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PERMEABILITY OF GUINEA-PIG SMOOTH MUSCLE TO NON-ELECTROLYTES

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In 1959, Bozler found that the non-electrolytes urea, thiourea and glycerol rapidly enter the smooth-muscle cells of the frog's stomach. Glycerol and other non-electrolytes including dimethyl sulphoxide have also been found to enter the intracellular space of many types of cells (Szent-Györgi, 1949; Davson & Danielli, 1952; Lovelock, 1953; Lovelock & Bishop, 1959).

Glycerol and dimethyl sulphoxide, but not urea and thiourea, possess the remarkable property of protecting living cells from damage during freezing to and thawing from very low temperatures. Dimethyl sulphoxide (DMSO; $CH_3.SO.CH_3$) is a polar substance which, like glycerol, forms hydrogen bonds with water. The use of these two compounds has allowed spermatozoa (Polge, Smith & Parkes, 1949), erythrocytes (Smith, 1950; Lovelock & Bishop, 1959) and bone marrow (Ashwood-Smith, 1960) to be stored at the temperature of solid CO_2 ($-79°$ C) or of liquid nitrogen ($-196°$ C) for long periods (see Smith, 1961).

From work with many types of cells, including erythrocytes and spermatozoa, it has become apparent that large concentrations of glycerol or of dimethyl sulphoxide must enter the cells in order to protect them from damage (Lovelock, 1953; Smith, 1961). On the basis of one series of experiments with mammalian eggs, however, Sherman (1963) has disputed that high intracellular concentrations of glycerol are necessary for protection against damage during freezing and thawing.

It was thought that some quantitative information on the rate of uptake of glycerol and dimethyl sulphoxide into cells was needed, especially when considering methods for storing intact organized tissues rather than cell suspensions. The time course of the entry of DMSO and glycerol into smooth muscle has therefore been investigated and possible factors affecting the permeability to DMSO have been studied. These experiments form part of an investigation into the action of DMSO in protecting smooth muscle from the harmful effects of freezing and thawing.

METHODS

Solutions. The composition of the modified Krebs's solution used was (mM): NaCl 118, KCl 4·5, $CaCl_2$ 1·4, $MgCl_2$ 1·16, NaH_2PO_4 1·16, $NaHCO_3$ 25, glucose 11·1. These concentrations of salts and glucose were the same in the Krebs's solutions containing dimethyl sulphoxide (DMSO) or glycerol. A highly purified sample of DMSO was kindly supplied by the Crown Zellerbach Co. The glycerol was Analar grade. ^{35}S-dimethyl sulphoxide and ^{14}C-glycerol were obtained from the Radiochemical Centre, Amersham. The concentration of inulin (when present) was 2·5 mg/ml. All solutions were bubbled with O_2 95 % + CO_2 5 %.

Incubations. Albino guinea-pigs of the Hartley strain, weighing 300–400 g, were used. Either two taeniae coli from the caecum of each animal, or both uterine horns, were placed in 4 ml. of Krebs's solution containing inulin in a small tube. In most experiments the tissues were kept at 37° C for 4 hr. The pieces of tissue were then transferred to identical tubes containing 4 ml. of inulin-Krebs's solution in which various concentrations of either DMSO or glycerol were present. Part of the DMSO or glycerol present was ^{35}S-DMSO or ^{14}C-glycerol (with an activity of about 0·04 μc/ml. of the final solution). In each group half the tissues were incubated with the labelled non-electrolyte for 5 min and the remainder for 20 min. After incubation the uteri were cut longitudinally, carefully drained on glass Petri dishes and then weighed separately. The taeniae coli were drained and weighed in the same way. After weighing, one uterine horn or one taenia coli from each incubation sample was dried to constant weight at 120° C, reweighed and the water content calculated.

The remaining tissue from each incubation sample was placed in 1·5 ml. of 0·85 N-NaOH in a 12·5 ml. calibrated test-tube and digested in a boiling water-bath for 5 min. After the extract had been neutralized with 2N-HCl the protein was precipitated with 2·5 ml. of 0·5 N-NaOH and 2·5 ml. of 10 % wt./vol. $ZnSO_4$. The contents of the tubes were then made up to 12·5 ml. with de-ionized water, stirred and filtered (Whatman No. 542).

Inulin estimation. The concentration of inulin in 3 ml. of the filtrate thus obtained was determined by the method of Ross & Mokotoff (1951), using the resorcinol reagent of Roe, Epstein & Goldstein (1949). Readings were made using a Unicam S.P. 500 spectrophotometer.

Liquid scintillation counting. A small amount (0·2 ml.) of the same filtrate was accurately pipetted and mixed with 4·8 ml. of dioxane scintillator and then counted for 10 min using a Tricarb liquid scintillation spectrometer. All samples were counted at least 4 times.

Standards and blanks. Small samples of both the individual and pooled inulin-Krebs's solutions containing ^{35}S-DMSO or ^{14}C-glycerol which had already been used for the incubation were added to a series of fresh muscles which had not been incubated. These muscles together with the samples were then digested in 1·5 ml. of 0·85 N-NaOH and put through the same procedure as before. The inulin concentration was estimated in various dilutions of the final filtrates from these extracts. The radioactivity of suitable dilutions of the same samples was also measured. Blank solutions were prepared and used for the estimations of both inulin concentration and radioactivity. These solutions contained muscles that had been incubated in a Krebs's solution without inulin or a labelled non-electrolyte.

Calculation of intracellular concentrations of DMSO or glycerol. It was initially assumed that for each tissue sample

$$\text{water content} = \text{inulin space} + \text{'intracellular water space'};$$

that is

$$[H_2O]_t = [H_2O]_e + [H_2O]_i.$$

These values have been expressed in ml./kg wet wt.

A second assumption was that the concentration of DMSO or glycerol in the inulin space was equal to that in the external solution. The amount of DMSO or of glycerol within the cell could then be estimated by subtracting the amount calculated to be in the inulin space

from that found on analysis of the whole muscle. The intracellular concentration of DMSO or glycerol was then determined by dividing this amount by the calculated value of $[H_2O]_i$.

However, since the majority of these experiments were carried out, Goodford & Leach (1964) have shown that the penetration of inulin into the extracellular space of smooth muscle may be impeded by intercellular mucopolysaccharides. A better estimate of the extracellular space may therefore be that obtained using monatomic ions (e.g. Na^+) instead of inulin. The intracellular concentrations of DMSO and glycerol were therefore recalculated using the value of extracellular space (45 %) mentioned by Goodford & Leach (1964).

V/A. This is the ratio of cell volume to cell surface area. Smooth-muscle cells were teased out from taeniae coli both in Krebs's solution and also after incubation in a Krebs's solution with streptokinase. It was difficult to obtain single cells by these methods but consistent measurements of length and diameter were obtained from those few cells that could be isolated.

Weight changes. Taeniae coli were weighed on a torsion balance at 3 min intervals before, during and after incubation in Krebs's solution containing DMSO. The surface fluid was carefully drained off before each weighing.

Computation of permeability constants. The basic equations describing the penetration of a substance into cells are those of Jacobs (1933):

$$\frac{d[S]_i}{dt} = P_1 \frac{A}{V}([S]_e - [S]_i), \tag{1}$$

$$\frac{dV}{dt} = P_2\left(\frac{S+a}{V} - [S]_e - [C]_e\right), \tag{2}$$

where

$[S]_i$ = intracellular concentration of penetrating substance S after time t,
$[S]_e$ = concentration of penetrating substance in the surrounding medium,
P_1 = permeability constant of substance,
P_2 = permeability constant of water,
A = surface area of cell,
V = volume of cell through which S is uniformly distributed,
a = amount of osmotically active substance originally present within the cell,
$[C]_e$ = concentration of non-penetrating substance in the external medium.

When osmotic forces are absent eqn. (1) can be used alone. However, although eqn. (2) was applicable to the experiments with the larger concentration of DMSO (1·4 M), it was not used. Instead, alterations to the term A/V in eqn. (1) due to osmotic effects have been calculated for each set of conditions using the expression

$$\frac{d(A/V)}{A/V} = -\frac{2}{5}\frac{dV}{V}. \tag{3}$$

Equation (3) can be derived and used if the following assumptions are made. First, that the muscle cell is a long thin cylinder with a uniformly elastic membrane over the curved surface, and, secondly, that when a movement of water changes the internal pressure by dp, it also changes V and A by dV and dA. One further assumption was made: the loss of weight of the muscle which was obtained experimentally for each set of conditions was assumed to be solely the result of a shift of intracellular water and thus could represent dV/V. The maximum correction to A/V was 7·3 % for the 5 min value in DMSO (1·4 M).

The term A/V was also corrected throughout, to allow for the volume of solids within the cell assumed to be inaccessible to the hydrophilic non-electrolytes (Davson & Danielli, 1952). The proportion of the cell volume available for penetration will approximate to

$$[H_2O]_i/(1000 - [H_2O]_e).$$

Thus

$$\frac{A}{V} = \frac{2}{r}\left(\frac{1000 - [H_2O]_e}{[H_2O]_i}\right).$$

Even after all these corrections had been made, the experimental values of $[S]_i$ after 5 and 20 min incubation could not be matched with eqn. (3) using an analogue computer, since the values of $[S]_i$ after 20 min incubation were greater than the external concentration $[S]_e$. The results could be matched, however, using an analogue of the following equation, modified from eqn. (1).

$$\frac{d[S]_i}{dt} = k_1^{in}\frac{A}{V}[S]_e - k_1^{out}\frac{A}{V}[S]_i, \quad (4)$$

where k_1^{in} = the apparent permeability constant for the substance entering the cell,
and k_1^{out} = the apparent permeability constant of the substance leaving the cell.

Both k_1^{in} and k_1^{out} are expressions which include the real permeability constant for the penetrating substance (P_1), but also include a term, which may or may not be linear, that reflects the difference between the state of the substance inside and outside the cell. Thus, k_1^{out}/k_1^{in} will show the relation between the different states of the non-electrolyte inside and outside the cells.

RESULTS

Inulin and water spaces in the uterus

The inulin space of the guinea-pig uterus during incubation in a Krebs's solution containing inulin is shown in Fig. 1. After 4 hr incubation the inulin space had reached 250·6 ± 20·6 ml./kg and the rate of uptake of inulin had decreased. After 24 hr incubation the inulin space had increased to 372·0 ± 18·0 ml./kg. In all subsequent experiments the time of the preliminary incubation in the inulin-Krebs's solution was kept constant

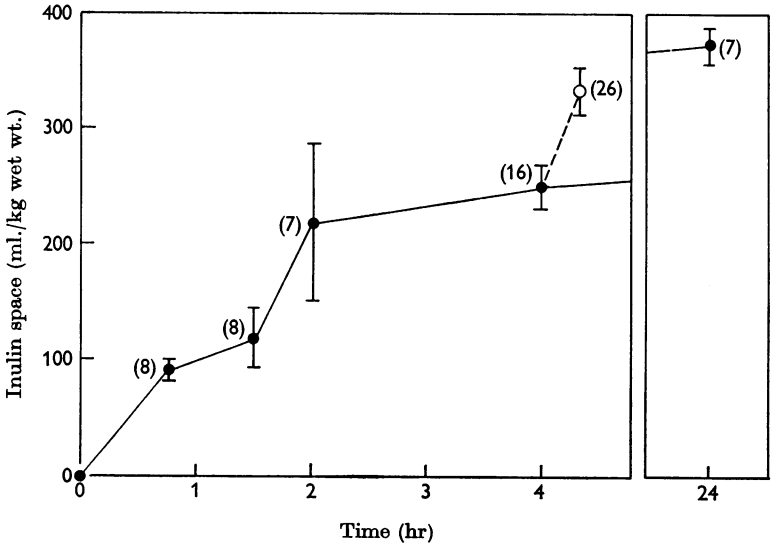

Fig. 1. The inulin space of guinea-pig uteri after various times of incubation at 37° C in inulin-Krebs's solution (●—●). The standard errors of the means are shown and the number of observations are given in parentheses. Incubation for 20 min in a Krebs's solution containing DMSO (1400 mM) (○---○) increased the 4 hr inulin space.

at 4 hr. Figure 1 also shows that when uteri were incubated for 4 hr in the inulin-Krebs's solution, and then for a further 20 min in an inulin-Krebs's solution containing DMSO (1400 mM), the inulin space increased to $333{\cdot}1 \pm 20{\cdot}7$ ml./kg. After the initial 4 hr incubation in the inulin-Krebs's solution the mean total-water content of the uteri was $831{\cdot}4 \pm 3{\cdot}8$ ml./kg ($n = 20$). When the mean inulin space was subtracted from this figure an approximation to the mean 'intracellular water space' ($[H_2O]_i$) could be made. This was found to be $580{\cdot}8 \pm 19{\cdot}2$ ml./kg ($n = 20$).

Inulin and water spaces in the taeniae coli

The mean inulin space of the taeniae coli after the initial 4 hr incubation in inulin-Krebs's solution was $344{\cdot}3 \pm 8{\cdot}5$ ml./kg ($n = 60$). During further incubation of the taeniae coli at 37° C with DMSO (either 14 or 1400 mM) the mean inulin spaces were not altered significantly between the fifth and twentieth minute of the incubation. The inulin space of a group of muscles which were incubated in a Krebs's solution with DMSO (1400 mM), but without calcium, rose significantly from $310{\cdot}2 \pm 14{\cdot}1$ to $387{\cdot}0 \pm 16{\cdot}5$ ml./kg between the fifth and twentieth minute of the incubation ($P = 0{\cdot}01$, $n = 20$).

After the initial 4 hr incubation in inulin-Krebs's solution the mean total water content of taenia was $807{\cdot}9 \pm 3{\cdot}2$ ml./kg ($n = 90$). The mean value of the intracellular water space ($[H_2O]_i$) after this preliminary incubation was $463{\cdot}6 \pm 10{\cdot}1$ ml./kg ($n = 60$). During the incubations at 37° C in solutions containing DMSO, the mean values of $[H_2O]_i$ did not alter significantly, except in the group of muscles incubated for 20 min at 37° C in the Krebs's solution containing DMSO (1400 mM) but no calcium. Here, the value of $[H_2O]_i$ fell from $494{\cdot}0 \pm 18{\cdot}9$ to $433{\cdot}0 \pm 18{\cdot}9$ ml./kg ($P = 0{\cdot}05$, $n = 20$) between the fifth and twentieth minute.

One experiment was carried out in which taeniae were incubated at 37° C for 5 and 20 min in a Krebs's solution which contained glycerol (13·68 mM). Under these conditions no significant change in the inulin space or intracellular water space was detected.

Intracellular concentrations of DMSO in the uterus

Figure 2 shows that, when uteri were incubated for 20 min at 37° C in an inulin-Krebs's solution containing DMSO (1400 mM), the intracellular concentration of DMSO varied with the weight of the uterus. When uteri weighed less than about 130 mg, positive values of the intracellular concentration of DMSO could be calculated, using the assumption that the DMSO concentration in the inulin space equalled that of the external solution. In contrast, when uteri weighed more than 130 mg, calculations based on the same assumption gave negative values for the intracellular

concentrations. These results indicated that the penetration of DMSO into the extracellular space in these larger uteri was not complete after 20 min incubation. For this reason, and also because these experiments do not allow a distinction to be made between the entry of DMSO into the myometrium and into the endometrium, all subsequent experiments were carried out using taeniae coli, weighing less than 60 mg. The taenia consists almost entirely of smooth muscle.

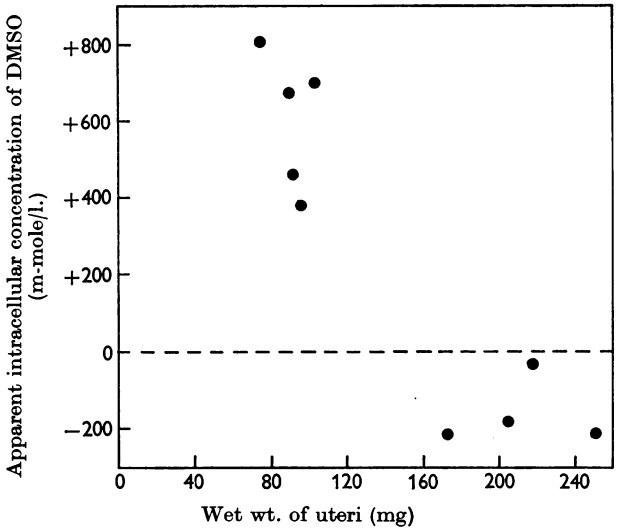

Fig. 2. Effect of the weight of uterus on the apparent intracellular concentration of DMSO after 20 min incubation at 37° C in a Krebs's solution containing DMSO (1400 mM). Each point represents a single observation.

Intracellular concentrations of DMSO in the taeniae coli

Intracellular concentrations of DMSO from the taeniae coli after 5 min and after 20 min incubation in DMSO solutions under various conditions are shown in Table 1. Each muscle weighed about 50 mg and the assumption was made that the concentration of DMSO in the extracellular space equalled that in the external solution.

The main finding shown in Table 1 is that the intracellular concentration of DMSO becomes higher than the external concentration. For example, after incubation for 5 min at 37° C in Krebs's solution containing DMSO (14 mM) the concentration of DMSO within the cell was $13{\cdot}02 \pm 0{\cdot}81$ m-mole/l. After 20 min incubation the concentration of DMSO within the cell was $17{\cdot}05 \pm 0{\cdot}46$ m-mole/l., that is, about 30% higher than that in the external solution. When the experiment was repeated in the presence of dinitrophenol (DNP, 0·1 mM) the concentration of DMSO within the cell after 20 min was again greater than 14 mM. On the other hand, after

20 min at +5° C the intracellular concentration of DMSO was only 12·35 ± 0·56 m-mole/l. Table 1 also gives, in parentheses, the intracellular concentrations of the non-electrolytes, calculated assuming that the extracellular space was as large as 450 ml./kg throughout. Goodford & Leach (1964) have shown that inulin may not penetrate into all the extracellular space and that higher values obtained with monovalent ions may be more correct estimates. Although the use of this calculation alters the values of the concentration of DMSO to some extent it will still be seen that the calculated concentration within the cell after 20 min is higher than in the external solution.

TABLE 1. The intracellular uptake of DMSO or glycerol in taeniae coli after incubation for 5 or 20 min in solutions containing DMSO. Muscles in all groups had previously been incubated in inulin-Krebs's solution at 37° C for 4 hr

	Conditions of incubation				Intracellular DMSO concentration, m-mole/l. cell water ± S.E.(*)	
	External concentration of non-electrolyte (mM)	Solution	Temp. (°C)	No. of observations in each group	5 min	20 min
DMSO	14	Krebs's	37	9	13·02 ± 0·81 (12·86)	17·05 ± 0·46 (17·16)
	14	Krebs's (+0·1 mM DNP)	37	5	13·23 ± 0·40 (12·51)	15·49 ± 1·17 (16·09)
	14	Krebs's	5	5	7·94 ± 1·20 (5·38)	12·35 ± 0·56 (11·58)
	1400	Krebs's	37	5	1393 ± 167 (1307)	1862 ± 114 (1956)
	1400	Krebs's (Ca^{2+} omitted)	37	10	1322 ± 76 (1286)	1766 ± 30 (1801)
Glycerol	13·68	Krebs's	37	5	7·32 ± 1·72 (5·72)	12·09 ± 3·51 (11·32)

* The intracellular concentrations given in parentheses are calculated using a value of 450 ml./kg throughout for the extracellular space (Goodford & Leach, 1964).

The uptake of DMSO from an external solution containing a higher concentration of DMSO (1400 mM) is also shown in Table 1. A concentration of this order is needed to protect cells from damage at very low temperatures (Smith, 1961). After 20 min incubation at 37° C the intracellular concentration of DMSO had reached 1862 m-mole/l., again exceeding the concentration in the external solution (1400 mM). When the concentrations are calculated using a value of 450 ml./kg for the extracellular space, the greater concentration of DMSO within the cell is still seen. The responses of smooth muscle after freezing to −79° C in the presence of DMSO (1·4 M) are altered by omitting the calcium from the freezing medium (Farrant, 1964). Table 1 shows that the omission of calcium from the incubation medium has little effect on the entry of DMSO into the cell.

Table 1 also shows that, even after 20 min incubation at 37° C in a 13·68 mM solution of glycerol, the concentration of non-electrolyte within the cell only reaches 12·09 m-mole/l.

V/A measurements of taeniae coli

The length of individual smooth muscle cells from the taeniae coli ranged between 95 and 110 μ, and their width was between 8 and 10 μ. V/A was found to be 2·25 μ.

Weight changes of taeniae coli in solutions containing DMSO

Taeniae which were incubated in Krebs's solution containing DMSO (14 mM) or glycerol (13·68 mM) did not lose weight. In contrast, when taeniae were incubated at 37° C in Krebs's solution containing DMSO (1400 mM) they lost up to 9% of their initial weight (Fig. 3). The omission of calcium ions from the Krebs's solution had no effect on these changes.

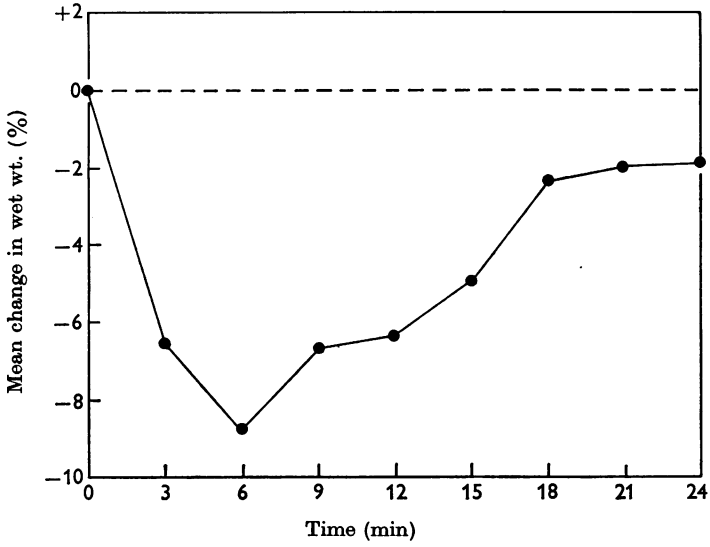

Fig. 3. Mean loss in weight of taeniae coli on immersion in Krebs's solution containing DMSO (1400 mM). Each point represents the mean of ten observations.

Apparent permeability constants

The intracellular concentrations of the two non-electrolytes obtained after 5 and 20 min incubation were matched with eqn. (4) using an analogue computer. This treatment enabled the values of the apparent permeability constants k_1^{in} and k_1^{out} to be computed for each set of conditions. These values are shown in Table 2. The ratio k_1^{out}/k_1^{in} was consistently less than 1·0 when taeniae were incubated at 37° C in solutions containing DMSO. If this ratio is directly related to the ratio of the activity coefficients of the non-electrolyte, then at this temperature the intracellular activity coefficient of DMSO is less than that of the external DMSO. In contrast,

when the experiment was carried out at +5° C, the ratio of the apparent permeability coefficients of DMSO became equal to 1·0. The results from the group of muscles incubated at 37° C in a solution containing glycerol showed that for this substance the ratio k_1^{out}/k_1^{in} was greater than 1·0.

TABLE 2. Apparent permeability constants of DMSO and of glycerol for the smooth-muscle cells of the taeniae coli

	Conditions of incubation			Apparent permeability constants ($\times 10^{-5}$ cm/sec)		
	External concentration of non-electrolyte (mM)	Solution	Temp. (°C)	k_1^{in}	k_1^{out}	k_1^{out}/k_1^{in}
DMSO	14	Krebs's	37	1·53	1·21	0·79
	14	Krebs's (+0·1 mM DNP)	37	1·92	1·72	0·90
	14	Krebs's	5	0·89	0·89	1·00
	1400	Krebs's	37	1·75	1·35	0·77
	1400	Krebs's (Ca^{2+} omitted)	37	1·64	1·32	0·81
Glycerol	13·68	Krebs's	37	0·75	0·85	1·13

DISCUSSION

The inulin space of guinea-pig uteri, measured after 4 hr incubation, was found to be 250·6 ml./kg. It is interesting to note that this value is lower than that of the cat uterus, but similar to that of the rabbit uterus (Daniel & Daniel, 1957). The increase in the inulin space which occurred in the uteri between 4 hr and 24 hr incubation may indicate either a greater penetration into the extracellular space or a slow intracellular penetration by the polysaccharide. The inulin space of toad sartorius is also reported to increase between the 2 hr and the 18 hr values (Tasker, Simon, Johnstone, Shankly & Shaw, 1959). A further point of interest was that incubation for 20 min in a Krebs's solution containing DMSO (1400 mM) increased the inulin space of the uteri from 250·5 ml./kg to 333·1 ml./kg, showing that a hypertonic solution not only alters the intracellular space of a tissue, but also increases the extracellular space. In the taeniae coli the mean inulin space was found to be 344·3 ml./kg after 4 hr incubation, which is close to the value of 330 ml./kg reported by Goodford & Hermansen (1961).

The main finding in this work is that the intracellular concentration of DMSO in taenia coli exceeded the concentration of DMSO in the external medium after 20 min incubation. The fact, that in guinea-pig uteri weighing more than 130 mg the mean concentration of DMSO in the extracellular space did not equal that in the external solution after as long as 20 min incubation, made it impossible to obtain quantitative results using this preparation. This finding again illustrates the importance of extracellular

diffusion in determining the kinetics of the intracellular uptake of a substance in a tissue (Harris & Burn, 1949). It becomes increasingly difficult for the penetrating substance to reach the innermost cells as the volume of the tissue and the length of the diffusion pathway increase. A complete analysis of extracellular diffusion, membrane penetration and protoplasmic diffusion will be essential if non-electrolytes are to be used to protect cells within large organs from damage during freezing and thawing. The equations for this treatment are available (Harris & Burn, 1949; Crank, 1956; Fenichel & Horowitz, 1963). Restriction of the present experiments to taeniae coli weighing less than 60 mg enabled a simpler approach to be taken because extracellular diffusion was assumed to take place at a much faster rate than surface penetration. The other main route of entry for protective compounds into organs is through the blood supply. However, a quantitative examination of this situation would be rendered difficult by the presence of the extra barrier to penetration presented by the capillary endothelium.

The greater concentration of DMSO inside the smooth muscle cells of the taenia coli was still apparent if Goodford and Leach's figure of 45% for the extracellular space was used in the calculation instead of the measured inulin space (Goodford & Leach, 1964). The finding that the intracellular concentration was greater supports the assumption that the concentration of DMSO in the extracellular space equalled that in the external medium. It is also in agreement with previous observations made with other non-electrolytes; for example, Bozler (1961) found that the intracellular concentration of urea and thiourea in smooth muscle from the frog's stomach was consistently higher than that in the external medium. In 1959 Bozler also suggested that only part of the intracellular space of smooth muscle is accessible to non-electrolytes. If this is so the local intracellular concentration of DMSO would be even higher.

The effect of lowering the calcium concentration on the permeability to DMSO was also examined because Bozler & Lavine (1958) have reported that a small amount of calcium slows or reverses the swelling of smooth muscle which is brought about by an isotonic solution of sucrose or by distilled water. The results showed, however, that the uptake of DMSO was little altered in the absence of calcium.

Although the main intention of this work was to determine the time course of the intracellular uptake of protective non-electrolytes by smooth muscle, several possible explanations of the apparently greater concentration of DMSO within the cells must be considered. The fact that the addition of DNP during the incubation did not alter the uptake of DMSO suggests that active transport was not involved. Furthermore, lowering the temperature from $+37°$ C to $+5°$ C reduced the entry of DMSO by

less than one half. If active transport were involved the Q_{10} would be much greater (Ussing, 1960).

It is possible that some of the DMSO is being removed from solution within the cell by being bound on to protein but this may not be a major effect. First, it is clear that the strength of the DMSO–water hydrogen bond is greater than that of the water–water hydrogen bond (Lindberg & Kenttämaa, 1960). This will tend to keep the DMSO in the bulk solution. Secondly, water can form hydrogen bonds with both hydrogen donors and hydrogen acceptors because it can both accept and donate hydrogen itself. Thus, with the peptide bond, water can both donate a hydrogen to the carbonyl oxygen and also accept the hydrogen from the NH group. In contrast, DMSO can only accept hydrogen: it cannot donate. Therefore a hydrogen bond between DMSO and the carbonyl group of the peptide bond is not possible. Also, the hydrogen-donating properties of the NH group are thought to be fairly weak (Cannon, 1958), compared with the hydrogen-accepting properties of the carbonyl group. However, DMSO will presumably form hydrogen bonds with hydrogen-donating groups on the protein side chains but a massive binding of the non-electrolyte appears unlikely.

Another possibility is that the activity coefficient of DMSO in solution within the smooth-muscle cells is for some reason less than that in the external solution. Although this is the most likely explanation it cannot be tested directly and other explanations cannot be excluded. An attempt has been made to measure the relative values of the factor, be it activity coefficient or not, describing the different state of DMSO inside and outside the cells. This has been done using the concept that the ratio of the apparent permeability constants into and out of the cells equals the ratio of the activity coefficients or other properties distinguishing the two situations. Table 2 shows that for DMSO at 37° C the ratio k_1^{out}/k_1^{in} is always less than unity. Thus, if a change in activity coefficient is involved then the intracellular activity coefficient is lower than that outside the cells. However, there are two curious points. First, the value of the ratio was similar whether the external concentration of DMSO was 14 or 1400 mM, and, secondly, the ratio became 1·0 when the experiment was carried out at +5° C. The finding that the ratio k_1^{out}/k_1^{in} for glycerol at 37° C was greater than 1·0 suggests that the behaviour of DMSO and glycerol may be qualitatively different within the cell.

In this work the primary objective was to obtain quantitative information on the time course of the intracellular uptake of DMSO by smooth-muscle cells. Later, it is hoped to correlate this uptake of DMSO with its capacity for protecting smooth muscle during freezing and thawing.

SUMMARY

1. The permeability of guinea-pig smooth muscle to dimethyl sulphoxide (DMSO) and glycerol has been studied. These non-electrolytes are known to protect erythrocytes and other cells from damage during freezing and thawing.

2. The intracellular concentrations of the non-electrolytes were measured by comparing the non-electrolyte space with the inulin space.

3. The penetration of DMSO into the extracellular space of uteri which weighed more than 130 mg was not complete even after 20 min incubation at 37° C.

4. After 20 min incubation at 37° C the intracellular concentration of DMSO in taeniae coli was higher than that in the external solution. Evidence was obtained that active transport of DMSO was not involved.

5. These results will enable comparisons to be made between the concentrations of DMSO or glycerol within the smooth-muscle cells and their capacity in protecting this organized tissue from damage during freezing and thawing.

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