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Received October 2nd, 1957

THE DIFFUSION OF VARIOUS SUBSTANCES THROUGH RAT DIAPHRAGM

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Earlier studies have reported that the diffusion constant holding for the movement of potassium in the extracellular space of rat muscle and brain appears reduced below the free solution value[1,2]. By contrast, the diffusion of other substances, both ions and neutral molecules, is unimpeded in this way. In the present series of experiments a

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thin membrane of muscle (diaphragm) has been set up to act as a diffusion barrier, and the rate of movement of the various test substances across it measured. With this muscle and using the technique employed earlier it has been confirmed that potassium ions appear to have a reduced mobility in the extracellular space of the tissue. However, it is now also shown that the effect is only observed under certain special circumstances. Using other experimental conditions, the diffusion of potassium ions in the extracellular space also appears within normal limits for free diffusion in solution. This finding removes the discrepancy before evident between the earlier results of the present author and those of other workers.

MATERIAL AND METHODS

The animals used were Swiss albino rats of 125 to 175 g in weight. They were killed by decapitation, and the diaphragms dissected out rapidly. After wiping free of blood, a portion of the muscle was placed over one end of a small plastic (Perspex) tube having internal and external diameters of 6 and 9 mm. respectively. The muscle was held in place by a loose-fitting plastic ring, which was designed so that when the diaphragm was in position it effectively sealed the end of the tube to leakage of water while at the same time loose enough so as not to cause stretching or tearing of the tissue. Within the tube was placed 1.5 ml of the solution containing the test substance, while in beakers below were placed 2 ml aliquots of the Ringer-type medium without the test substance. The beakers were withdrawn at intervals and replaced with fresh samples, and the amounts of test substance appearing in the solution in the beakers assayed. In about one-half of the experiments the diaphragms were incubated for three or four hours in the solution afterwards used to fill the tube. This variation made no difference to the equilibrium values of the amount of substance diffusing through the muscle sheet.

Further details need to be given for the experiments involving the potassium ion. It was pointed out earlier[1] that measurement of the diffusion of this ion using a radioactive tracer is complicated by the exchange which occurs with the intracellular contents, and also by the relatively much greater quantity of intracellular K compared with that in the extracellular space. It was for these reasons that the following modification of the procedure given above was used, adapted from earlier work. The medium in which the tissue was incubated before the start of the experimental period was an isotonic solution of potassium phosphate (154 mmoles/l with respect to K) in which a proportion of the ions were labelled with radioactive tracer. The solution in the beakers was the Ringer-type medium containing labelled K of the same specific activity as that of phosphate solution. Incubation of a muscle in K phosphate brings about complete exchange of the cellular and extracellular K^3, and a replacement of the extracellular sodium by potassium. When a muscle so treated is placed in contact with the Ringer-type medium containing labelled K, the high extracellular K diffuses out in return for Na, but the cellular radioactivity does not change[1]. Other experiments involving K were done with the aid of a flame photometer and no radioactive material was used. Thus the uncertainty introduced by K exchange with the intracellular contents was avoided. In these cases the solution placed in the beakers was the Ringer-type medium, but one which had initially no K ion in it. The K which appeared in this fluid by diffusion through the muscle was measured with the photometer. In this series of experiments, in addition to some involving potassium phosphate analogous to those described above, it was possible also to measure the diffusion of K from the Ringer-type medium, and as well from solutions containing other concentrations of K and of phosphate. K phosphate experiments done in this way gave results identical with those obtained by the isotope method; however, differences to be described below appeared when other solutions were employed.

The radioactive isotopes used were ^{22}Na, ^{42}K and ^{36}Cl, and all were obtained from Atomic Energy of Canada, Ltd., Chalk River, Ontario. Radioactive media were prepared by substituting for part of the inactive ionic species of the Ringer-type medium, which contained, in mmole/l, Na 144, K 5.5, Ca 2, Mg 1, Cl 153.5, SO_4 1, glucose 12. The solutions were kept stirred by passing in bubbles of O_2 gas and all measurements were carried out at 20°C. Radioactive assay was performed on 1 ml aliquots from the beakers, which were counted beneath an end-window Geiger-Mueller tube connected to a scaler-counter combination.

The diffusion of sucrose was followed by placing in the tube closed by the diaphragm a solution containing 1% (w/v) of the substance. In this case 2.5 ml of medium was placed in the beakers, and duplicate assays on 1 ml aliquots were made for sucrose by the method of HUBBARD AND LOOMIS[4].

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RESULTS

The differential equation governing the movement of a substance in a plane sheet is:

$$\frac{dS}{dt} = \frac{D}{\lambda^2} \cdot \frac{d^2S}{dx^2},$$

where "S" is the concentration of tracer substance in the sheet, "D" the diffusion constant, and "λ" a factor by which the geometrical diffusion path is increased by reason of impenetrable obstacles, *i.e.* the muscle cells.

The particular solution of this equation applicable in the present case, where the quantity measured is the amount of substance appearing in the solution bathing the sheet on one side is:

$$S_1 = S_0 \operatorname{erfc} \left\{ \frac{l}{2\sqrt{\frac{Dt_1}{\lambda^2}}} \right\}, \tag{5}$$

where "S_1" is the concentration of tracer in the solution at time "t_1", "S_0" the initial concentration in the sheet, and "l", one-half the total thickness of the diaphragm. CREESE[6], has reported that for rats weighing 120 g the thickness of the muscle averages about 0.06 cm, and this is the value adopted in the present work.

The derivation and calculation of λ^2 have been given in detail in the preceding paper[2]. Using CREESE's figure of 26.5% for the proportion of the extracellular space in rat diaphragm, λ^2 works out at 2.173.

Using the integrated equation above, the values for the diffusion constants in diaphragm for sodium, sucrose and chloride have been estimated, and are shown in Table I. The figures range from almost twice the free-solution value for sucrose, down to one-fifth of the free solution level for Na. The variability between individual determinations on a single substance is high: however, the values for the extracellular diffusion constants obtained are at least of the same order of magnitude as those reported for free diffusion of the substances under the same conditions of temperature and concentration. These results were expected on the basis of experiments reported earlier where a leg muscle was used[1,2].

TABLE I

THE DIFFUSION OF Na, Cl, AND SUCROSE IN RAT DIAPHRAGM AT 20°C

Substance	*D in diaphragm* $cm^2/min \times 10^4 \pm$ S.D.	*D in free solution* $cm^2/min \pm 10^4$	*Ratio D muscle / D free solution*
Na	1.36 ± 0.44 (8)*	7.2	0.19
Cl	3.08 ± 1.72 (7)	8.5	0.36
Sucrose	5.57 ± 4.07 (4)	3.1	1.80

* Number of determinations.

The results for potassium diffusion are presented in Table II. The last line of the Table shows the result when experiments were performed in the same way as in the earlier report; that is, after incubation of the muscle in a medium containing high concentrations of K and phosphate. In this case the same results as then obtained are repeated, in that the diffusion constant measured is less than 10% of the free solution value. However, when the conditions of incubation are changed, it becomes clear that

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under suitable circumstances the extracellular diffusion of K is relatively unimpeded. These circumstances are apparently that the extracellular concentration of K should be in the physiological range, and that there should not be a large concentration of phosphate in the extracellular space.

That a high concentration of phosphate has an impeding effect is shown by the fact that incubation in KCl solution, where the K concentration is the same as in the phosphate medium, gives a higher diffusion constant than occurs with the phosphate solution.

The difference may in fact be greater than appears in Table II, since incubation in KCl results in such swelling of the muscle cells that the value of λ^2 used may be too small. The indication also is that replacing chloride by phosphate in the ordinary Ringer-type medium reduces the measured diffusion constant for K, although the number of determinations is too small to be certain of this point.

TABLE II

THE DIFFUSION OF K IN RAT DIAPHRAGM AT 20°C

D in free solution = $9.9 \cdot 10^{-4}$ cm²/min.

Conditions	*K concentration in medium mmole/l*	*D* $cm^2/min \pm S.D. \times 10^4$	*Ratio D muscle / D free solution*
Ringer-type solution	5.5	12.44 ± 2.66 (3)*	1.26
Ringer-type, phosphate replacing Cl	5.5	9.23 ± 2.90 (2)	0.93
Ringer-type, 4 × usual [K]	22	4.61 (1)	0.47
KCl	154	2.62 ± 0.14 (2)	0.26
K phosphate, pH 7.0	154	0.67 ± 0.48 (9)	0.07

* Number of determinations.

Ionic mobility in an applied electric field

The effect of a polarizing current on the movement of various ionic species was tested by placing small platinum electrodes in the tube above the muscle, and in the beaker beneath it. Current from a 1.5 volt dry cell was passed between them. Suitable resistors and a 1 mA meter were inserted in the circuit as well.

Table III summarizes the results of individual experiments of this kind. It can be seen that for Na, Cl and K ions, (in the latter case when diffusion from a Ringer-type medium is measured), application of the current gives the expected change in the

TABLE III

Ion.		*(1) Measured current, amperes*	*(2) Theoretical charge carried by ion, coulombs*	*(3) μequiv./sec extra ionic movement due to current*	*(4) Charge carried by measured extra ionic movement, coulombs*	*Ratio column 2 / column 4*
Cl		$8 \cdot 10^{-4}$	$4.0 \cdot 10^{-4}$	$3.4 \cdot 10^{-3}$	$3.3 \cdot 10^{-4}$	0.83
		$6 \cdot 10^{-4}$	$3.0 \cdot 10^{-4}$	$1.3 \cdot 10^{-3}$	$1.3 \cdot 10^{-4}$	0.43
		$6 \cdot 10^{-5}$	$3.0 \cdot 10^{-5}$	$3.8 \cdot 10^{-4}$	$3.7 \cdot 10^{-5}$	1.23
Na		$5 \cdot 10^{-4}$	$2.4 \cdot 10^{-4}$	$2.2 \cdot 10^{-3}$	$2.1 \cdot 10^{-4}$	0.88
		$4 \cdot 10^{-4}$	$1.9 \cdot 10^{-4}$	$1.6 \cdot 10^{-3}$	$1.5 \cdot 10^{-4}$	0.78
K Ringer		$8 \cdot 10^{-4}$	$1.4 \cdot 10^{-5}$	$1.3 \cdot 10^{-4}$	$1.3 \cdot 10^{-5}$	0.93
		$9 \cdot 10^{-4}$	$1.6 \cdot 10^{-5}$	$1.5 \cdot 10^{-4}$	$1.4 \cdot 10^{-5}$	0.88
K Phosphate		$4 \cdot 10^{-4}$	$2.0 \cdot 10^{-4}$	$7.5 \cdot 10^{-4}$	$0.7 \cdot 10^{-4}$	0.35
		$6 \cdot 10^{-5}$	$3.0 \cdot 10^{-5}$	$4.2 \cdot 10^{-5}$	$0.4 \cdot 10^{-5}$	0.13

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amount of ion movement. However, when K phosphate is used, the expected increase in ionic transfer is not observed. Presumably in this case, since the solution in the beaker below the muscle membrane was always a Ringer-type medium, the current is carried not by K and phosphate ions but by Na and Cl ions from this medium. The implication once again is that it is the phosphate part of the salt which is causing this apparent reduction in the freedom of mobility of K ions.

DISCUSSION

The results in the present paper go far towards providing a possible explanation of the apparently slow diffusion of K in the extracellular space of muscle which has been reported in earlier work. It has now been shown that the effect is only observable where diffusion from a solution containing a high concentration of both K and phosphate is measured, and that if a low concentration of K (as in a Ringer-type medium) is employed, or if another ion (as Cl) is used with a high K concentration, the K apparently diffuses at about the free solution rate.

HARRIS[7] has reported that if phosphate ion is applied to a point on a frog sartorius muscle, that a portion of it becomes fixed and no longer mobile under an applied electric field. He has shown further that the un-ionized complex formed between phosphate and, presumably, the muscle proteins, does dissociate slowly. Such a finding appears to agree well with the present results, for binding of the phosphate would have the effect of diminishing the diffusion of K, both with and without an applied electric field, when these are the only ionic species present in appreciable concentration.

It remains only to rescind those conclusions drawn as a consequence of the earlier results, which dealt with the effect of the apparent slow diffusion of K on observed fluxes across the muscle cell membrane[8]. Since it now appears that K is able to diffuse freely except under special circumstances, those conclusions are no longer valid.

ACKNOWLEDGEMENTS

I am indebted to the Muscular Dystrophy Association of Canada for their continued generous support of this work. I also wish to thank Miss JEAN THOMASSON for her excellent technical assistance.

SUMMARY

1. The diffusion constants for sodium, potassium and chloride ions, and for sucrose, moving in the extracellular space of a thin muscle membrane (diaphragm) have been measured. Under conditions where the ionic species are present in physiological concentrations, the diffusion constants for all of them are within the free solution range. The diffusion constant for sucrose is also within the limits of the free solution value.

2. If the composition of the extracellular fluid of the muscle is altered such that it contains a high concentration of both K and phosphate ions, the diffusion of K from the extracellular space now appears to be very much slower than normal. This effect does not appear if the high concentration of K is associated with another anion such as chloride.

3. It is concluded that the slow diffusion of K when it is present in combination with phosphate ion, is due possibly to a binding of the latter ion with the proteins of the muscle. The finding that the mobility of the K ion in an applied electric field is reduced when it is associated with phosphate, tends to support this view.

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Received October 10th, 1957

OVERSHOOT AND BLOCK OF CONDUCTION BY LIPID-SOLUBLE ACETYLCHOLINE ANALOGUES*

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It was suggested, in 1941, in modification of the original hypothesis of neurohumoral transmission, that the release and action of acetylcholine are intracellular processes taking place within the conducting membrane along the entire length of the nerve fiber[1-3]. Acetylcholine was postulated to be responsible for the change in electrical polarity of the membrane which occurs during the passage of the nerve impulse. This view was substantiated during the following decade. In particular, the inseparability of acetylcholinesterase and conduction was demonstrated. Moreover, when the sequence of energy transformations occurring during nerve activity was established and acetylcholine was integrated into the metabolic pathways of conducting cells, it became apparent that the activity of acetylcholine precedes the other events, suggesting that it is the specific operative substance in nerve conduction in the sense applied by MEYERHOF to ATP in muscle contraction[4,5].

Later it was suggested that acetylcholine combines with a receptor substance (probably a protein which resembles acetylcholinesterase) and that this reaction brings about a change in conformation which alters the membrane permeability. This suggestion introduces the possibility of receptor activators—substances which combine reversibly with the receptor and evoke a change in membrane potentials—and receptor inhibitors—substances which combine reversibly with the receptor but are unable to evoke activity. In general, it has been noted that while simple quaternary ammonium ions are receptor activators, the tertiary ammonium ions, which are

* This work was supported in part by the Division of Research Grants and Fellowships of the National Institutes of Health, Grants No. B-400 and B-573, United States Public Health Service, by the Atomic Energy Commission, Contract No. AT(30-1)-1503, and by the National Science Foundation, Grant No. G-4331.

** Fulbright Fellow from the U.S. Educational Foundation in Belgium.

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