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THE TOTAL INTRACELLULAR CONCENTRATION OF MAMMALIAN TISSUES COMPARED WITH THAT OF THE EXTRACELLULAR FLUID

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Since animal tissue cells have easily distensible membranes it is to be expected that the total intra- and extracellular concentrations are the same. If the concentration within the cell were maintained appreciably higher than that outside, this would involve a continuous active extrusion of water, with an unremitting expenditure of energy directed thereto throughout the normal life of the cell.

The view that there is no such continuous expenditure of energy is supported by the fact that, from the analytical data for the rat muscle expressing the amounts of known constituents of quantitative significance (Conway & Hingerty, 1946), the total molecular concentration within the fibres would appear to be identical with that in the extracellular fluid.

On the other hand, Robinson (1950) has described experiments with mammalian tissues, which indicate, as he believes, that not only is the molecular concentration within the cells appreciably higher than that outside, but it is even 50–100% higher. This would imply that about 150–300 m.mole of unrecognized material exist in the fibre water of skeletal muscle. Such material would exceed the total potassium content, also that of the total phosphate, but it has so far escaped notice.

Robinson considers that his observations can be explained only if energy derived from respiration is used to expel water from the cells. In a steady state the higher internal osmotic pressure causes water to diffuse into the cells as fast as it is pumped out. His conclusions are based on facts of the following kind. Slices of rat kidney immersed in isotonic Ringer's fluid, either oxygen-free or containing cyanide, were found to take up water rapidly. Increase of weight had been previously observed by Stern, Eggleston, Hems & Krebs (1949) for slices of kidney, liver, brain, lung and spleen under anaerobic conditions. Similar observations were made for slices of rabbit kidney by Conway & FitzGerald (1942). Also, the swelling of the isolated frog kidney in

cyanide-Ringer was investigated by Conway, FitzGerald & MacDougald (1946), but this was attributable to changes in the second convoluted tubule. Robinson found (1950) that the tissue slices of kidney or muscle which had taken up much water under anaerobic conditions lost this water again when restored to an oxygen atmosphere. The reversal was rapid, taking only a few minutes.

Concerning intracellular concentrations in mammalian tissues he refers to Sabbatani (1901) and other workers following the same procedure, who found that the osmotic pressures of the cells of parenchymatous organs were not the same as those of the extracellular fluids, but some 50–100% greater. Thus Gömöri & Molnár (1932) found the freezing-points of a number of organs from the rabbit to be considerably lower than that of the serum. However, Höber (1922) has effectively criticized the Sabbatani method in which the freezing-point of unbroken cells is examined, and emphasized the large degree of supercooling that would arise.

The question of the molecular concentrations in mammalian tissue cells would be finally decided, if by reliable cryoscopic measurements the freezing-points of the intracellular fluid could be measured or with certainty inferred for *in vivo* conditions. This question is investigated here, and the results described appear to show conclusively that the normal intracellular concentrations of mammalian tissues is the same as that of the extracellular fluid within the sampling error involved. In other words, no energy would appear to be normally wasted in the active extrusion of water from the cells.

METHODS

In the microcryoscopic method used, one thermocouple was immersed in an ice-water mixture at 0° C and the other in the tissue mixture (prepared as described below), which, having been supercooled, was made to freeze by seeding with a tiny crystal of frozen 0·95% (w/v) NaCl. The thermocouples were connected to a mirror galvanometer.

Apparatus

A sketch of the apparatus is shown in Fig. 1. *A* and *B* are two large and rather heavily constructed Dewar flasks, well insulated by surrounding with several layers of cotton-wool. The flask *B* contained a mixture of distilled water and crushed ice made from distilled water. The flask was filled almost completely with the ice-water mixture, and the thermocouple kept well below the surface. The stirrer *S* was also frequently used, and in this way the temperature of the thermocouple was maintained exactly at 0° C throughout the freezing-point measurements.

The second flask contained a freezing mixture of ice and saline maintained at about −1·5° C. Immersed in this mixture was a glass chamber which could hold several 'freezing-point tubes' (*C*).

A more detailed sketch of this arrangement is shown in Fig. 2. The tube *C* (about 1 ml. capacity) contained the 'tissue mixture' *U*, and this tube could be slipped on rapidly and easily over a small piece of rubber tubing *P* attached to the end of the forked tube *L*. This is held in position by the cork *M*, and the thermocouple T_1 is introduced through it, dipping into the tissue mixture. The thermocouple was made of thin copper and eureka wire, and in order to insulate one from the other the copper is brought up through a glass tube of narrow bore and the eureka wire wound around the outside. The tube makes a good stirrer for the tissue mixture.

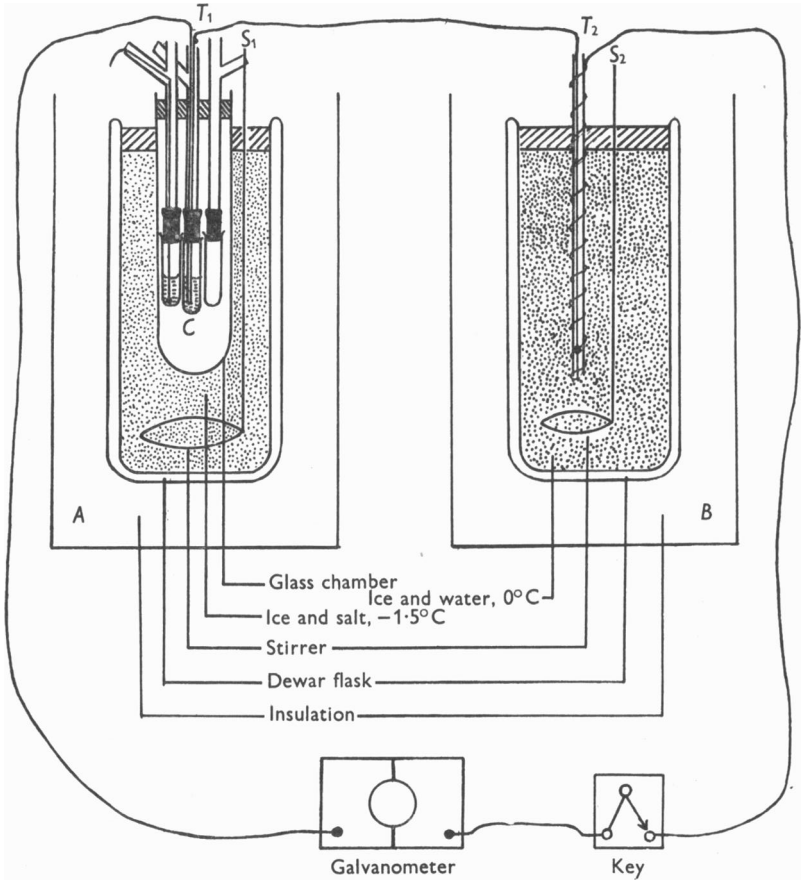

Fig. 1. The microcryoscopic arrangement is shown in diagrammatic cross-section.

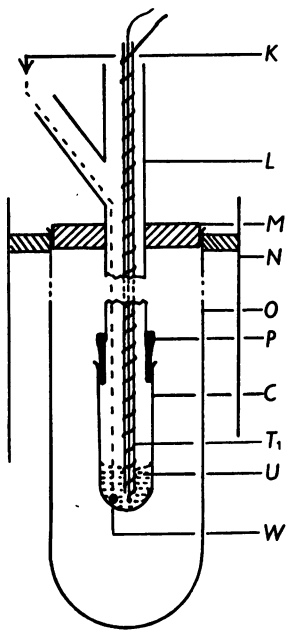

Fig. 2. An enlarged diagrammatic cross-section of a freezing tube in position.

For seeding, small perforated glass beads, W, are frozen in 0·95% NaCl (i.e. a saline with approximately the same freezing-point as the tissue mixture). These can be introduced through the fork of the tube L at K.

Using this method it is possible to work accurately with as little as 0·2–0·4 g of the mixture or 0·1–0·2 g of the ground tissue. Each one-hundredth of a degree Centigrade was represented on the scale by a displacement of 2·5 mm. Repeated determinations of the freezing-point of a standard solution did not differ by more than one-hundredth of a degree, which was sufficiently accurate for the purposes of the investigation.

Preparation of the tissues

In nearly all cases the experimental animal (guinea-pig or rat) was anaesthetized with ether; the abdominal muscle, kidney or other tissue was then removed and dropped immediately into liquid oxygen. After this the animal was bled from the jugular vein, a sample of the plasma or serum being kept for analysis. In some experiments the tissues were not frozen but ground at room temperature.

The frozen tissue was ground in a mortar, cooled by pouring in liquid oxygen from time to time. A rapidly weighed amount of the powdered tissue was then quickly but well ground with an equal volume of 0·95% (w/v) NaCl at 0° C. This saline contained M/500-sodium azide and M/500-monoiodoacetate, with the object of inhibiting or delaying the increase in the concentration of metabolites after grinding. (Such inclusions were found to have only a small delaying effect.) The saline was added to the ground tissue to ensure sufficient fluidity for the subsequent cryoscopic procedure.

When the mixture was sufficiently fluid and mixed, due to such grinding, it was then either transferred as quickly as possible to a freezing-point tube and a determination carried out, or it was maintained at approximately 0° C for varying periods.

Determination of the freezing-point

The tissue mixture in tube C was allowed to supercool by about 0·5° C, being stirred from time to time with the thermocouple holder. The freezing mixture in the bath outside the cooling tube was maintained at $-1·5$ to $-1·7$° C, being frequently stirred. When the mixture in tube C was sufficiently supercooled a perforated glass bead containing frozen 0·95% NaCl was introduced at K and at once the light image of the scale moved back to the position of the true freezing-point.

The procedure was standardized using standard NaCl solutions at and around 0·95% NaCl. From the scale readings corresponding to the freezing-point of the tissue mixture and of the saline, that of the tissue could be determined by the following calculation.

Calculation of the freezing-point (0° C) of the tissue

It will be convenient to consider here the freezing-point depression rather than the freezing-point. It will be written simply as F_t; the depressions for the tissue mixture, the saline used and the tissue being given as F_m, F_s and F_t.

The value of F_t is 0·57° C, being the freezing-point depression for 0·95% NaCl containing the inhibitors as described above.

For each gramme weight of tissue taken, an equal number of millilitres of saline is added. (Here millilitres of saline will be regarded as equal to grammes of saline.) If f is the weight of tissue water per g tissue, this can be regarded as forming a solution in the ground tissue with freezing-point depression $= F_t$. The total water in the ground tissue and saline mixture is $(wf + w)$, where w is the weight of ground tissue taken. The following may then be written:

$$F_m(wf + w) = F_t wf + F_s w. \tag{1}$$

From this

$$F_t = F_m\left(\frac{1+f}{f}\right) - \frac{F_s}{f} \tag{1a}$$

$$= F_m\left(\frac{1+f}{f}\right) - \frac{0·57}{f}. \tag{2}$$

This, however, takes no account of possible condensation of water during the grinding of the frozen tissue, and mixing with the saline. This was assumed to be a negligible quantity, but was investigated later (after being represented to the authors as possibly significant). The investigation was carried out by determining the dry weights of tissue ground at room temperature with a volume of water equal in millilitres to the grammes weight of tissue; and, similarly, the dry weights after freezing in liquid oxygen then grinding and mixing with a volume of water at 0° C equal in millilitres to the grammes weight of the frozen and ground sample. The procedure was the same as that described above before determining the freezing-points, except that water was here used instead of saline.

In calculating the condensed water, the total volume of frozen and ground mixture with water, containing the same dry weight as 100 g tissue plus 100 ml. water ground at room temperature is obtained. Subtracting 200 g from this gives the weight of the condensed water per 100 g original tissue.

Guinea-pig liver was selected for the investigation, a number of small portions of the fresh liver being taken for freezing, and a similar number for grinding at room temperature, the total weight being approximately the same as that used in the freezing-point determinations recorded, and of the order of 1 g. The samples of the mixtures were weighed in platinum crucibles and dried overnight at 105° C.

From a series of twelve experiments the mean condensation was $1{\cdot}6 \pm 1{\cdot}5$ (S.E.) g/100 g original tissue. This is not significantly different from zero, but could in an individual instance be as high as 6%. Such variability, however, includes the variability in dry weight of the tissue samples.

In considering how such condensation of water may enter into the calculations of the freezing-point depressions, if it is assumed that w is the original weight of the tissue in the sample of frozen and ground powder, and that a is the grammes water condensed into the powder, then the weight of the powder sample $=(w+a)$. To this $(w+a)$ g of saline are added, the total weight of water being now $(wf+w+2a)$. On mixing with the saline some more fluid is condensed $(=b)$, so that the total weight of water is $(wf+w+c)$, where $c=(2a+b)$, or the total condensed water.

Equation (1) may then be rewritten as

$$F_m(wf+w+c)=F_t wf+F_s(w+a), \tag{3}$$

from which, on rearranging

$$F_t=\left\{F_m\left(\frac{1+f}{f}\right)-\frac{F_s}{f}\right\}+\left\{\frac{1}{wf}\left(F_m c-F_s a\right)\right\}. \tag{4}$$

The first expression within large brackets on the right corresponds to that in equation (1*a*) above. The second expression amounts to an additional and relatively small mean correction for a freezing-point depression of F_m. For approximate calculation, it may be noted that the value of b is likely to be much less than a, so that in effect $a=\frac{1}{2}c$, and with F_m and F_s nearly the same in value the correction in equation (4) amounts to about $\dfrac{0{\cdot}57\times 0{\cdot}016}{2f}$. With f near to 0·8 it is approximately 1% of the F_t value.

This small correction will be neglected in the ensuing calculations, but it may be noted in any final consideration that the mean F_t figures found for various tissues are probably 1%, and possibly some few per cent, too low because of condensation during grinding after freezing in liquid oxygen.

The value of f for muscle, liver, kidney and serum is 0·77, 0·72, 0·76 and 0·92 respectively, and equation (2) becomes:

$$F_t=2{\cdot}30\ F_m-0{\cdot}74 \text{ (for muscle)}, \tag{2a}$$

$$F_t=2{\cdot}99\ F_m-0{\cdot}79 \text{ (for liver)}, \tag{2b}$$

$$F_t=2{\cdot}32\ F_m-0{\cdot}75 \text{ (for kidney)}, \tag{2c}$$

$$F_t=2{\cdot}08\ F_m-0{\cdot}62 \text{ (for serum)}. \tag{2d}$$

Effect of deep freezing on the F_t values. It is conceivable that deep freezing may alter the F_t value because of some protein change. It was considered desirable, therefore, to investigate this as

follows. A tissue suspension was made at room temperature by grinding with an equal weight of saline in the usual manner. After leaving for an hour, samples were taken and their freezing-points determined in the small freezing tubes. These were then immediately introduced into liquid oxygen, and after some time the tubes were removed, the mixture thawed out and its freezing-point determined once more.

The tissue chosen was guinea-pig kidney. Six alternate determinations before and after deep freezing were carried out on different kidney suspensions. No difference appeared due to the immersion in liquid oxygen. Thus the mean F_t of the six determinations before deep freezing was 0·712° C and after it was also 0·712° C. The mean difference between each set of determinations was $0·000 \pm 0·003$.

RESULTS

F_t values after grinding at room temperature

Table 1 shows the results obtained after grinding the tissue at room temperature with an equal volume of saline, calculating F_t as if the tissue solids were uniformly dissolved in the total tissue water, as described in Methods. It will be seen that such F_t values are in general much greater than those of the plasma, that for the diaphragm being about twice as great, and for the abdominal muscle about 50% greater.

F_t values after freezing and grinding in the cold

The various tissue values as given in Table 1 are considerably less than those obtained after grinding at room temperature. Owing to the marked differences obtained the results from each tissue will be considered separately.

TABLE 1. Average freezing-point depressions (F_t) for mammalian tissues (guinea-pig) ground at room temperature, or first frozen in liquid oxygen and then ground.

	Freezing-point depressions			
	Ground at room temperature		Frozen and ground	
Tissue	$F_t \pm$ S.E. (° C)	No. of observations	$F_t \pm$ S.E. (° C)	No. of observations
Liver	$0·65 \pm 0·03$	8	$0·55 \pm 0·01$	5
Kidney	$0·69 \pm 0·02$	8	$0·55 \pm 0·02$	8
Abdominal muscle	$0·80 \pm 0·02$	5	$0·61 \pm 0·01$	18
Diaphragm	1·06	4	0·62	2

Mean freezing-point depression for serum $= 0·55 \pm 0·005$ (14).

Liver. The mean value found for guinea-pig liver was $0·55 \pm 0·01$° C (S.E.) showing therefore no difference from the mean serum value of $0·55 \pm 0·005$° C within the sampling error. Maintaining the ground liver mixture at 0° C for upwards of 60 min also caused no appreciable change.

Kidney (guinea-pig). Table 2 summarizes fifteen results with guinea-pig kidney. Maintaining the ground mixture up to 24 min at 0° C showed no appreciable change, and from 24 min to upwards of 50 min the mean change was but little. The mean result within 24 min at 0° C after grinding was $0·566 \pm 0·01$° C, and thus showed no significant change from the plasma value.

Abdominal muscle (*guinea-pig or rat*). In marked contrast to liver and to guinea-pig kidney, a rapid increase of F_t occurs after freezing, grinding and maintaining at 0° C. It will appear from Fig. 3 that the mean value of F_t determined as rapidly as possible after freezing, grinding and mixing (taking about 4–5 min) will almost certainly give a figure somewhat greater than the value for zero time or that presumably corresponding to the living tissue. A better approximation, therefore, to the true *in vivo* value may be expected by extrapolating to zero time, and this would seem best done by way of a regression equation of F_t against time (t) based on the results under 20 min maintenance at 0° C after grinding and mixing. Up to this time the increase

TABLE 2. Average freezing-point depressions (F_t) for the guinea-pig kidney, subsequent to freezing in liquid oxygen and grinding, then mixing with equal volume of saline ($F_t = 0{\cdot}57$) and maintaining for different times at 0° C

Time in min		No. of	Mean F_t
Range	Average	determinations	(° C)
0–6	4·7	3	0·55
6–12	8·0	2	0·57
12–18	14·0	2	0·55
18–24	19·0	1	0·54
>24	58·0	7	0·58

The average F_t after less than 24 min maintenance at 0° C $= 0{\cdot}556 \pm 0{\cdot}01$.

in F_t is approximately linear. Such an equation has been obtained for fourteen corresponding sets of F_t and t for the abdominal muscle of the guinea-pig. The coefficient of correlation was $+0{\cdot}88$, the standard deviations being 0·0634° C and 3·84 min, and the means 0·651° C and 9·1 min respectively. The equation is therefore

$$F_t = 0{\cdot}0146t + 0{\cdot}52. \tag{5}$$

When t is zero, F_t results as $0{\cdot}52 \pm 0{\cdot}02$° C.

The mean value is somewhat lower than that of the serum ($= 0{\cdot}55 \pm 0{\cdot}005$), but the difference ($-0{\cdot}03 \pm 0{\cdot}021$) is not statistically significant. Assuming a range of three times the standard deviation to include all values that might be practically significant, the upper level amounts to about 5% above the mean plasma value. The thick line in Fig. 3 is drawn in accordance with equation (5).

The diaphragm. Like the abdominal muscle, this shows a rapid increase in F_t on maintaining the ground mixture in the cold. In about 12 min a level of 0·72 is reached, and in about 35 min as high as 0·98° C.

The rat kidney. Unlike that of the guinea-pig, the F_t for the frozen and ground kidney mixed with saline and maintained at 0° C shows a rapid increase with time of maintenance. Fig. 4 gives the results of a series of observations on different animals. Here, as with the abdominal muscle and diaphragm, the average of the most rapidly determined F_t values will be almost certainly

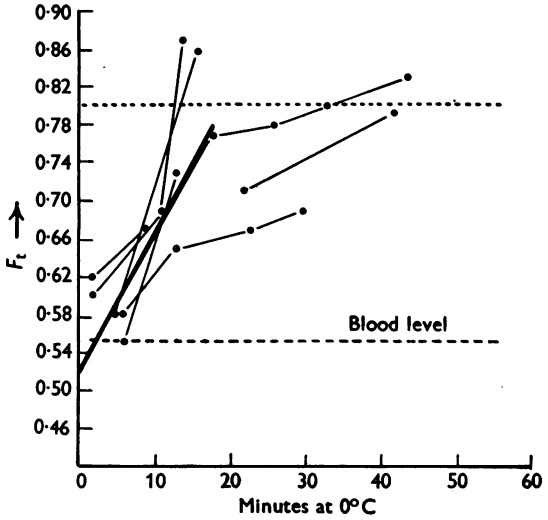

Fig. 3. Freezing-point depressions for abdominal muscle. The tissues were frozen and ground in liquid oxygen as described in text, the mixture being maintained at 0° C for varying times. Points joined in the diagram represent observations on the same tissue mixture. The thick line is from the regression equation (5) in text. The lower dotted line gives the mean blood level and the upper gives the mean level after grinding and mixing at room temperature.

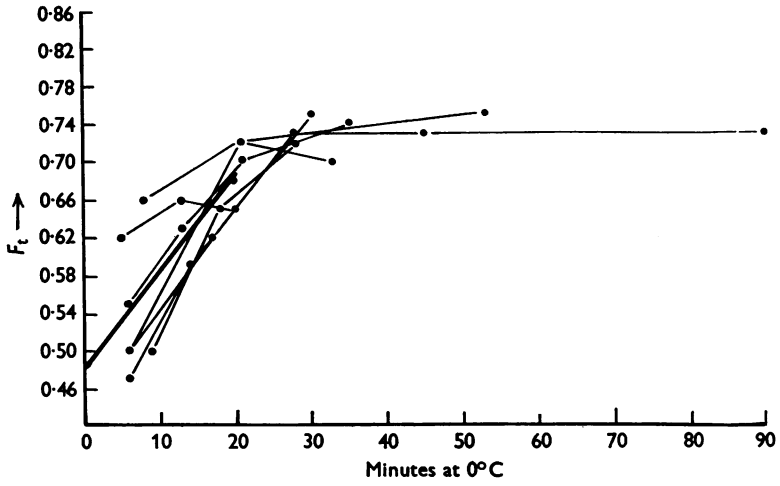

Fig. 4. Freezing-point depressions for rat kidney, frozen in liquid oxygen and ground as described in text, the conditions being the same as for Fig. 3. The thick line is from the regression equation (6) in text.

higher than the true value for the tissue *in vivo*. Up to and including a maintenance time of 21 min, sixteen observations have been made on the kidneys of seven different animals. The correlation coefficient between F_t and t is $+0{\cdot}78$; the standard deviations being 0·080° C and 6·04 min, and the means 0·61° C and 13 min respectively. From such data the regression equation may be given as

$$F_t = 0{\cdot}0103t + 0{\cdot}48. \qquad (6)$$

At zero time $F_t = 0{\cdot}48 \pm 0{\cdot}027$° C. This value of F_t is somewhat lower than that of the serum ($= 0{\cdot}507 \pm 0{\cdot}006$° C). However, the difference ($-0{\cdot}027 \pm 0{\cdot}028$) is not statistically significant. The thick line in Fig. 4 is drawn from equation (6).

The use of 0·1 % *mercuric chloride as inhibitor of the rise of molecular concentration after grinding the frozen tissue*

The variability in determining the F_t values for the tissue *in vivo* introduced by the rapid increase with time of maintenance at 0° C for such tissues as rat kidney and diaphragm can be much reduced by the use of mercuric chloride of the order of 0·1 %, introduced in the saline used for mixing.

TABLE 3. Effect on F_t values of maintaining the ground tissue mixture at 0° C, when 0·1 % $HgCl_2$ is incorporated in the saline

	Minutes at 0° C						
	10	15	25	30	40	70	
	F_t values (° C)						Mean F_t values
Abdominal muscle	0·57	0·54	0·56	0·56	0·52	—	0·550
Diaphragm	—	0·53	0·54	—	0·56	0·55	0·545
Rat kidney	0·52	—	—	—	0·52	0·51	0·517

The effect of $HgCl_2$ itself on the freezing-point of tissue mixtures was investigated in a series of experiments with the abdominal muscle of the guinea-pig and also with the rat kidney. These tissues were frozen in liquid oxygen and ground as usual. A sample of the ground tissue was maintained at 0° C for 60 min. Having reached a practically steady state one portion of the sample was mixed with an equal volume of 0·95 % (w/v) NaCl containing 0·1 % (w/v) $HgCl_2$, and another portion with an equal volume of 0·95% (w/v) NaCl.

It was found that the presence of the small amount of mercuric chloride caused an increased depression of the freezing-point equivalent in the calculated F_t values to 0·03° C for the kidney and 0·02° C for the abdominal muscle. These relatively small corrections were then used in finding the F_t values of the tissues to which the saline with mercury was added at once after grinding and maintained for different periods at 0° C.

The results for guinea-pig abdominal muscle and diaphragm, as well as for rat kidney, are summarized in Table 3. It will be seen from the table that the mercuric chloride inhibits the increase of F_t on maintaining the mixture at 0° C. The mean of the nine values in Table 3 (up to 70 min standing) for the

abdominal muscle and diaphragm is 0·55 ± 0·005° C or the same as that of the serum.

For the rat kidney the mean of the three observations up to 70 min was 0·517, or about 2% higher than the mean serum value of 0·507, but this excess is not significant. In a further series on rat kidney the mercuric chloride did not prevent the F_t value from increasing, but in this experiment the freezing-point determinations of saline containing the mercuric chloride were variable and the results are somewhat doubtful.

DISCUSSION

From the above account it appears that using guinea-pig and rat as experimental animals the lowering of the freezing-point of water by the tissue solutes, regarded as in solution in the total tissue water, does not differ appreciably from that of the intercellular fluid. If an appreciable difference existed, it would be necessary then to allow for the intercellular fluid in the tissue in calculating the F_t value for the intracellular water and hence the molecular concentration of the intracellular fluid. This correction, however, does not arise.

After grinding tissues frozen in liquid oxygen and mixing with 0·95% sodium chloride there may be a marked increase in the F_t values with the time of maintenance even at 0° C, but throughout the F_t values were referred to total tissue water for the reason that intracellular water no longer exists as such on grinding the frozen tissues, whereas the total tissue water may still be regarded as a real figure.

Some of the tissues examined, such as the liver and kidney of the guinea-pig, showed no appreciable change in the F_t values on maintaining the ground mixture at 0° C up to about 30 min. Other tissues such as guinea-pig or rat diaphragm and abdominal muscle showed a rapid increase to values 50–100% above the normal or serum levels. Such rapid increase of solutes in the ground mixture even when maintained at 0° C appears to be quite in harmony with Robinson's observations (1950) on slices of the renal cortex of the rat which were rendered anaerobic. Robinson has sought to interpret his findings of such rapid uptake of water by the hypothesis that there exists under normal average conditions a much higher molecular concentration within the cells than in the external fluid. This necessarily involves a continuous pumping out of water which is inhibited by cyanide or oxygen lack. The present experiments show that the normal intracellular concentration of the kidney as a whole does not differ significantly from that of the intercellular fluid.

From his experiments with rat kidney slices and preliminary experiments on liver and diaphragm which behaved qualitatively in a similar way, Robinson, in a somewhat lengthy discussion, considered the extension of his hypothesis to the tissue cells. These in general, he thought, might have a much

higher concentration (50–100% higher) than that of the extracellular fluid. The cryoscopic experiments described above lend no support to such generalization and show that within the sampling error involved (up to some few per cent of the mean values) the intracellular molecular concentration of tissue cells does not exceed that of the extracellular fluid.

With respect to the osmotic relations of mammalian tissue cells to the extracellular fluid, account may be taken here of some recent experiments of Eggleton (1951). She injected intravenously into cats (with renal pedicles tied) 2 g of NaCl per kg using a 20% solution. Urea was injected at the same time (1 g/kg). After 4 hr samples of blood were taken and the plasma analysed for chloride and urea, the results being compared with those of samples taken before the injections. Assuming urea to distribute itself freely in all the body water, then the volume of this water is given by the grammes injected divided by the increase in grammes per 100 ml. plasma water, the result being multiplied by 100. The figure obtained was 63% of the body weight.

She then writes with respect to the NaCl injection: 'If the cell contents behaved osmotically as an "ideal" solution, the amount of water withdrawn would be such that the final concentration of chloride in the extracellular fluid would be the same as that which would have obtained if the chloride had penetrated freely into the cell contents.' Thus the increase of chloride in the plasma water divided into the amount injected and multiplied by 100 could be expected to give 63% of the body weight like urea. But in fact it gave only 49%.

Various considerations were discussed for the apparent discrepancy, Robinson's work and conclusions with large active excretion of water from the cells complicating ordinary osmotic exchanges being cited as affording a possible explanation. From the work described here, it is obvious that such an explanation is no longer tenable. With regard to the true reason for the difference found between the chloride figure for the body water (49% body weight) and that using urea (63%), it may be pointed out that the assumption contained in the above quotation from Eggleton (1951) is not justified. The true relation may be indicated as follows. The cells are considered to be impermeable (or virtually impermeable) to the injected substance. Then if this already exists in the extracellular water as α times the total moles of impermeable substances therein, and if after injection the total molarity of these impermeable substances is increased to i times that before injection, and further if S represents the total water available for solution to the injected substance (calculated by the method of Eggleton) then it may be shown that S is given by the following relation

$$S = \frac{(V_c + V_e)}{1 + \frac{V_c}{iV_e}(1-\alpha)}, \tag{7}$$

where V_c and V_e are the volumes of the cellular and extracellular water respectively. (The derivation of equation (7) is given in the Appendix.) It is only when $\alpha = 1{\cdot}0$ that S is equal to the total body water. When, on the other hand, α is zero and the amount injected is relatively small so that i does not differ appreciably from 1·0, then $S = V_e$ or the volume of the extracellular water. The injection of thiocyanate (or perhaps better, of inulin) may be given as an example.

When the injected substance is NaCl, then α is 0·73 (Conway, 1950*a*, *b*). Expressing V_c and V_e as percentages of the body weight, then from Eggleton's data for urea and for thiocyanate, $V_c = 38\%$ of the body weight and $V_e = 25\%$; $(V_c + V_e)$, or the total body water, being 63%, so that equation (7) becomes

$$S = \frac{63}{1 + 0{\cdot}41/i}. \tag{8}$$

With the injection of 2 g NaCl per kg this means the addition of 68·4 m.osmole to 0·25 l. of extracellular fluid already containing approximately 77 m.osmole of solute material. The total of solute moles in the extracellular fluid is thus raised from 77 to 145·4, i.e. by 1·89 times. Putting in this value for i in equation (8), $S = 51{\cdot}8\%$ of the body weight which is very similar to Eggleton's result, and some small refinements could be suggested in the calculation which would make the agreement a little closer.

It follows then that the water or osmotic changes resulting from the injection of large amounts of NaCl into a cat with renal pedicles tied are in accordance with expectation on the basis of the virtual impermeability of the cells to NaCl, and the exchange of water following simple physical relationships.

SUMMARY

1. A microcryoscopic method is described whereby multiple freezing-point determinations can be carried out on ground tissue samples as small as 0·1–0·2 g.

2. The average freezing-point depression of guinea-pig serum was found to be 0·55° C ± 0·005 S.E. (fourteen samples), whereas the freezing-point depressions (here designated F_t) of various tissues of the guinea-pig and rat, ground at room temperature, ranged from 1·06 for the diaphragm to 0·65° C for the liver.

3. Tissues first frozen in liquid oxygen immediately after excision, then ground to powder and mixed with an equal volume of 0·95% NaCl at 0° C, gave F_t values of 0·61° C for the diaphragm and 0·56° C for the kidney.

4. If the tissue-saline mixtures were maintained at 0° C, there was a rapid increase in the F_t value. Extrapolation to zero time yielded values identical with that of serum.

5. Sodium azide and monoiodoacetate incorporated in the saline had little

effect in delaying this increase in F_t at 0° C, but mercuric chloride inhibited the rise for a relatively long time. The mean of twelve determinations on guinea-pig abdominal muscle and diaphragm gave $0{\cdot}55 \pm 0{\cdot}007$ which does not differ significantly from the serum value.

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APPENDIX

Derivation of equation (8) in text, for the water space available to an injected substance with renal pedicles tied. Symbols used:

N_c = total number of solute moles in the cells which are non-diffusible or are held by electrostatic forces.

N_e = total number of solute moles in the extracellular fluid, to which the cell walls are impermeable or which are held from penetrating appreciably into the cells by electrostatic forces.

V_c = litres of total water in the cells.

V_e = litres of water in the extracellular fluid.

αN_e = number of moles of a special kind (X) in the group N_e in the extracellular water.

iN_e = new total in extracellular fluid due to the injection of the substance X, to which the cells are impermeable or virtually impermeable.

$N_e(i-1)$ = moles of X injected.

jV_e = new volume of extracellular fluid arising from the injection of X.

The increase in concentration of X in the extracellular fluid is equal to

$$\frac{\alpha N_e + (i-1)N_e}{jV_e} - \frac{\alpha N_e}{V_e}, \tag{9}$$

and the ratio (S) of injected substance (X) to its increase in concentration is given by

$$S = N_e(i-1) \Big/ \left(\frac{\alpha N_e + (i-1)N_e - \alpha j N_e}{jV_e} \right)$$

$$= \frac{(i-1) \times jV_e}{\alpha + i - 1 - \alpha j}. \tag{10}$$

A relation between i and j may be derived from a consideration of osmotic equality across the cell membranes before and after injecting substance X.

Thus

$$\frac{N_e}{V_e}=\frac{N_c}{V_c}, \tag{11}$$

and

$$\frac{iN_e}{jV_e}=\frac{N_c}{V_e-(j-1)V_e}. \tag{12}$$

From equations (11) and (12)

$$j=\frac{i(V_e+V_c)}{(V_c+iV_e)}. \tag{13}$$

Inserting the value of j in equation (10), there finally results

$$S=\frac{(V_e+V_c)}{1+\frac{V_c}{iV_e}(1-\alpha)}. \tag{14}$$