 0.04 M for *p*-aminohippurate accumulation by rabbit kidney slices (5) and, as in the case of sulfate uptake, there is a correlation between the tissue potassium content and the accumulation process (25). It has been shown that phenol red is taken up by flounder tubule cells only in the presence of K^+ , but is not discharged into the tubular lumina unless Ca^{++} is also present in the medium (23, 24). This observation has been interpreted as evidence for a two-stage process in phenol red transport, the first step (uptake by cells) having a requirement for K^+ , whereas the second step (transfer from cells to tubular lumina) is Ca^{++} -dependent. Although, in the case of sulfate accumulation, the events are far less fully described, it seems possible that a two-stage process may also be involved here. The first step might, again, represent sulfate uptake at some specific site or sites within the kidney tissue, and would depend on the presence of K^+ . Release from such sites—evidenced by prevention or reversal of significant accumulation—might, according to the hypothesis, require the presence of non- K^+ cations; *e.g.*, Na^+ , Ca^{++} , Mg^{++} , and choline. Like phenol red and PAH accumulation *in vitro*, sulfate uptake is prevented or rapidly reversed by CN^- , and fails also with other respiratory impairment. PAH uptake is known to be specifically accelerated in the presence of acetate as substrate (5), but a similar acceleration could not be demonstrated for phenol red accumulation (24) nor, in the present study, sulfate uptake. The wide range of effects of metabolites, poisons, etc. which has been used to characterize the PAH and phenol red uptake processes *in vitro* (26, 27 and others) has not been established as yet for the process of sulfate accumulation. In essence, the interest of these methods for studying renal accumulative processes *in vitro* lies primarily in the possibility of manipulating and defining relatively exactly the conditions under which tubular transport of specific compounds may occur (5).

Only limited information as to the site of renal sulfate accumulation is available as yet. Based on an autoradiographic technique, experiments have been made indicating that a fairly definite localization of S^{35} within the kidney slices may be established under certain conditions, and that glomeruli and other vascular elements are not involved in this localization. These findings will be treated in more detail elsewhere (28).

SUMMARY

Rat kidney slices incubated *in vitro* may show, in parallel with other shifts in electrolyte content, a striking capacity for accumulating sulfate ion (sulfate labelled with S^{35}). The uptake is reversed or reduced by CN^- , cooling to room temperature, and by interference with adequate oxygenation. Under the conditions of the experiment, the presence in the medium of sodium acetate and glucose as substrates was found to be without measurable effect on the accumulation. The extent of sulfate uptake is related to the ionic composition of the medium in which the tissue is incubated, for the uptake occurs optimally only in the presence of a K^+ level of about 0.04 M, and is decreased as the concentration of Na^+ rises. Likewise, when Ca^{++} , Mg^{++} , or choline is present in the medium, sulfate accumulation may be depressed. In addition to renal cortex, kidney medulla and liver showed capacity for sulfate accumulation, whereas no convincing evidence for significant uptake was obtained with strips of aorta, colon, or diaphragm.

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