THE DIFFUSION OF POTASSIUM, SODIUM, SUCROSE AND INULIN IN THE EXTRACELLULAR SPACES OF MAMMALIAN TISSUES

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In a previous paper[1] the diffusion constants governing the movement of potassium, inulin and thiocyanate in the extracellular space of mammalian muscle were measured. It was found that when an appropriate correction for the increase in the mean diffusion path due to the presence in the tissue of the impermeable muscle fibres was applied, the diffusion constants for inulin and thiocyanate approximated to those holding in free solution, but that the diffusion of potassium occurred at about one-fifteenth of the free solution rate. It was suggested that a possible explanation of this phenomenon might be the adsorption of the K onto the surfaces of the muscle fibres, and that this might be a necessary preliminary to the uptake of K across the muscle fibre membranes.

This investigation has now been extended to include measurements of the diffusion constant for sodium ions, both in muscle and in other mammalian tissues, brain, peripheral nerve, and liver. Brain and nerve are similar to muscle in that there exist large electrical asymmetries between their intra- and extracellular phases; while in the case of liver no such potential gradient is present. Since the penetration of inulin into the extracellular space of brain tissue is small (for references see [2]), the diffusion of sucrose has been measured as well. It has been found that the diffusion of K in brain and nerve is much slowed, as it is in muscle; while in liver it is approximately the same as in free solution. The diffusion of Na, sucrose and inulin is also the same as the free solution rate in the extracellular spaces of muscle, brain and liver. In nerve the diffusion of all the tested substances is slowed; there apparently being in this tissue some non-specific diffusion barrier between the extracellular space and the surrounding medium.

METHODS

The tissues used in this study were obtained from albino rats. The animals were killed by decapitation, and the tissues rapidly excised. The extensor digitorum longus muscle and a portion of the sciatic nerve trunk were used; these were weighed on a torsion balance and then placed in the appropriate incubation medium. At the end of incubation they were carefully dried by blotting with filter paper and reweighed in order to determine the amount of swelling, before being subjected to the desired analytical procedure. Brain and liver slices were prepared with a razor blade: average brain slices were 2 mm in thickness, and liver slices 3 mm. They were weighed and placed on fine screens of silver-plated brass before being introduced into the incubation media. The screens insured that the slices remained flat during the course of the incubation, and also permitted them to be removed from the solutions without damage.

The techniques involved in the use of radioactive ^{42}K have been described in detail in earlier communications[1,3], and the method by which diffusion of K in the extracellular space of muscle

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was measured[1] has been applied without modification to the other tissues studied. Na diffusion was followed by placing the tissue for a period of 3–4 hours in a saline solution containing a proportion of radioactive ^{22}Na, after which it was transferred to a non-radioactive solution of identical composition, and the loss of radioactivity from the tissue followed. The results obtained are complicated by the fact that the loss of labelled Na from the inside of the cells is also measured; however, the time constants governing diffusion and cellular exchange are sufficiently different to permit a separation of the two processes to be made. All measurements were carried out at a temperature of 20° C. HARRIS AND BURN[4] and JOHNSON[5] have employed a similar method to determine Na diffusion in frog muscle extracellular space.

The uptake of inulin or sucrose was followed by incubating the tissues in saline media which contained 1% (w/v) of the tracer substances. At the end of the desired period of incubation the tissue, after drying and weighing, was ground with sand, suspended in 3 ml of water, and 1 ml of 12% trichloroacetic acid added. The mixture was centrifuged, and aliquots of the supernatant assayed for the tracer. Standard solutions used were dilutions of the soaking solutions. Both inulin and sucrose were determined by the method of HUBBARD AND LOOMIS[6], as adapted by PAPPIUS AND ELLIOTT[2]. Results were calculated with due allowance made for the amounts of inulin or sucrose in the water of swelling, as described by the latter authors.

THEORY

In the earlier paper[1] the equation governing the diffusion of a substance from or into a semi-infinite cylinder was derived, and the extensor digitorum longus muscle corresponds approximately to this shape. The same equation can be employed for diffusion into the sections of nerve used in the present work, since they too are many times longer than their maximum radius, and are roughly circular in cross-section. The derivation of λ, the fractional increase in the mean diffusion path due to the nerve or muscle fibres, will also be the same for nerve as for the muscle as previously discussed. The diffusion equation for a cylinder of radius "a" is:

$$f = 1 - 4\left[\frac{e^{-\frac{D j_{0_1}^2 t}{\lambda^2 a^2}}}{j_{0_1}^2} + \frac{e^{-\frac{D j_{0_2}^2 t}{\lambda^2 a^2}}}{j_{0_2}^2} + \ldots\right] \quad (1)$$

where "f" is the fraction of the equilibrium amount of substance which has entered the cylinder; "D" is the diffusion constant; "t" the time; and j_{0_1}, j_{0_2}, ... are the zeroes of the Bessel function $J_0(x)$.

λ is given by:

$$\lambda = 1 + 4/\pi\,[V_c/V_t]\,[1 - \pi/4], \quad (2)$$

where V_c and V_t are the volumes of the cells and of the whole tissue respectively.

The slices of brain and liver tissue used in this work are most appropriately considered as plane sheets exposed on both sides to the bathing solution, having a total thickness of $2b$. The differential equation describing the diffusion of a substance into a sheet is:

$$dS/dt = D/\lambda^2 \cdot d^2S/dx^2, \quad (3)$$

where "S" is the concentration of tracer substance in the sheet; "D" the diffusion constant; and λ a factor, analogous to that described above, by which the distance that the substance has to travel between the outside and the inside of the tissue is increased by reason of impermeable obstacles, *i.e.* the tissue cells.

Placing the tissue sheet at zero time into a solution containing a concentration of substance S_0, the following boundary conditions prevail:

$$\text{At } x = b,\ S = S_0 \text{ for all times;}$$
$$\text{At } t = 0,\ S = 0 \text{ for all } x\text{'s.}$$

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Then [7]:

$$\frac{S}{S_0} = 1 - \frac{4}{\pi}\left[e^{-\frac{D\pi^2 t}{4b^2\lambda^2}} \sin\frac{\pi x}{2b} + \frac{1}{3} e^{-\frac{9D\pi^2 t}{4b^2\lambda^2}} \sin\frac{3\pi x}{2b} + \ldots\right], \quad (4)$$

Integrating (4) for all values of "x", the fraction of the equilibrium amount of substance which has entered the tissue, that is, the average saturation "f" is given by:

$$\frac{\overline{S}}{S_0} = f = 1 - \frac{8}{\pi}\left[e^{-\frac{D\pi^2 t}{4b^2\lambda^2}} + \frac{1}{9} e^{-\frac{9D\pi^2 t}{4b^2\lambda^2}} + \ldots\right] \quad (5)$$

This equation has been used for the evaluation of $D/b^2\lambda^2$ when "f", is measured against time.

The derivation of λ for a plane sheet is analagous to that described in the preceding paper for the cylindrical muscles. Consider a plane slice of tissue, circular in cross-section, of radius "R" and thickness "h". Within this slice there are "N" spherical cells of radius "r". Consider now a cylindrical section cut through the slice, of height "h" and infinitely small radius. The number of spherical cells which will be cut by this section is equal to the number of centres of the spheres which lie within a cylinder of radius "r" surrounding the section; that is, in the volume $\pi r^2 h$.

The total density of spheres in the whole sheet is $N/(\pi R^2 h)$; therefore the number contained in the volume $\pi r^2 h$ is Nr^2/R^2. In a precisely similar manner to the case of the muscle fibres discussed in the earlier work, it can be shown that the extra diffusion path caused by the presence of a cell of radius "r" is given by:

$$2r \cdot [1 - \pi/4].$$

Therefore in the present case the total extra distance for all the cut spheres is:

$$2r \cdot [1 - \pi/4][Nr^2/R^2]. \quad (6)$$

Now the ratio of the volume of the spheres, V_c, to the total volume of the sheet, V_t, is:

$$V_c/V_t = (4/3\ \pi\ r^3\ N)/(\pi R^2\ h) = (4r^3\ N)/(3R^2\ h); \quad (7)$$

and substituting in (6) above, the increase in the mean diffusion path is:

$$3h/2\ [1 - \pi/4]\ V_c/V_t. \quad (8)$$

But "h" is twice the geometrical diffusion path, and therefore

$$\lambda = 1 + 3V_c/V_t\ [1 - \pi/4]. \quad (9)$$

RESULTS

Inulin

Fig. 1 shows the time course of the uptake of inulin by brain and nerve at 20° C. The plotted values are the averages of a number of individual determinations, which are given in parentheses above each point. For the purposes of calculation the curves have been replotted as log $(1 - f)$ against time, that for nerve being shown in Fig. 2. Extrapolation of the straight portion of curve A of Fig. 2 back to zero time permits estimation of the contribution of the first term of the diffusion equation (1) to the total. The contribution of the second term in the expansion to the earlier part of the experimental curve is shown by curve B in Fig. 2, where the first term values in the extrapolated portion have been subtracted from the experimental curve, and the differences plotted. From the linear portions of these two curves, the times to

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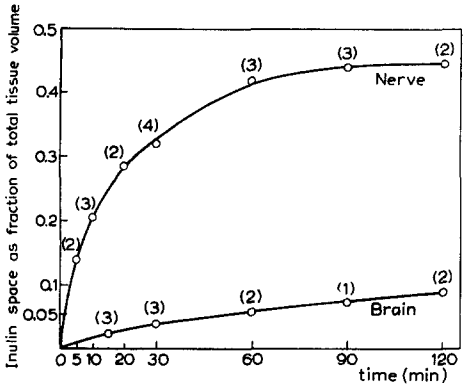

Fig. 1. The time course of the uptake of inulin by brain and nerve. The plotted points are the averages of the numbers of determinations shown in parentheses.

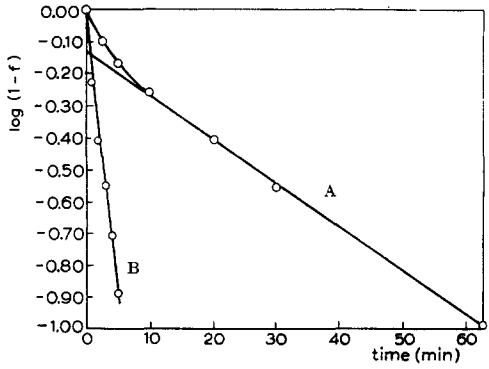

Fig. 2. The uptake of inulin by nerve replotted from the data of Fig. 1. Curve A represents the experimentally determined points replotted as log $(1 - f)$ against time. Curve B shows the difference between the linear and non-linear portions of A. For further details see text.

half saturation for the first two terms of the expansion may be read off, and the results, together with the appropriate value of λ^2 calculated from (2), inserted in (1) as follows:

$$f = 0.5 = 1 - 4\left[\frac{e^{-\frac{5.78 \times 21.5 \times D}{1.323 \times 0.0025}}}{5.78} + \frac{e^{-\frac{30.5 \times 1.8 \times D}{1.323 \times 0.0025}}}{30.5}\right]$$

whence $D = 1.5 \cdot 10^{-5}$ cm²/min.

As reported by other authors, the extent of penetration of inulin into brain tissue is small, such that the apparent extracellular space measured with this substance as tracer was found to be only 0.10 of the total tissue volume. In contrast, the sucrose space in both brain and nerve was 0.45, and in nerve the inulin space was also 0.45. The figures for both tissues are larger than normally quoted in the literature for the extracellular space, and presumably reflect the extent of cellular damage caused by the excision and preparation of the tissues.

Treating the experimental curve of Fig. 1 for the uptake of inulin by brain in the same manner as described above, and substituting the times to half saturation in equation (5), with λ^2 calculated from (9), the following is obtained:

$$f = 0.5 = 1 - \frac{8}{\pi}\left[e^{-\frac{\pi^2 \times 50 \times D}{2.500 \times 0.16}} + \frac{1}{9} e^{-\frac{9\pi^2 \times 0.7 \times D}{2.500 \times 0.16}}\right],$$

whence $D = 1.9 \cdot 10^{-4}$ cm²/min.

These results, together with that for muscle, are given in Table I. The values obtained for the diffusion constant for inulin in the extracellular spaces of brain and muscle are close to that reported for diffusion in free solution at the same temperature; however, in nerve the diffusion is considerably slower. This last point will be further mentioned below.

Sucrose

Table I also gives the results obtained when sucrose was used in place of inulin as a tracer substance. The experimental curves obtained were analysed in the same way

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as those for inulin, and the final results were substantially similar. Diffusion in muscle and brain was approximately the same as in free solution, while in nerve it was much reduced.

TABLE I

THE DIFFUSION OF INULIN AND SUCROSE IN EXTRACELLULAR SPACE

		Apparent e.c. space	$\frac{Vc}{Vt}$	*Radius (a) or thickness (b) cm*	λ^2	*Observed* cm^2D/min	*Free solution* cm^2D/min
Inulin	Muscle	0.15	0.85	0.175	1.543	$1.2 \cdot 10^{-4}$ *	
	Brain	0.10	0.90	0.10	2.500	$1.9 \cdot 10^{-4}$	$1.0 \cdot 10^{-4}$ **
	Nerve	0.45	0.55	0.05	1.323	$1.5 \cdot 10^{-5}$	
Sucrose	Muscle	0.15	0.85	0.175	1.543	$3.4 \cdot 10^{-4}$	
	Brain	0.45	0.55	0.10	1.838	$2.1 \cdot 10^{-4}$	$3.1 \cdot 10^{-4}$ ***
	Nerve	0.45	0.55	0.05	1.323	$4.3 \cdot 10^{-5}$	

* This result from previous paper[1].
** Reference[8].
*** Reference[9].

Sodium

The diffusion constants calculated for the movement of Na ions in the extracellular spaces of the tissues examined are given in Table II. Roughly normal values were obtained in muscle, brain and liver, while again diffusion in peripheral nerve is low.

TABLE II

THE DIFFUSION OF SODIUM IN EXTRACELLULAR SPACE

	Apparent e.c. space	$\frac{Vc}{Vt}$	*Radius (a) or thickness (b) cm*	λ^2	*Observed* cm^2D/min	*Free solution* cm^2D/min
Muscle	0.15	0.85	0.175	1.543	$2.7 \cdot 10^{-4}$	$7.2 \cdot 10^{-4}$ *
					$2.4 \cdot 10^{-4}$	
					$1.1 \cdot 10^{-4}$	
					$3.2 \cdot 10^{-4}$	
					$3.1 \cdot 10^{-4}$	
Brain	0.45	0.55	0.10	1.838	$14.0 \cdot 10^{-4}$	
					$6.8 \cdot 10^{-4}$	
					$5.6 \cdot 10^{-4}$	
					$5.6 \cdot 10^{-4}$	
Nerve	0.45	0.55	0.05	1.323	$1.2 \cdot 10^{-5}$	
					$0.7 \cdot 10^{-5}$	
Liver	0.14	0.86	0.15	2.420	$8.3 \cdot 10^{-4}$	
					$12.7 \cdot 10^{-4}$	

* Reference[10].

Potassium

It was previously reported that the movement of muscle extracellular K is relatively slow compared with diffusion in free solution[1]. This result has been confirmed in the course of the present work, and the same phenomenon is observed when diffusion

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in brain and nerve is followed (Table III). It is true that in nerve the diffusion of all the test substances is low; but in muscle and brain K movement alone has been found to be impeded. By contrast, however, the measured extracellular diffusion constant for K in liver slices was of the same order as the free solution value, behaving in the same way as Na in this tissue.

TABLE III

THE DIFFUSION OF POTASSIUM IN EXTRACELLULAR SPACE

The values for the fractional volume of the extracellular space, for V_e/V_t, for the radius (a) or thickness (b), and for λ^2, are the same as those given in Table II.

	Observed cm^2D/min	*Free solution* cm^2D/min
Muscle	$3.8 \cdot 10^{-5}$ *	$9.9 \cdot 10^{-4}$ **
	$4.3 \cdot 10^{-5}$ *	
	$4.9 \cdot 10^{-5}$ *	
	$8.4 \cdot 10^{-5}$	
	$10.9 \cdot 10^{-5}$	
	$4.1 \cdot 10^{-5}$	
Brain	$7.2 \cdot 10^{-5}$	
	$3.2 \cdot 10^{-5}$	
	$5.2 \cdot 10^{-5}$	
Nerve	$0.8 \cdot 10^{-5}$	
	$1.0 \cdot 10^{-5}$	
	$5.0 \cdot 10^{-5}$	
	$4.0 \cdot 10^{-5}$	
	$4.1 \cdot 10^{-5}$ ***	
	$2.0 \cdot 10^{-5}$	
Liver	$3.4 \cdot 10^{-4}$	
	$4.0 \cdot 10^{-4}$	
	$3.4 \cdot 10^{-4}$	
	$4.0 \cdot 10^{-4}$	

* These results from previous paper[1].
** Calculated in[1].
*** Perineureum removed.

DISCUSSION

The findings earlier reported[1] on the diffusion of various substances in the extracellular fluid of mammalian muscle have now been extended to include other tissues and other tracer substances. In muscle the previous demonstration that the diffusion of K ions was much slowed, while the movement of thiocyanate ions and of inulin was essentially the same as in free solution has been extended. The diffusion constants for Na ions and for sucrose have now been measured, and are found to be of the same order as the diffusion constants of these materials in free solution.

There are in the literature a number of estimates of effective diffusion constants in extracellular fluids, especially for Na in frog muscle. These have been summarized in Table IV, and appropriate values for λ^2 calculated from the data given by the various authors. The agreement between the various estimates is good, and support the conclusion presented in this paper that Na is able to move freely in the extra-

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TABLE IV

EXTRACELLULAR DIFFUSION CONSTANTS FROM THE LITERATURE

Tissue	*Substance*	*Temperature*	$cm^2D/\lambda^2 min$	λ^2 *(calculated)*	cm^2D/min	*Reference*
Rat diaphragm	Na	37	$2.5 \cdot 10^{-4}$	2.22	$5.6 \cdot 10^{-4}$	11
Frog M. extensor longus dig. IV	Na	20	$1.9 \cdot 10^{-4}$	1.44	$2.7 \cdot 10^{-4}$	12
Frog M. sartorius	Na	20	$2.1 \cdot 10^{-4}$	2.40	$4.9 \cdot 10^{-4}$	13
Frog M. sartorius	Na	18	$1.5 \cdot 10^{-4}$	2.40	$3.6 \cdot 10^{-4}$	4, 14
Frog M. sartorius	Na	20	$3.4 \cdot 10^{-4}$	2.40	$8.2 \cdot 10^{-4}$	12
Frog M. sartorius	Na	22	$1.8 \cdot 10^{-4}$	2.40	$4.3 \cdot 10^{-4}$	5
Rat diaphragm	K	37	$3.1 \cdot 10^{-4}$	2.22	$6.9 \cdot 10^{-4}$	11
Frog M. sartorius	K	18	$3.3 \cdot 10^{-4}$	2.40	$7.9 \cdot 10^{-4}$	15
Rat diaphragm	Inulin	37	$3.4 \cdot 10^{-5}$	2.22	$0.8 \cdot 10^{-4}$	11
Frog M. sartorius	Inulin	20	$6.0 \cdot 10^{-5}$	2.40	$1.4 \cdot 10^{-4}$	16
Frog M. sartorius	Sucrose	22	$7.2 \cdot 10^{-5}$	2.40	$1.7 \cdot 10^{-4}$	5
Cat nerve (intact)	Na	37	$9.0 \cdot 10^{-5}$	1.32	$1.2 \cdot 10^{-4}$	17
Bullfrog nerve (intact)	Na	25	$8.0 \cdot 10^{-7}$	1.32	$1.1 \cdot 10^{-6}$	18
Toad nerve (desheathed)	Na	25	$4.0 \cdot 10^{-5}$	1.32	$5.3 \cdot 10^{-5}$	19

cellular space of muscle tissue. The same is true of the older estimates of inulin and sucrose diffusion in muscle (Table IV). Previous calculations of the rate of movement of K in muscle do not, however, agree with those found here. Earlier authors have concluded that the diffusion of K is impeded in the extracellular space of frog muscle and rat diaphragm to no greater an extent than is the diffusion of Na; whereas the present results would indicate a reduction to about one-fifteenth of the free solution value. There do appear to be differences between diaphragm and the extensor digitorum muscle used in this work other than that brought out here: thus all the K of diaphragm appears to be free to undergo exchange with the K of a bathing solution[11], while this does not seem to be the case with the extensor[3]. A similar discrepancy as regards K exchangeability has been reported by different authors using frog sartorius muscle[12, 20]. A possible explanation of the differences between the older K diffusion measurements and those reported here may be that previous workers did not attempt to prevent exchange between the external fluid and the intracellular K. Under this circumstance the slow diffusion of K could be misinterpreted as K exchange.

The diffusion of substances within slices of brain tissue is similar to that in muscle. K diffusion is much slowed compared with free solution; while Na, sucrose and inulin apparently move at about the normal rate. In the case of peripheral nerve, the measured diffusion of all the test substances was low. In one experiment where K diffusion was followed, removal of the perineureum had no obvious effect (Table III). However, SHANES[18] and SHANES AND BERMAN[21] have reported that the epineureum of bullfrog sciatic nerve constitutes an effective barrier to the free passage of solutes from the external medium into the nerve, and it seems possible that a similar effect has been observed here.

In contrast to the tissues mentioned above, the diffusion of K as well as of all the other test substances, is essentially normal in the extracellular space of liver.

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In a non-excitable tissue, therefore, the diffusion of K in the extracellular fluid seems to be unimpeded, while in excitable tissues such as muscle and brain it is slowed. This effect is not attributable to the high intracellular concentration of K in nerve and muscle cells, since the concentration is as high in the cells of the liver[22]. It seems specific for K ions, since the present work shows that Na ions at least move freely in the extracellular fluids of liver, muscle, and brain. It was suggested in the previous paper[1] that adsorption of K ions onto the surfaces of the cells might be responsible for the slow diffusion; and it would seem, in the light of the present extension of the work, that the electrical conditions applying in excitable tissues are concerned in the effect.

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SUMMARY

The diffusion of K, previously reported to be low in the extracellular fluid of mammalian muscle, has been shown to be slowed also in the extracellular space of brain tissue; whereas in liver K diffuses at about the free solution rate. Diffusion constants have been estimated for Na ions, inulin and sucrose in these three tissues, and are all approximately at the free solution level.

In peripheral nerve the diffusion of all test substances is low, possibly due to the epineureum.

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