The Effect of Temperature on the Uptake of Radiosulfate by Rat Renal Tissue from Radiosulfate-Containing Solutions *in Vitro*

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ABSTRACT Kidney cortex slices incubated *in vitro* at 0°C. accumulate radiosulfate from the incubation medium. This process differs from the previously described uptake of radiosulfate by renal tissue incubated at 38°C., for instance, in the lesser sensitivity of the uptake at 0°C. to differential effects of Na^+ as compared with K^+ ions, and of sucrose as compared with glucose. Phlorizin inhibits radiosulfate accumulation at 0°C., whereas it enhances the uptake at 38°C. Effects of the cations K^+ and Na^+ and of phlorizin at temperatures intermediate between 0° and 38°C. have been studied. Parallels have been noted between the accumulative processes for radiosulfate of kidney slices maintained at 0°C. and of mitochondria isolated from rat liver and kidney cortex. These similarities may be attributed to an important role of radiosulfate uptake by mitochondria in slice accumulation of radiosulfate in the cold.

INTRODUCTION

It has been known for some time that slices of mamalian kidney cortex can accumulate S^{35}-labeled sulfate ions *in vitro* at 38°C., and more recently it was shown that radiosulfate uptake in this system occurs even at 0°C. (1, 2). The latter result was somewhat surprising, in view of the failure of renal tissue *in vitro* to accumulate in the cold other substances, such as dyes and *p*-aminohippurate (3–5). Forster and Hong (4) have found that isolated tubules of the flounder, when incubated at 2–4°C., show intracellular accumulation of certain dyes (bromphenol blue, bromcresol green). This occurs also at higher temperatures, even in the presence of metabolic inhibitors. However, the properties of the over-all process of uptake of radiosulfate by rat kidney slices differ from the uptake of these dyes which are apparently "trapped" intracellularly by the fish tubule. Thus, for instance, sulfate accumulation at rela-

tively elevated temperatures is depressed or abolished under conditions unfavorable for cellular oxidations and phosphorylations.

In the experiments described in the present paper, detailed studies were made of the effects on sulfate uptake of ambient temperature and medium composition. The results are of interest because they indicate the complexity of the processes involved in kidney accumulation of sulfate *in vitro*. Close analogies were found between sulfate uptake by slices at 0°C. and sulfate uptake by mitochondria isolated from rat liver and kidney (6), whereas the accumulative process at 38°C. was strikingly different from these. Variation of incubation temperature has been used to help analyze the mechanism of sulfate accumulation as it occurs *in vitro* under the conditions of the experiment.

METHODS

The technique for preparation of rat kidney cortex slices, and incubation of these slices in defined, radiosulfate-containing media has been described in detail elsewhere (1). The slices were about 0.35 mm. thick, and were trimmed by hand to contain cortical tissue only. Incubation media were mixtures of KCl or NaCl solutions (0.04 M) with sucrose or glucose solutions (0.22 M), and contained tracer concentrations of $S^{35}O_4^=$ (about 5×10^{-9} M, maximal activity about 2.5×10^{-7} mc./ml.), with total sulfate concentration of 10^{-7} M. Phlorizin, when used, was present at the concentration (4.5×10^{-3} M) previously shown to be optimal for enhancement of S^{35} gradients developed by kidney slices incubated at 38°C. (7). Slices were incubated in a conventional Warburg apparatus, with oxygen atmosphere, at temperatures ranging from 5 to 38°C. When incubations were carried out at 0°C., the slices were placed in Warburg flasks or test tubes immersed in an ice bath, and shaken, or alternatively agitated with a stream of air or oxygen which was bubbled through 0.15 M NaCl or 0.3 M sucrose before being led into the incubation medium through polyethylene tubing.

At the end of specified periods of incubation in the presence or absence of $S^{35}O_4$, the tissue samples were withdrawn from the media and blotted lightly with Whatman No. 40 filter paper. Portions were weighed and dried (2 hours at 100–110°C.) for water content and dry weight determinations, or extracted in 2 ml. portions of water for subsequent measurement of S^{35} content. Sample extracts and suitable dilutions of incubation media were analyzed for S^{35} in a helium-isobutane flow counter, using a technique described in detail previously (1, 7). From the results, virtual gradients of S^{35} in the tissue slices, as compared with media (S/M gradients), were calculated from the relationship:

$$\text{S/M gradient} = \frac{\text{Total tissue } S^{35} \text{ content (counts per min.)}}{\text{Total tissue water } (\mu\text{l.})} \div \text{Medium } S^{35} \text{ concentration (counts per min. per } \mu\text{l.)}$$

As in other studies of this sort (5, 8, 9), S/M gradients of 1.0 would be expected if radiosulfate were distributed uniformly throughout the tissue water, whereas, in fact, higher gradients were obtained under many of the conditions used. Further details of individual experimental procedures will be outlined below, in connection with the description of the results of the experiments.

RESULTS

Fig. 1 shows that there was a progressive increase in S^{35} content during the course of 8 or more hours in kidney slices incubated at 0°C. in solutions containing 0.04 M KCl, 0.2 M sucrose, and 10^{-7} M sulfate with tracer concentrations of $S^{35}O_4^{=}$. Even after 24 hours, most of the radioactivity remained in the tissues. At 38°C. the virtual S^{35} gradients increased faster than at 0°C., but the tissue radiosulfate content declined after 8 hours and was reduced to a low level by the end of 12 hours of incubation. Moreover, the maximal virtual gradients of S^{35} found at 38°C. were not as high as at 0°C. Thus, virtual gradients higher than 20 were rarely seen in the series of experiments carried out at 38°C., but at 0° gradients above this level (up to 38) were noted after 8 or more hours in all the experiments performed.

Experiments summarized in Fig. 2 were designed to compare in further detail the process of S^{35} accumulation by kidney slices at 0° with the process at 38°C. Thus, it has been established that isotopic sulfate accumulation by renal tissue at 38°C. is depressed if Na^+ and various other cations replace K^+ in the medium (1, 10), or if sucrose is replaced by glucose, whereas the net uptake is enhanced by phlorizin (7). In contrast, it may be noted, mitochondria from liver and kidney tissue, when incubated at 0 to 38°C., take up sulfate rapidly even when K^+ is replaced by Na^+ in the incubation medium (2). Mitochondrial uptake is not differentially depressed by glucose as compared with sucrose, and it is reduced significantly by phlorizin (2). When kidney cortex slices were incubated in the presence of $S^{35}O_4^{=}$ at 0°C., it was found that the over-all process determining their radiosulfate uptake resembled that of mitochondria, rather than the net process involved in sulfate accumulation by kidney slices at 38°C. Thus, as shown in Fig. 2, kidney tissue incubated for half an hour at 0°C. showed no differential effects of Na^+, K^+, glucose, and sucrose, and there was a striking depression of radiosulfate uptake when phlorizin was present in the incubation medium. Although these observations were not extended consistently throughout periods longer than 30 minutes, it may be noted that fairly high virtual S^{35} gradients (7.18 to 15.39) were found in tissues which were incubated for 8 hours at 0°C. in NaCl–sucrose media. These values were somewhat lower than the gradients usually found in KCl–sucrose–incubated slices after 8 hours at 0°C. (Fig. 1), yet the differential effects of the cations were much less striking at 0°C. than when the incubation temperature was maintained at 38°C. Moreover, it was found

that at 0°C. the inhibitory effect of phlorizin was demonstrable for at least 25 hours, whereas at 38°C. the enhancing effect on S^{35} gradients was as consistently maintained for at least 8 hours.

The maximal S/M gradients for S^{35} which were developed in KCl-sucrose media at 0°C. during the course of 24 hours could be as high as 38 (Fig. 1). If, in slices incubated at 0°C., mitochondria represent the actual site of S^{35} uptake, and the properties of the process of accumulation in mitochondria determine the characteristics of the slice uptake, then the close parallels be-

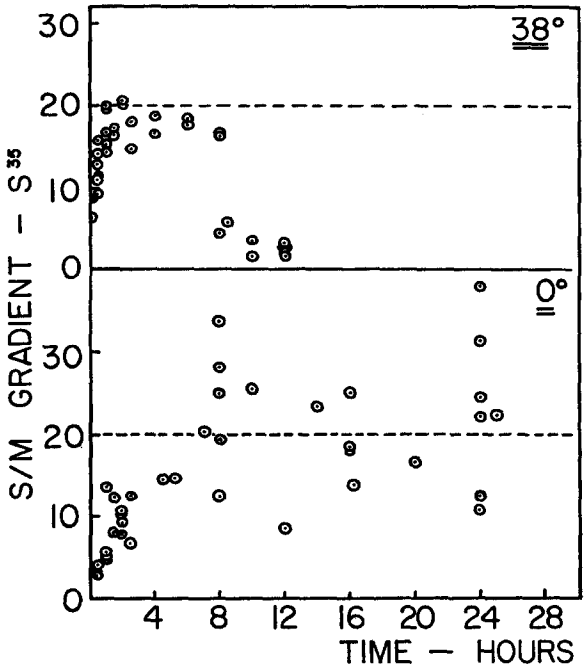

FIGURE 1. Virtual gradients for S^{35} developed in kidney tissue during prolonged incubation at 38°C. and at 0°C. Incubation medium 0.04 M KCl, 0.2 M sucrose, $S^{35}O_4^=$ (10^{-7} M sulfate). Summary of results of twenty experiments.

tween the effects of medium composition on S^{35} content of slices and mitochondria at 0°C. could be understood quite readily. A rough calculation was made to determine whether the uptake by slices could be accounted for quantitatively by reasonable mitochondrial gradients. Assuming that slice intracellular water is about 60 per cent of the total tissue water, and that mitochondrial water represents at least one-quarter of the intracellular water (12, 13), slice/medium virtual gradients for S^{35} of about 40 would represent a virtual gradient of approximately 250 in mitochondrial water. This is about the same as the average maximal gradient found in mitochondrial water *in vitro* (14).

In corroboration of the hypothesis that mitochondria may represent an important site of intracellular accumulation of $S^{35}O_4^-$ at 0°C., it was found that mitochondria isolated from kidney slices which had been incubated for 8 to 18 hours at 0°C. in KCl–sucrose–$S^{35}O_4^-$ media contained more S^{35} than did mitochondria separated from tissue incubated in radiosulfate-free media

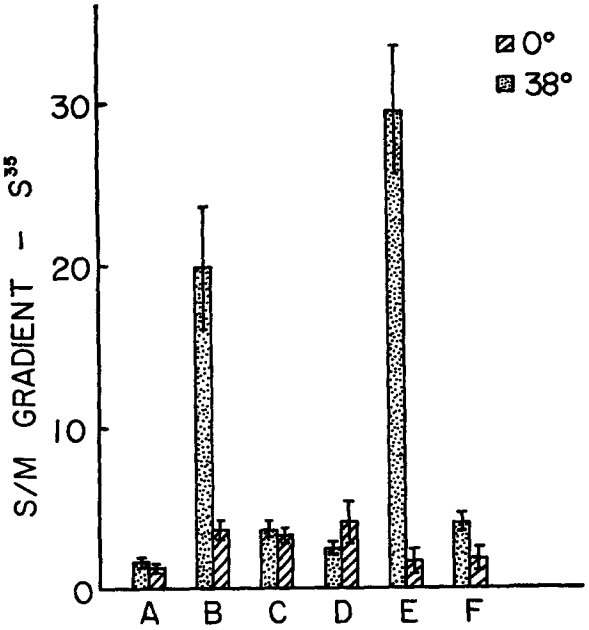

FIGURE 2. Virtual gradients for S^{35} developed in kidney tissue during 30 minutes of incubation at 0°C. or 38°C. in various media. The media were as follows: *A*, 0.3 M sucrose; *B*, 0.04 M KCl, 0.20 M sucrose; *C*, 0.04 M KCl, 0.20 M glucose; *D*, 0.04 M sucrose 0.20 M sucrose; *E*, 0.04 M KCl, 0.20 M sucrose, 4.5×10^{-3} M phlorizin; *F*, 0.04 M KCl, 0.20 M glucose, 4.5×10^{-3} M phlorizin. Over-all sulfate concentration about 10^{-7} M in all experiments. The heights of the columns indicate the magnitude of the means obtained under each set of conditions. The vertical lines represent twice the magnitude of the standard deviation in each series.

(Table I). Kidney cortex slices were prepared and incubated for various periods of time in solutions containing 0.04 M KCl, 0.2 M sucrose, and either no added sulfate, or radiosulfate with a total SO_4^- concentration of 10^{-7} M. At the end of the incubation period, tissue which had been incubated with radiosulfate, and slices which had not been exposed previously to radiosulfate, were placed in separate tissue homogenizing vessels (pyrex, with teflon pestle) containing 2 ml. portions of KCl-sucrose with $S^{35}O_4^-$ (10^{-7} M sulfate) at 0°C. Each lot of slices was ground gently by hand, so that the tissues were macerated with minimal rise in temperature. Subsequently, the tissue brei in each vessel was transferred to a polyethylene centrifuge tube, and the samples were

separated by 10 minutes of centrifugation at 700 × *g* at 0°C. into supernatant fractions and sediments (designated "First sediment" in Table I) consisting of nuclei, cell fragments, some mitochondria, etc. The supernatant fractions were then recentrifuged at 6000 × *g* at 0°C. for 10 minutes. The second supernatant fluid was then removed from the sedimented mitochondria. Samples of the first sediment and the mitochondrial sediment were then weighed in aluminum foil cups, and later extracted in 1 or 2 ml. volumes of water. Wet and dry weight determinations were made on samples of both

TABLE I

RADIOSULFATE CONTENT OF FRACTIONS OF KIDNEY HOMOGENATE PREPARED FROM KIDNEY CORTEX SLICES INCUBATED IN THE PRESENCE AND ABSENCE OF RADIOSULFATE

The slices were incubated in KCl-sucrose with or without 10^{-7} M sulfate containing radiosulfate. They were homogenized in KCl-sucrose containing 10^{-7} M sulfate with radiosulfate.

		Virtual S^{35} gradient of			
		First sediment incubated with		Mitochondrial sediment incubated with	
	Experiment No.	No S^{35}	S^{35}	No S^{35}	S^{35}
A. Tissue incubated for 8 to 18 hrs at 0°C.	161	3.9	8.4	4.9	12.8
	162	3.2	8.2	16.4	23.9
	166	2.1	5.2	7.6	23.4
B. Tissue incubated for 8 to 24 hrs. at 0°C., but warmed briefly at various stages of preparation*	167 (1)	5.0	3.5	12.9	7.7
	166 (2)	—	3.6	—	5.1
	163 (3)	3.1	4.1	8.4	7.6
C. Tissue incubated for ½ hr. at 38°C.	164	3.0	3.2	3.7	2.5
	168	2.8	4.4	6.8	9.3

* Tissue warmed briefly during preparative stages as follows: (1) half-way through incubation period; (2) during tissue homogenization; (3) during centrifugation.

first and mitochondrial sediments. Using the sediment extracts and samples of the supernatant fluids suitably diluted with water, measurements were made of relative S^{35} contents of each fraction of the original tissue brei. With these data, it was possible to estimate virtual S^{35} concentrations in the first sediment and in the mitochondrial sediment using the formal relationship:

$$\text{Virtual } S^{35} \text{ concentration} = \frac{S^{35} \text{ content per unit wet weight}}{\text{water content per unit wet weight}}$$

Virtual S^{35} gradients between the fractions and supernatant fluids were then calculated. The results are summarized in part A of Table I. It may be seen that the highest gradients were observed in mitochondrial fractions isolated after prolonged incubation of tissue in the presence of radiosulfate. The finding that mitochondria which had not been exposed previously to $S^{35}O_4^=$ also

showed some S^{35} after maceration of the tissue in the presence of radiosulfate was consistent with previous observations (6, 11).

The high virtual gradients for S^{35} in mitochondria were not seen if the slices or tissue brei had been exposed to elevated temperature during the course of

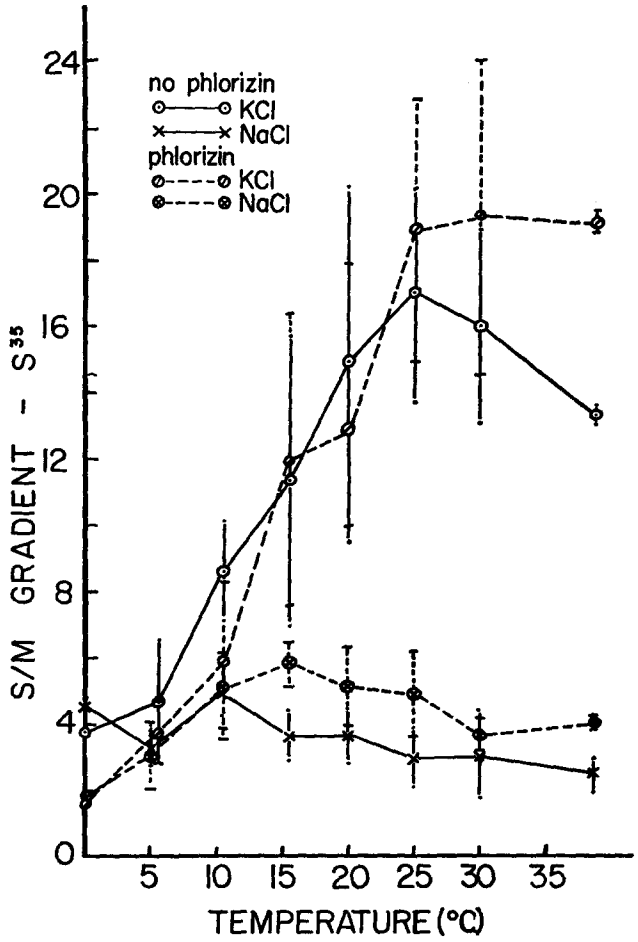

FIGURE 3. Effects of temperature on virtual S^{35} gradients developed in tissues during 30 minutes of incubation in 0.04 M KCl–0.2 M sucrose or in 0.04 M NaCl–0.2 M sucrose media. Phlorizin, when present, was at a concentration of 4.5×10^{-3} M. Points indicate mean values and lengths of vertical lines represent twice the magnitude of the standard deviations. Solid vertical lines represent standard deviations of the measurements made in the absence of phlorizin, dashed vertical lines represent standard deviations of measurements made when phlorizin was present in the medium.

the procedure, as shown in part B of Table I. For instance, in the experiment designated (2) in this table, tissues incubated at 0°C. for 18 hours were placed in a KCl–sucrose–radiosulfate medium at 25°C. and kept there during the 30 seconds during which the tissue was macerated. The brei was then rapidly

cooled to 0°C., and maintained at that temperature for the rest of the experiment. In these circumstances, the virtual S^{35} gradient of the mitochondrial sediment was only 5.1, whereas it was 23.4 in the case of slices kept at 0°C. throughout the procedure of mitochondrial separation. It may be noted, too, that when kidney slices were incubated at 38°C. for ½ hour in the presence of S^{35}, high S^{35} gradients were not seen in the mitochondria isolated after maceration of the tissues.

Since the over-all phenomena of S^{35} uptake by tissues appear to have strikingly different physiological characteristics at 0° and at 38°C., a series of

TABLE II

EFFECT OF PHLORIZIN ON S/M GRADIENTS FOR S^{35} DEVELOPED BY KIDNEY CORTEX SLICES DURING 30 MINUTE INCUBATION AT VARIOUS TEMPERATURES, IN NaCL-SUCROSE OR KCl-SUCROSE SOLUTIONS

Mean values and standard deviations are given for each set of experimental conditions. Numbers in parentheses are numbers of experiments from which each figure was derived.

Temperature	Ratio of S/M gradients in the presence of phlorizin to control gradients in	
	NaCl-sucrose medium	KCl-sucrose medium
°C.		
0	0.42±0.11 (4)	0.44±0.10 (4)
5–6	0.95±0.46 (5)	0.85±0.19 (5)
10–11	1.04±0.31 (4)	0.68±0.08 (4)
15–16	1.62±0.55 (5)	0.86±0.19 (5)
20	1.43±0.33 (5)	0.97±0.16 (5)
25	1.68±0.44 (4)	1.10±0.16 (4)
30	1.29±0.29 (4)	1.21±0.16 (4)
38	1.71±0.11 (5)	1.47±0.43 (6)

experiments was carried out to assess the effects of ambient temperature intermediate between these extremes (Fig. 3). Whereas virtual S/M gradients changed relatively little with rising temperature in media containing NaCl and sucrose as exogenous solutes, when K^+ replaced Na^+ the gradients increased at about 5°C., reaching a maximum at about 25°C. Previously, it had been shown that addition of Na^+ to media containing K^+ resulted in depression of tissue S/M gradients for S^{35} at 38°C. (10). These findings may be explained if the net uptake of radiosulfate into kidney tissue *in vitro* is the resultant of at least two processes. The first of the two postulated processes is dependent on K^+, inhibited by Na^+, and accelerated as temperature is increased. The second process may be demonstrated clearly at 0°C. It is much less affected differentially by Na^+ and K^+ than is the first process. It may be recalled that earlier experiments established the fact that the effects of the

cations Na^+ and K^+ are fully reversible. Thus, at 38°C. the depression of S^{35} uptake in Na^+-containing solutions is followed by enhancement on transfer to KCl–sucrose media, and S^{35} accumulated during incubation of slices in KCl–sucrose is lost rapidly when tissues are placed in media containing Na^+ in place of K^+ (10).

The effects of phlorizin (4.5×10^{-3} M) yielded additional evidence on the complexity of radiosulfate uptake. As shown in Table II, the marked phlorizin inhibition seen at 0°C. was not present at 5°C. Enhancement of tissue S^{35} content was noted at 15°C. and higher temperatures when Na^+ was the

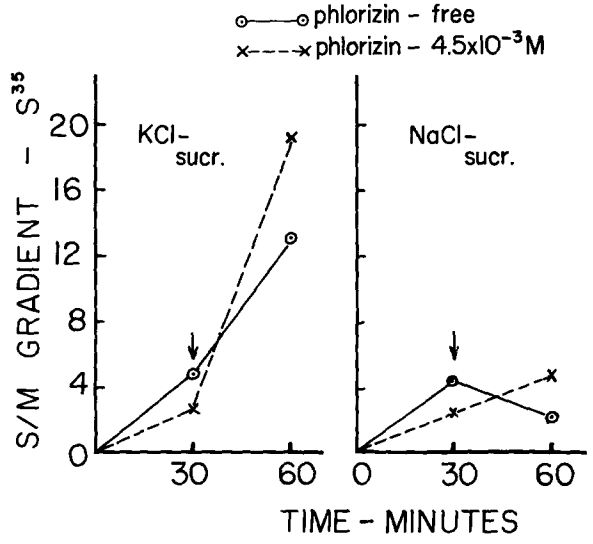

FIGURE 4. Virtual S^{35} gradients of tissues incubated in 0.04 M KCl–0.2 M sucrose or 0.04 M NaCl–0.2 M sucrose. In the 0 to 30 minute incubation period, the temperature was 0°C. At the arrow, the tissues were warmed rapidly to 38°C. and maintained at that temperature during the 30 to 60 minute period of incubation.

major cation of the medium, but there was no significant enhancement in KCl–sucrose solutions until the ambient temperature was elevated to 30°C. Fig. 4 shows that the depression of sulfate uptake at 0°C. by phlorizin was reversible in both KCl–sucrose and NaCl–sucrose media. Further investigations showed that the net rate of $S^{35}O_4^=$ uptake was similarly inhibited at 0°C. and enhanced at 38°C. in KCl–sucrose solutions.

Because it has been suggested that the complicated effects of phlorizin on renal carbohydrate transport include an action on cellular permeability (15), thirty-four experiments were carried out to determine whether phlorizin (4.5×10^{-3} M) would affect the rate of loss of radiosulfate from tissues transferred from sulfate-containing to sulfate-free media. Renal cortical slices were incubated for 30 minutes at 0°C. or at 38°C. in 0.04 M KCl–0.2 M sucrose media,

containing $S^{35}O_4^=$ and $S^{32}O_4^=$ (total $SO_4^=$ concentration = 10^{-7} M). The slices were then transferred to 2 ml. volumes of sulfate-free media otherwise identical in salt and sucrose composition with the incubation media in which they had been exposed to radiosulfate. The media to which the slices were transferred, which will be designated as the leaching media in the discussion to follow, were maintained at 0° or 38°C. Tissues were leached, for periods up to 60 minutes, with oxygenation and agitation. In each experiment, the loss of radiosulfate from the tissues in the presence and absence of phlorizin (4.5×10^{-3} M) was measured by comparison of the slice S^{35} content (expressed as counts per minute per milligram tissue dry weight) after incubation but

TABLE III

S^{35} CONTENT OF KIDNEY CORTEX SLICES FOLLOWING LEACHING IN SULFATE-FREE MEDIA

Initial incubation was carried out at 0° or 38°C. in KCl-sucrose media, 10^{-7} M with respect to $SO_4^=$($S^{35}O_4^=$ + $S^{32}O_4^=$). Subsequent leaching in $SO_4^=$ media with or without phlorizin (4.5×10^{-3} M) at 0° or 38°C. S^{35} content is expressed as percentage of content (counts per minute per milligram dry weight of tissue) before the start of leaching. The means and range (in parentheses) of values obtained from four separate experiments are given for each set of conditions.

	S^{35} content after 10 min. leaching at		S^{35} content after 60 min. leaching at	
	0°C.	38°C.	0°C.	38°C.
Initial incubation at 38°C.				
No phlorizin	96 (94–111)	58 (51–72)*	57 (45–87)	13 (8–21)
Phlorizin	94 (69–128)	59 (56–62)	65 (43–83)	18 (5–42)
Initial incubation at 0°C.				
No phlorizin	82 (60–100)	34 (14–52)	54 (44–65)	16 (10–22)
Phlorizin	66 (53–84)	41 (32–50)	58 (48–80)	23 (15–31)

* Three experiments only.

before leaching with the S^{35} content after leaching. Thus, the experimental system was somewhat analogous to the system used to observe *p*-aminohippurate loss from kidney slices in the careful study of Foulkes and Miller (5). In the conditions of the experiment, a variety of factors may affect the measured loss of isotope during the leaching process. These factors include dissociation of S^{35} from any intracellular accumulative site, permeability of cell membranes to sulfate, diffusion of sulfate within the extracellular fluids to the surrounding leaching medium, and reaccumulation of $S^{35}O_4^=$ from the leaching medium into the cells. Because of the complexity of the experimental situation, it was recognized at the outset that any observations on differences between rates of loss of sulfate in phlorizin-free and phlorizin-containing leaching media would be subject to several interpretations. When very brief

periods of leaching were used, however, it seemed unlikely that sulfate reaccumulation would occur to a significant extent, because of the relatively large ratios (about 100 to 250) of leaching medium to tissue water. Moreover, it seemed improbable that passive diffusion of radiosulfate within the extracellular spaces would be affected by the presence or absence of phlorizin. Thus, at least in the case of short leaching times, marked differences in rates of loss by phlorizin-treated slices as compared with untreated controls might

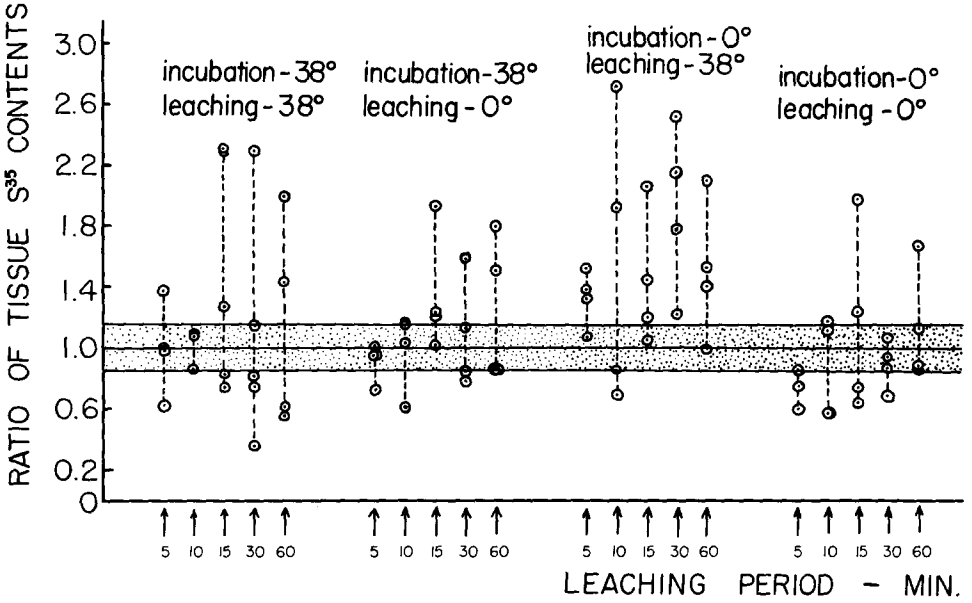

FIGURE 5. Kidney cortex slices which had accumulated radiosulfate during 30 minute incubation in 0.04 M KCl + 0.2 M sucrose were transferred to sulfate-free media and leached for 5 to 60 minutes. The ordinate gives the ratio of S^{35} content of slices leached in the presence of phlorizin to the S^{35} content of slices leached in phlorizin-free media. The duration of leaching in each case is indicated on the abscissa. Taking into account the errors of the measurements, a difference of ± 15 per cent between phlorizin-treated and untreated slices may be considered significant. This range from the ratio 1.00 is indicated by the stippled band.

be attributed to effects of phlorizin on cell membrane permeability to sulfate, or on intracellular accumulative sites.

In point of fact, the results of this series of experiments, summarized in Table III and Fig. 5, proved to be highly variable and difficult to interpret. Within the first 10 minutes of leaching, tissues lost 28 to 49 per cent of their accumulated sulfate at 38°C. In confirmation of earlier findings (16), the loss was much less at 0°C., as shown in Table III. During this early period, phlorizin did not affect consistently the S^{35} content in any of the tissues except those leached at 38°C. after incubation at 0°C. In the latter case, the relatively

higher tissue sulfate values might be accounted for by cell uptake of $S^{35}O_4^=$ present in the extracellular fluids. When leaching was carried out for longer periods (15 to 60 minutes) kidney slices tended to have higher S^{35} contents in the presence than in the absence of phlorizin. Even these results were by no means consistent, however, as shown in Fig. 5. Thus, in the early period of leaching when reaccumulation of radiosulfate would be unlikely, and it might be expected that loss would be differentially determined largely by membrane permeability to sulfate, phlorizin did not appear to alter the rate of sulfate loss from the tissues.

DISCUSSION

Some mammals have been shown to have a stable renal reabsorptive mechanism for sulfate ions (17, 18). In the dog, this mechanism has been localized in the proximal convoluted tubules (19). Recently, Becker and Thompson (20) have described sulfate flux from peritubular to intraluminal fluid in the dog kidney. To a large extent, the detailed analyses of radiosulfate uptake by kidney tissue *in vitro*, of which the present study is a part, are motivated by interest in possible relationships between this phenomenon and renal sulfate exchange and transport as seen in the intact animal. In this objective, the work parallels experiments on *in vitro* accumulative processes for dyes, *p*-aminohippurate, tetraethylammonium ions, and related compounds (3–5, 8, 9 and others). Yet the observations on sulfate may also bear on more general aspects of sulfate metabolism. Enzyme systems, such as those involved in sulfate activation and the splitting of aryl sulfates, may be involved in radiosulfate exchange. It must be acknowledged, however, that continued search has not revealed the presence in kidney slices of radiosulfur-labeled compounds other than sulfate ions, under the conditions of the present experiment (14). A specific source of interest has been the finding of analogies in the pattern of radiosulfate uptake by isolated liver and kidney mitochondria and by kidney slices at 0°C. (2).

The observations described in the present paper could be accounted for as follows: Sulfate ions may enter all, or certain specific renal cortical cells *in vitro* by simple diffusion across the cell membranes. At 0°C., radiosulfate does this, and may become concentrated by association with mitochondria by a process similar to that which occurs *in vitro*. There exists, in addition, a second manner of sulfate entry into cells. This process is energy-dependent, and requires the presence of K^+, Rb^+, or Cs^+ (10). It is inhibited by Na^+, as well as by some other cations (Li^+, Ca^{++}, Mg^{++}, choline) (1, 10). It is also sensitive to all the metabolic inhibitors so far tested. This K^+-dependent process, which allows for the rapid establishment of high virtual radiosulfate gradients and an over-all increase in total sulfate content (21), is sensitive to temperature,

so that it does not occur measurably below 5°C. It is still not established whether the two processes postulated above are, in fact, distinct, or are fractions of a single process artificially separated by the conditions of the experiment. Nor is it possible to state, as yet, what relationship either or both of the processes may have to renal sulfate exchange and transport *in vivo*. Circumstantial evidence suggests close parallels between kidney sulfate reabsorption and kidney cortex slice accumulation of radiosulfate at 38°C., as has been stressed previously (1).

Effects of cooling kidney tissue on accumulation of dyes and *p*-aminohippurate have been described by Forster and coworkers (4, 22) and by Foulkes and Miller (5), and may be compared with the effects of cold seen in the case of sulfate uptake *in vitro*. *p*-Aminohippurate is lost by kidney tissue chilled to 0°C. after accumulation of the compound at higher temperature, although the rate of efflux is lower than at 20–37°C. The dyes, bromphenol blue and bromcresol green, which are taken up intracellularly even in the cold and in the presence of metabolic inhibitors, but which do not appear in the lumina of isolated flounder tubules, are not lost after accumulation when the kidney tissue is chilled to 0°C. Thus, all these compounds differ from sulfate with respect to the differential effects of temperature on *in vitro* accumulation and/or retention. Only a single compound is known to parallel sulfate ion in its pattern of uptake and retention by renal tissue *in vitro*. This is the disulfonic acid dye indigo carmine which Forster and Hong (4) have found to be accumulated by cells of the isolated renal tubules of the flounder at 20°C., with slower transfer intraluminally. Indigo carmine uptake did not occur in the presence of DNP, but when renal tubules were chilled to 2 to 4°C., there was no loss of dye from the cells. No information exists as to the behavior of indigo carmine in the case of mammalian kidney slices incubated under conditions comparable with those used in the present study with radioactive sulfate.

The effects of phlorizin on kidney sulfate uptake add to the evidence for complexity of the processes involved. As yet, however, they cannot be explained in terms of basic actions of phlorizin on cellular systems, for these are varied, complicated, and still only partially defined. In relatively high concentrations, comparable with those used in the present study, phlorizin not only blocks cellular transport of glucose by a variety of tissues, but it also inhibits various oxidative enzymes of kidney tissue (23), and disrupts mitochondrial organization and metabolism *in vitro*, with attendant swelling in 0.3 M sucrose (24) and depression of mitochondrial radioactive sulfate uptake (11). Phlorizin appears to decrease cell membrane permeability to non-metabolized carbohydrates and carbohydrate derivatives (15), both directly and indirectly by reversing the increase in cell permeability caused by insulin (25). Lotspeich (26) has summarized evidence for association of phlorizin with proteins of cell

membranes and has stressed the possible roles of the reactive groupings of the glycoside in effecting such membrane attachments which might alter both passive permeability and active transport mechanisms for a variety of compounds.

In view of these known and inferred actions of phlorizin, it might be expected that the glycoside could depress net tissue uptake of S^{35} at 0°C., either by decreasing membrane permeability to sulfate and thus limiting its access to the mitochondrial site, or by altering the mitochondria themselves. Although phlorizin may enter and actually undergo active secretion by kidney cells *in vivo*, it is not known whether, under the conditions of the present *in vitro* experiments, phlorizin can penetrate the cell membranes to reach the mitochondria. At temperatures above 0°C., where the specifically K^+-dependent process for sulfate accumulation may be observed, the enhancing effect of phlorizin on the S/M gradients developed could be explained by reduction in the passive permeability of the cells to sulfate, with consequent increased tendency of the sulfate transported by energy-dependent processes into the cells to remain "trapped" within them. However, the results in Table III and Fig. 5 do not support this view. Alternatively, it is quite possible that the action of phlorizin on sulfate transport is more specific, and related to its well established, though still unexplained, action on the mechanisms of glucose transport. *In vivo*, phlorizin reverses the inhibitory effects of high concentrations of glucose on sulfate reabsorption, and it has been suggested that sulfate, like phosphate, may share with glucose a part of the complex renal pathway for transport (18). Finally, and independently of possible effects on glucose, phlorizin may either enhance an active process whereby sulfate enters the cells, or depress a specific mechanism involved in the movement of sulfate out of renal cells. Experiments are planned to attempt to distinguish between these various possibilities. Although sulfate accumulation *in vitro* appears to be a highly complicated phenomenon, the results of experiments using temperature differences, inhibitors, and other variables in incubation conditions suggest the existence of separable steps in the mechanisms involved. These may be analogous to the discrete processes in dye and *p*-aminohippurate transport *in vitro* described by Puck *et al.* (27), Forster and coworkers (3, 4), and Foulkes and Miller (5).

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