A STUDY OF THE FLUID UPTAKE OF RAT KIDNEY SLICES IN VITRO*

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INTRODUCTION

Observations of Sperry and Brand (1), Elliott (2), Opie (3, 4), and others (5–9) have established the somewhat surprising fact that surviving slices of mammalian liver, brain, kidney, and certain other tissues take up relatively large amounts of fluid from a variety of solutions which are considered to be isosmotic with mammalian interstitial fluids (*e.g.*, homologous serum and various Ringer's solutions). The fluid uptake is evidenced by increased weight (3, 4), relative water content (5, 6), and swelling revealed by histological study (3). When mammalian tissues are incubated in the presence of an adequate oxygen supply at temperatures of approximately 38°C., they do not take up fluid from isosmotic or even markedly hypotonic solutions (6, 7). Swelling does occur, however, even under conditions of optimal temperature and oxygen supply, in the presence of respiratory inhibitors and some other compounds affecting metabolism (6, 9, 10). Although there is good agreement in the literature as to the findings outlined above, the mechanism accounting for them is far from established. Robinson and McCance (8) have formulated clearly an hypothesis explaining the occurrence on the basis that the fluids within mammalian cells may be strongly hypertonic with respect to the extracellular fluids. This postulated hypertonicity has been considered to be a physiological condition (6, 8) or might be caused by respiratory impairment with consequent accumulation of osmotically active metabolities. In addition, fluid uptake by certain tissues has been attributed in part to a process similar to, or identical with, colloidal imbibition of water (3, 4). Finally, the phenomenon might be ascribed to influx of water accompanying ions such as sodium and chloride penetrating through cell membranes which were partially permeable to solutes present in the media in which the tissues were immersed *in vitro*. Thus, Mudge has shown that rabbit renal cortex undergoes reversible exchanges of potassium for sodium ions, accompanied by alterations in relative tissue water content, under conditions of depressed respiration (9).

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The occurrence of cellular fluid uptake from "physiological" solutions *in vitro* is of considerable interest in connection with the description of membrane permeability of mammalian cells, in the study of over-all water and electrolyte balance of tissues, and possibly also in connection with active water and ion transport by renal and other secretory cells (8). In addition, it has been pointed out that the experimental situation may parallel the frequently occurring pathological conditions in which cellular water and electrolyte balance are disturbed (7, 8). Consequently, experiments have been undertaken in order to obtain further information about the problem. The water uptake from a range of different solutions, varying with respect to the identity and concentration of their solutes, has been studied to test the hypothesis that the intracellular fluids of rat renal tissues are hypertonic as compared with the extracellular fluids. The results of these experiments are summarized in the present report.

Methods

Experiments were carried out on kidney slices obtained from albino rats. These animals were chosen as the source of experimental material since they have been used previously by a number of workers in studies of water and electrolyte exchanges *in vivo* and *in vitro*. The rats were of both sexes, ranged in weight from 140 to 455 gm., and were obtained from the Carworth Farms, New York. They were maintained on a diet of Purina laboratory chow, usually with water *ad libitum*. The animals were sacrificed by concussion, and the chest was quickly opened to allow exsanguination from the heart. The kidneys were removed and placed on a sheet of wax paper in a small moist chamber. Each kidney was then divided in half and slices were prepared using a Terry tissue microtome (11). The slices were approximately 0.3 to 0.5 mm. thick, and were cut in a plane at right angles to the long axis of the kidney. As each slice was cut, it was transferred to a sheet of wax paper and divided, by means of a razor blade, into cortical and medullary sections. The fragments of cortex and medulla were then placed separately in designated immersion fluids, or used for control studies. Approximately 20 seconds was required for the preparation of each tissue sample. The time which elapsed between sacrifice of the animal and completion of preparation of all the kidney slices was of the order of 12 to 21 minutes.

The immersion fluids used included heparinized homologous blood and various salt and carbohydrate solutions which will be described in the section on results. The error of preparation of the solutions was estimated as less than ± 1 per cent. The pH of the solutions was measured with a Beckman pH meter. In general, observations were made with the fluids at room temperature. When temperature was studied explicitly as a factor affecting tissue water exchange, beakers containing kidney slices were placed in water baths at varied temperatures controlled within ± 0.05°C. The volumes of solutions used for immersion ranged from 5 to 25 ml. The depth of fluid in the containing beakers was of the order of 1 to 2 cm., and in all experiments the solutions were open to atmospheric air and unstirred.

Control slices, or slices which had been immersed for known periods of time (usually 30 to 60 minutes) in specific fluids, were blotted with Whatman No. 40 filter paper and

rapidly weighed in tared aluminum foil cups on a Roller-Smith precision balance. The samples were then allowed to stand in a desiccator over activated alumina for 10 or more hours, and subsequently were dried in an oven at 104–110°C. for 2 hours and cooled in the desiccator before they were reweighed. From the results, dry weights and wet tissue water contents were obtained. The data presented below were calculated in terms of relative water content, defined as the ratio of water content to dry weight of the tissue sample.[1] The minimum precision of the weighings was about ±0.05 mg., and the tissue samples had a wet weight range of 7 to 45 mg., whereas the dry weights ranged from about 2 to 12 mg.

RESULTS

In confirmation of the work of earlier investigators, it was found that kidney slices increased in relative water content after immersion in so called isosmotic fluids, such as heparinized homologous blood and Krebs-Ringer-phosphate solution, at room temperature. A measurable change in relative tissue hydration occurred within 2 minutes and became greater with continued immersion. Characteristically, cortical tissue showed a progressively increasing water content during the course of an hour or more, whereas medulla gained more rapidly during the first half-hour of exposure to the solutions *in vitro*, and then tended to decrease in relative water content. Results of a representative experiment are shown in Fig. 1. In this, as in other experiments, it was noted that the change in relative water content in Krebs-Ringer-phosphate solution was marked. In the experiment illustrated in Fig. 1, for instance, after ½ hour the increase in relative water content was 35.9 per cent for renal cortex and 47.3 per cent for medullary tissue. These figures are comparable with the data of Robinson (6), Stern *et al.* (5), and others. An elevation of relative water content does not, of course, signify unequivocally uptake of water, since instead it may represent loss of solids from the tissue slices. In accordance with the findings of Opie (3) and Elliott (2), however, repeated weighings of individual slices demonstrated that fluid was actually taken up by renal cortex under the conditions of the experiment.

Because of the relatively more consistent behavior of cortical tissue on

[1] The use of the expression *relative water content* appears to facilitate the interpretation of the data, since it does not depend on the explicit or implicit assumption that dry weight is constant. Other workers (*e.g.*, references 5, 6) have computed the apparent water uptake of tissues on the assumption that dry weight is constant. It may be noted that the apparent uptake calculated in this way may range up to 50 to 70 per cent of the initial water content, in the work reported in the literature and in the present series of experiments. The data given here as *relative water content* may be converted to the more familiar form *percentage water content* by use of the following relationship: Let R = relative water content, (mg. tissue water)/(mg. tissue dry weight); and P = percentage water content, or 100 × (mg. tissue water)/(mg. tissue wet weight). Then $P = 100\,R/(1 + R)$.

immersion, only results of experiments using renal cortex will be discussed in detail in the material presented below. It may be noted, however, that the observed changes in water content of medullary slices, in spite of greater variability, were similar qualitatively to the variations observed in the case of renal cortex. The results of experiments in which cortical samples were immersed in a variety of "isosmotic" solutions are summarized in Table I. Fluids used were heparinized blood obtained from the animal which served as the donor of the kidney tissue; Krebs-Ringer-phosphate solution (pH 7.1; total

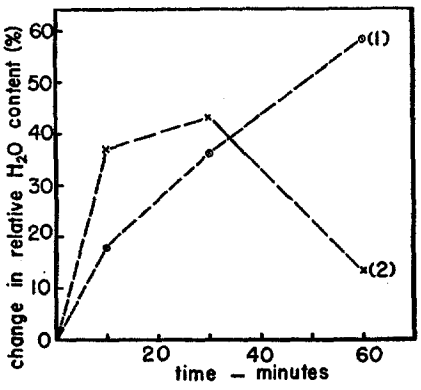

FIG. 1

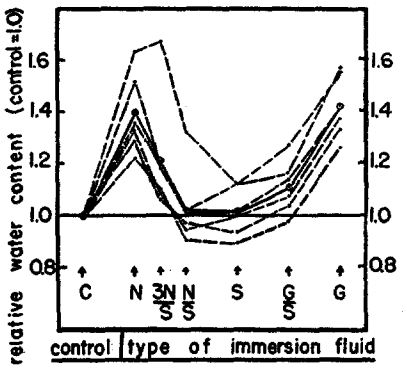

FIG. 2

FIG. 1. Percentage change from control level of water content of rat renal tissues immersed in Krebs-Ringer-phosphate solution for 1 hour. Curve (1), cortex; curve (2), medulla. Temperature = 24.5°C.

FIG. 2. Diagram summarizing results of 6 experiments in which "isotonic solutions" of pure or mixed solutes were used. Rat renal cortex immersed for ½ hour at room temperature. Explanation of symbols used: C = control (non-immersed tissue); N = 0.15 M NaCl; $3N/S$ = 3 parts 0.15 M NaCl + 1 part 0.3 M sucrose; N/S = 1 part of 0.15 M NaCl + 1 part 0.3 M sucrose; S = 0.3 M sucrose; G/S = 1 part of 0.3 M glucose + 1 part of 0.3 M sucrose; G = 0.3 M glucose.

concentration approximately 300 m.osM[2]/liter); 0.15 M solutions of NaCl and $NaNO_3$; and 0.3 M solutions of glucose, fructose, galactose, sucrose, maltose, and lactose. It may be seen that a marked increase in relative water content occurred in all the immersion fluids used with the exception of the solutions of disaccharides. An interesting comparison may be made, for instance, between the fluid contents of tissues in equally concentrated solutions of mono- and disaccharides. Kidney cortex appeared to behave as though its intracellular

[2] The term milliosmol (m.osM) is used in the present paper, as elsewhere (see, for instance, Mudge, G., *Am. J. Physiol.*, 1951, **167**, 207) to represent the sum of millimoles of undissociated solutes and milliequivalents of dissociated solutes. Thus, the concentration of solutes measured in terms of milliosmols is directly related to the osmotic pressure of the solution under consideration.

fluids were isosmotic or slightly hypotonic with respect to the 0.3 M disaccharide solutions, but strongly hypertonic with respect to the 0.3 M monosaccharide solutions. This finding is inconsistent with the view that the observed water transfer into the tissues is determined by its own concentration gradient alone under the conditions of the experiment. Thus, the evidence does not lend support to the hypothesis of Robinson and McCance that intracellular fluids of mammalian kidney cells are hypertonic to the extracellular fluids except by virtue of dynamic water adjustments.

It might be supposed that the failure of kidney cortical slices to take up fluid from disaccharide solutions was the result of changes in the properties of the cell membrane caused by removal of the tissues from their normal electrolyte-containing environment. This is rendered unlikely, however, by the fact that water content did increase during immersion of the tissues in the equally

TABLE I

Summary of Changes in Relative Water Content of Renal Cortical Tissue Immersed for ½ Hour in Various Solutions

Solution used	No. of experiments	Average change in relative water content from control value
		per cent
Rat whole blood (with heparin)	4	+24.4 ± 15.8
Krebs-Ringer-phosphate (pH 7.1)	22	+29.4 ± 18.5
Sodium chloride, 0.15 M	18	+45.9 ± 27.6
Sodium nitrate, 0.15 M	6	+61.5 ± 31.6
Glucose, 0.3 M	11	+42.4 ± 13.5
Fructose, 0.3 M	6	+45.8 ± 28.6
Galactose, 0.3 M	6	+57.9 ± 31.4
Sucrose, 0.3 M	33	−2.4 ± 17.3
Maltose, 0.3 M	4	−19.3 ± 10.4
Lactose, 0.3 M	6	−10.6 ± 15.4

electrolyte-free solutions of monosaccharides. Fluid uptake from 0.3 M sucrose was not altered by repeated rinsings of the tissue in sucrose solutions, although this procedure might be expected to remove more completely traces of extracellular liquids adhering to the slices. Thus, in 4 experiments, cortical tissue was placed in 0.3 M sucrose, allowed to remain there for 10 minutes, then transferred to a fresh lot of sucrose solution. The procedure was then repeated, so that the tissue was exposed to 3 fresh lots of the immersion fluid during the course of ½ hour. In these circumstances, the relative water content changed by −4.3 ± 6.2 per cent, as compared with the control. This value did not differ significantly from the average change following continuous immersion of kidney cortex in a single lot of sucrose solution (Table I). No evidence of gross damage to the cells maintained in sucrose solution was found in preliminary experiments showing that the oxygen uptake in Krebs-Ringer-phosphate (pH 7.1, no substrate added, temperature 37.5°C.) was essentially the same for slices which had

been immersed for ½ hour in 0.3 M sucrose as for slices exposed during the same time to Krebs-Ringer-phosphate. Furthermore, any changes occurring in the cell membrane must have been rapidly reversible. Thus, slices immersed in 0.3 M sucrose gained in relative water content on subsequent immersion in Krebs-Ringer-phosphate, whereas the water content of cortical tissues soaked in Krebs-Ringer-phosphate decreased when the slices were transferred to 0.3 M sucrose (Table II).

As a result of the findings presented above, it does not seem possible to accept the hypothesis that the fluid uptake of rat kidney tissue in isosmotic solutions *in vitro* is the result of the hypertonicity of the intracellular fluids.

TABLE II

Effects on Relative Water Content of Rat Kidney Cortex Resulting from Transfer of Tissue from Krebs-Ringer-Phosphate to 0.3 M Sucrose; or from 0.3 M Sucrose to Krebs-Ringer-Phosphate

Experiment No.	Relative water content of tissue		Change in water content on transfer from KRP to sucrose	Relative water content of tissue		Change in water content on transfer from sucrose to KRP
	(1) Immersed for ½ hr. in KRP*	(2) Transferred to sucrose for ½ hr.		(1) Immersed for ½ hr. in sucrose	(2) Transferred to KRP for ½ hr.	
			per cent			*per cent*
T87	4.00	2.74	−31.5	2.30	3.30	+43.5
T98	3.90	2.22	−43.1	2.81	4.24	+94.5
T101	3.61	3.17	−12.2	3.06	3.18	+3.9
T102	3.94	2.84	−27.9	2.44	3.45	+41.4
T108				1.80	3.59	+99.4
T110				3.53	5.03	+42.5
T111				3.42	4.15	+21.3

* Krebs-Ringer-phosphate.

It may, then, represent ingress of water accompanying solutes such as sodium, chloride, and nitrate ions, or the relatively small monosaccharide molecules. It must be noted that the situation may be more complex than this statement implies. In "isosmotic" solutions composed of equal parts of 0.15 M NaCl and 0.3 M sucrose, little or no fluid uptake occurred (Fig. 2). Water was taken up, however, from a solution of tonicity approximately equal to 0.3 M sucrose which was prepared by mixing 3 parts of 0.15 M NaCl with 1 part of 0.3 M sucrose. Two hypotheses may be considered to explain the observed failure of increased hydration of tissues soaked in mixtures of equal parts of 0.15 M NaCl and 0.3 M sucrose. It is possible that the presence of sucrose in relatively high concentration (0.15 M) changed the characteristics of the cell membrane so that penetration of water in the direction of the postulated osmotic gradient could not occur during the time of the experiment. Alternatively, if fluid uptake accompanied

penetration of sodium and/or other ions according to their concentration gradients, reduction in concentration of these ions on dilution of the 0.15 M NaCl with 0.3 M sucrose might be the cause of the failure of net fluid uptake by the tissues. Against the first hypothesis is the evidence that fluid does enter renal tissue from mixtures of equal parts of 0.3 M glucose and 0.3 M sucrose, although, as might be expected, to an appreciably lesser extent than from pure solutions of 0.3 M glucose (Fig. 2).

Sources of Error.—Since the results of studies on the penetration of fluids into rat renal tissue *in vitro* diverge rather sharply from predictions based on current ideas about cell–extracellular fluid exchanges, it appears desirable to discuss in detail sources of error inherent in the experimental method used. Undoubtedly some damage to the tissue cells resulted from their removal from a normal environment, possible relative anoxia, and direct trauma accompanying the process of tissue slicing and maintenance *in vitro*. Further, no attempt has been made to evaluate the extent of intracellular, as compared with extracellular uptake of fluids by a given tissue, though studies by Opie (3) and Robinson (6) have shown that much of the water taken up by tissues in the circumstances of the experiment actually enters the cells.

Additional sources of error which may be considered relatively minor, random, and therefore unlikely to contribute to the observed result of a clear-cut differentiation between the degrees of hydration of tissue exposed to various "isosmotic solutions," included the following: (*a*) Non-homogeneity of the tissue used. In 32 experiments, the water content of the first pair of cortical slices prepared in each experiment was compared with the relative hydration of the last pair to be cut. The average deviation between these paired initial and final samples was ± 5.6 per cent. Part of this deviation may have resulted from combined weighing and drying errors, which, in test studies, were estimated as averaging about ± 2 per cent. In addition, the water content of medullary tissue is higher than that of cortical tissue (relative water contents—average of 32 experiments: cortex, 2.79 ± 0.30; medulla, 3.58 ± 0.48). This could have affected the findings since cortical and medullary tissues were separated simply by dividing the whole kidney slice with a razor blade and in studies on cortical tissue, for instance, contamination of cortical with medullary tissue may have existed occasionally. (*b*) The pH of the solutions used ranged from about 5.7 (for non-buffered pure solutions) to 7.4 (for buffered solutions—blood, Krebs-Ringer-phosphate). This variation could not affect qualitatively the conclusions which may be drawn from the findings. Thus, the characteristic difference in behavior between slices in mono- and in disaccharide solutions could not be attributed to variations in pH. (*c*) The temperature of the solutions used was determined by ambient room temperature, and ranged from 21.4 to 32.0°C. Additional experiments showed that fluid uptake is somewhat higher for a given time interval at the upper extreme of this temperature range than

at the lower. Yet this variation could not have affected the direction of the results, since the difference between the fluid uptake of tissues immersed in disaccharide solutions on the one hand, and slices soaked in any of the additional solutions tested on the other, was demonstrable within a single experiment at constant temperature as well as in the entire series of experiments in which the temperature range occurred. (*d*) Errors in preparing the solutions, and failure to correct for activity coefficients of dissociable solutes existed but could not be critical factors in determining the results obtained. Thus, in Fig. 3 are presented the results of 5 experiments in which the effect of variation in solute

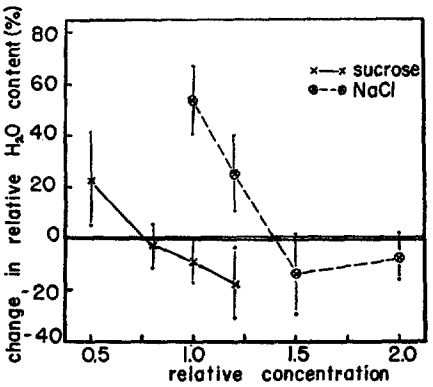

FIG. 3. The effect of solute concentration in the immersion fluid upon relative tissue water content of kidney cortex. Data from 5 experiments. Abscissa, relative concentration of immersion fluids, with the value 1.00 representing solutions isosmotic with rat blood plasma (0.15 M for NaCl, and 0.3 M for sucrose). Ordinate, percentage change in relative water content from control value. Immersion time ½ hour. Solutions at room temperature. Average values are indicated by the X's and circled X's. The standard deviation for each average is represented by one-half the length of the vertical lines passing through the plotted averages.

(NaCl and sucrose) concentration upon tissue fluid uptake was studied. The data summarized here show that even extremely gross errors, such as ±10 per cent, in the estimation of the concentration, and therefore of effective osmotic pressure, of sucrose molecules and Na^+ and Cl^- ions would not affect qualitatively the conclusions reached as a result of the present study. (*e*) Two additional sources of random variation may be noted. Slice thickness varied, and differences must have existed also in the amounts of immersion fluid adhering to surfaces of the slices after blotting with filter paper.

DISCUSSION

Observations on the increased fluid content of renal tissue *in vitro* appear to be consistent with the simple hypothesis that water penetrates renal cells

accompanying ions (*e.g.*, Na^+ and Cl^-) or relatively small molecules (monosaccharides), whereas no net water uptake occurs in solutions of the relatively large and presumably non-penetrating disaccharide molecules. As independent supporting evidence for this hypothesis, it may be noted that Na^+ and K^+ ions exchange readily across renal cell membranes under comparable experimental conditions (9, 14), whereas sucrose has a limited and presumably primarily extracellular distribution in kidney tissue *in vitro* (6) as it does *in vivo*. Furthermore, neither of the two other mechanisms which have been advanced to explain the highly consistent occurrence of fluid uptake by various tissues from isosmotic solutions *in vitro* appears to explain satisfactorily the findings in the case of the kidney presented here. Thus, the hypothesis that the cells may be hypertonic with respect to the extracellular fluids could not account for the absence of uptake in disaccharide solutions isotonic with the ion-containing solutions in which marked uptake does occur. Further, processes akin to colloidal imbibition of water could hardly be invoked to explain the difference between uptake in monosaccharide solutions and in fluids containing disaccharides. Opie and Rothbard (4), Robinson (6), and others have found that certain mammalian tissues decrease in relative water content when they are immersed in highly concentrated solutions of sodium chloride, and this observation has been confirmed for kidney in the present study (Fig. 3). It might be supposed that this would constitute evidence against the hypothesis that sodium and chloride ions, as well as a variety of other solutes, are able to penetrate renal cells under the conditions of the experiment. Such is not necessarily the case, however, for the observed loss of fluid from kidney tissue to highly concentrated solutions could be explained readily by supposing that the solutes traverse the barrier at the cell membrane much more slowly than water. Then if the time of observation was short enough, and the external solute concentration sufficiently high, the net osmotic driving force would be outwards even though solute continued to penetrate gradually into the intracellular phase.

It is probable that the phenomenon of tissue uptake of water from fluids approximately isosmotic with blood plasma and other known extracellular fluids is of considerable theoretical significance, since it constitutes evidence that the fluid content of certain tissues of mammals is subject to dynamic regulation. This regulation appears to be disrupted when optimal respiratory conditions are impaired, as by decreased oxygen supply, fall in environmental temperature, or the presence of various chemical inhibitors of respiration (6, 15). Little is known of the mechanism of the postulated control, although the work of Robinson (10) and Mudge (9) suggests that tissue hydration and/or electrolyte balance may depend specifically upon conversion of energy through the high energy phosphate bonds of the ATP system. Thus, the presence of 2,4-dinitrophenol increases the hydration without decreasing oxygen consumption of tissues *in vitro*. In addition, renal tissues hyperhydrated in the

presence of cyanide return toward normal water content when the cyanide concentration in the medium is reduced (6). This fact is of interest, since it suggests that relatively stable mechanisms operate to readjust cellular water content when the physiological stress state is corrected. Although no direct experimental work has as yet been presented to indicate whether phenomena paralleling the *in vitro* occurrences take place *in vivo* as well, Opie (13) and Robinson and McCance (8) have discussed the evidence that excessive cellular fluid uptake can occur pathologically in intact animals. Finally, it should be stressed that the phenomenon of hyperhydration in isosomotic solutions *in vitro* is not peculiar to kidney, but is seen also in the case of liver, pancreas, striated and smooth muscle, nerves and brain, and other mammalian tissues (1–6). Among the relatively smaller group of cells and tissues showing less or negligible fluid uptake under the specified *in vitro* conditions are thymus, areolar tissue, erythrocytes, certain tumors, and tissues from otherwise normal animals poisoned with various chemical agents (*e.g.*, carbon tetrachloride, chloroform) (3, 12, 13).

The described osmotic behavior of mammalian tissues appears somewhat erratic as compared with the more predictable response of algae, marine invertebrate eggs, mammalian erythrocytes, and other classical material for the study of osmotic and permeability problems. Probably the complete analysis of mammalian fluid uptake *in vitro* will require detailed and specific study of the exchange of water and individual solutes. Such an analysis may prove to be well worth the effort because of the light which it promises to cast on the difficult problems of extracellular-intracellular fluid exchanges *in vivo*.

SUMMARY

Rat renal cortical and medullary tissues show a marked elevation of relative water content when immersed in "physiological solutions" containing sodium and chloride ions, or in equally concentrated solutions of monosaccharides. In contrast to this, no increase in relative water content occurs in isosmotic solutions of three disaccharides studied. It appears to be unlikely that the fluid uptake is a result of intracellular hypertonicity existing either physiologically or pathologically. A more satisfactory alternative hypothesis is that ingress of water accompanies entrance of solutes (ions, monosaccharide molecules) into the tissue cells.

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