CIX. MAGNESIUM AND CHLORIDE "PERMEATIONS" IN MUSCLE

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IN a previous paper [Conway & Kane, 1934] the diffusion relations of a number of anions and of urea through tissues were studied. Using an equation which took account of the equilibrium value to which the diffusion tended, the same coefficient was found for 4 different anions—sulphate, chloride, phosphate and lactate—at comparatively low concentrations) into or out of muscle, and for sulphate into or out of liver and kidney. This value of 6.0×10^{-5} (cm.²/min.) was shown to be independent of the degree of "permeation" of the tissue, by which term was understood the amount per 100 g. of tissue water as a percentage of the external concentration. With low "permeation", the interspace water or water between the tissue elements must account for a large fraction of this value and with a high "permeation" it accounts for a comparatively small fraction. The interspace water must therefore have practically no influence on the diffusion rates through tissues and this is intelligible only if we suppose that it is locked off into compartments by tissue elements in contact with each other. That this is so would seem necessary when we consider that the diffusion rate through such water is ten times as fast as the total diffusion through the tissue.

Such a view supposes that anions penetrate the tissue elements in muscle, and as against this much weight has been given to evidence of the following kind [e.g. Fenn, 1936]. The "permeation" of chloride in normal freshly excised muscle, with reference to the plasma chloride is only about 19%. It is held that the interspace water must account for most if not all of this figure. The interspace water of such an excised tissue may represent, however, only 9% or less of the whole tissue water.

Also the potential changes of muscle in varying solutions seem at present more easily interpreted by assuming impermeability to anions and permeability to potassium but not to sodium [e.g. Mond & Netter, 1930; 1932]. The observed potentials are a net result of a complex series of events and can be differently interpreted.

From the view that anions such as chloride enter only the interspace water, it is supposed that this water is open to free diffusion from outside and is therefore free for diffusion of anions and cations. Cations such as magnesium and sodium should enter here as fast as the chloride and sulphate anions with which they are associated. It is shown in the present communication that for magnesium this is far from being true. In about 15 min. chloride and sulphate have practically reached their full equilibrium value of 30–40% permeation, when the free magnesium cation "permeation" is only 10%. After 60 min. the magnesium value is about one-third of the chloride, when no change or a small increase of the muscle volume has taken place. Even with a marked diminution of volume due to a high concentration of magnesium chloride outside the value

is only about one-half of the chloride. On a basis of anion impermeability it would be impossible to account for these differences by any reference to diffusion rates *in vitro*. As given in the *International Critical Tables* (vol. 5) the diffusion coefficients of magnesium chloride and sodium chloride *in vitro* at 20° and 1 g. equiv. per l. are 6.72×10^{-4} and 7.62×10^{-4} (cm.²/min.) respectively, and the observed diffusions will vary as the square root of these values.

Some barrier exists for the magnesium which the chloride anion can pass much more freely and this must be the tissue membrane or substance. A certain fraction of diffusible anions exists therefore within the tissue with which the chloride anions can freely interchange. It is possible that the chloride and sulphate anions pass only through part of the muscle substance and this would make the interpretation of other facts somewhat simpler. It has been found here however, in a preliminary investigation of similar "permeations" in primitive cells such as yeast, that the samples so far examined show very close parallelism with muscle, and for such cells no question of interspace water arises for consideration.

In relation to the chloride and magnesium diffusion it was also thought of interest to discover what equilibria might be reached on prolonged contact at low temperatures over many days with isolated sartorii at 2–3°. The results have been surprising, for whereas the magnesium diffusion is very much slower than the chloride it gradually reaches and passes the chloride equilibrium at about the fourth to the fifth hour and it finally reaches a "permeation" of about 100. The total magnesium is then far higher within the tissue than outside, the excess being due to the bound magnesium, as will be shown. Of the 26.8 mg. (mean value) magnesium per 100 g. sartorius only 1–5 mg. are present as the ionized or diffusible substance.

METHODS

Experimental. The sartorii or other tissues used in the diffusion were prepared and weighed after suitable excision of extraneous tissue and drying of the surface, then immersed in Locke's solution or such solution in which part of the sodium chloride was replaced by magnesium sulphate or chloride. Stirring was carried out by a continuous stream of oxygen which was previously bubbled through a magnesium carbonate-phosphate mixture, thereby carrying away carbon dioxide sufficient to maintain a constant pH in the tissue vessel with Locke's or Ringer's fluid of approximately 7.5. Blood, where examined, was obtained through a cannula in the aorta and urine from the frog's bladder.

Analytical. Magnesium. The tissue was dried in a platinum crucible and then ignited so that no charred particles remained. The residual inorganic material was taken up by 3 successive small volumes of dilute hydrochloric acid and the magnesium determined by the method described by Cruess-Callaghan [1935] in which the complex with 8-hydroxyquinoline is brominated with a known excess of bromine and this excess determined iodimetrically. In this method, using duplicates, quantities as low as 20 γ magnesium could be determined to within 7%. As a rule about 50–150 γ magnesium were analysed in duplicate with a standard deviation of approximately 2 γ .

Chloride. The chloride was determined according to a method previously published [Conway, 1935], involving the oxidation of the chloride to chlorine in the protein filtrate and its determination iodimetrically. The chloride in a single sartorius of the frog can be determined by this method to within a few per cent.

RESULTS

(1) *The distribution of magnesium in tissues*

In Table I is given a summary of analyses carried out by the hydroxy-quinoline method on muscle, gland tissue and plasma of frogs (*Rana temporaria*). For the analyses of muscle and kidney 2-4 tissues were usually taken so that the amount of magnesium analysed lay, as a rule, in the region of 30-120 γ . The mean

Table I. *Distribution of magnesium in tissues of frog*

Tissue	No. of determinations	Mean conc. mg./100 g.	S.D. of the single analysis
Muscle (sartorius)	35	26.8	5.7
Kidney	25	28.4	9.4
			Range
Liver	5	18.1	(11.1-29.1)
Stomach	4	15.2	(15.1-15.4)
Heart	5	20.3	(17.6-21.8)
Plasma	3	2.9	(3.9-2.2)
Urine	2	1.8	(2.1, 1.5)

The plasma and urine figures are given per 100 ml.

value for the kidney is somewhat higher than in muscle, being 28.4 mg./100 g. tissue as compared with 26.8 mg./100 g. The liver, stomach and heart values run somewhat lower. Particular attention may be drawn to the plasma value since the three results given were obtained from three 1 ml. volumes of plasma, perfectly clear and free from contamination by haemoglobin. Each ml. plasma analysed represented a mixed fluid from 6 to 8 frogs and gave 2.9 mg./100 ml. as an average of the three determinations. The two urine determinations (2.1 and 1.5 mg./100 ml.) were also carried out on 1 ml. volumes collected from a number of frogs of the same consignment from which the blood was taken.

(2) *Long period (1-7 days) magnesium equilibria at 2-3°*

From these long period equilibria it becomes certain that of the mean 26.8 mg./100 g. sartorius, by far the greater part is unionized and indiffusible. In the first experimental series the muscles (2 or 3) were immersed in 25 ml. of magnesium-free Ringer in a stoppered Erlenmeyer flask as a unit group for each analysis. The companion sartorii were in many instances grouped and analysed directly. The results are given in Table II. From the table it appears that the muscle retains about 80 % of its magnesium up to the fifth day. After this a marked fall sets in and the greater part of the magnesium is lost about the seventh day. When the four long period immersions (24-28 hr.), for which there are also analyses for the companion tissues are considered, only a mean value of 1.4 mg. out of the original 24.7 mg./100 g. tissue have diffused out. While the long retention is being maintained the muscle is in fact freely permeable to the magnesium cation as is shown by the following experiments.

In these the technique of the first was repeated except that the Ringer now contained 20 or 40 mg. magnesium per 100 ml. The results are given in Table II. The average from 6 experiments with 20 mg./100 ml. external magnesium with immersions ranging from 1 to 5 days is 37.9 mg./100 g. of the muscle after immersion. Expressed as mg./100 g. tissue water this is 47.8 mg. (The tissue water is calculated throughout on the basis that in 100 g. of the original or freshly excised muscle there are 80 g. of water, and that no appreciable weight of solids is lost on immersion.) The indiffusible magnesium from 8 experiments

Table II. *Magnesium in sartorii after long period immersions at 2-3°*

Fluid for immersion	Duration of immersion hr.	Weight of immersed sartorii after and before (mg.)	Magnesium in companion tissues mg./100 g.	Magnesium in immersed tissues	
				mg./100 g.	mg./100 g. of tissue water
Locke's solution	24	168, 160	25.3	21.9	28.4
	24	257, 244	15.5	14.3	18.1
	25	250, 243	—	23.1	29.0
	25	338, 308	—	23.0	29.6
	28	243, 231	27.0	26.4	33.5
	28	243, 231	31.1	26.0	33.0
	49	274, 277	—	17.7	22.1
	116	320, 300	—	18.0	22.9
	143	288, 308	—	17.9	22.0
	143	257, 281	—	12.1	14.8
	144	217, 206	21.6	12.1	15.3
	144	208, 198	19.8	11.6	14.7
	167	281, 310	—	5.2	6.3
		Mean up to 116 hr.		21.3	27.1
		Mean change of weight		5.2%	
Locke's solution containing 20 mg./100 ml. Mg added as sulphate	25	250, 255	—	37.5	46.8
	25	335, 322	—	36.9	46.6
	26	297, 288	23.9	34.7	43.6
	27	284, 276	29.8	36.9	46.6
	49	252, 236	—	36.8	46.7
	116	307, 296	—	44.8	56.6
		Mean value		37.9	47.8
Locke's solution containing 40 mg./100 ml. Mg	24	137, 116	27.3	37.9	49.6
	24	140, 123	32.5	53.6	69.4
	28	132, 114	31.1	46.4	60.6
	28	213, 184	36.9	54.3	70.7
	48	137, 118	39.1	50.8	66.2
	48	127, 106	27.1	50.0	65.8
	92	102, 109	26.7	48.6	59.8
	92	83, 90	31.7	54.4	66.7
		Mean value		49.5	63.6

was 27.1 mg./100 g. of tissue water, therefore 20.8 were present as the magnesium cation which gives a "permeation" of 104 with respect to the outside fluid. With the 8 experiments where 40 mg./100 ml. magnesium were present in the external fluid the mean value for 1-5 days was 63.6 mg./100 g. tissue water (Table II). Subtracting as above the bound magnesium, 36.5 mg. free magnesium cation are present in 100 g. tissue water reaching therefore a "permeation" of 91. It is clear therefore from these experiments that when 80-90 % of the total magnesium is retained in the absence of external magnesium, the muscle is at the same time freely permeable to the magnesium cation, which reaches a full "permeation". The retained magnesium is bound in a complex probably of an organic type. The amount of this bound magnesium has a mean value of 22 ± 1.0 mg./100 g. muscle.

Apart from the question of the bound magnesium it might be thought that at the same time the muscle membrane had become freely permeable to all ions over this period and that it resembled entirely dead tissue. While such an idea does not affect the determination of the bound magnesium as above, it is a fact that the muscle membrane is behaving in a selective and surprisingly constant manner, as may be seen from the next section giving the chloride equilibria over similar long periods. (In Fig. 1 the "permeation" of the two ions is shown for several days immersion.)

(3) *Long period (1-7 days) chloride equilibria at 2-3°*

The long period chloride equilibria were investigated like those of magnesium. Groups of sartorii were immersed in Ringer solution containing 0.6 % NaCl. This Ringer solution was varied by the introduction of 20 mg. magnesium per 100 ml.

Table III. *Chloride "permeations" in long period sartorii immersions at 2-3°*

Solution for immersions	Time of immersion hr.	Weights before and after immersion mg.	Chloride in immersed tissue "permeation"
Mg-Ringer	19	221, 216	39.8
"	19	219, 196	45.6
Ringer	24	147, 138	37.9
Mg-Ringer	24	195, 178	35.8
Ringer	72	177, 178	38.4
Mg-Ringer	72	143, 135	50.6
Ringer	96	117, 102	73.4
Mg-Ringer	96	133, 121	79.5
Ringer	144	118, 93	93.2
Mg-Ringer	144	148, 118	89.4

The magnesium-Ringer solution for 19 hr. immersion contained 200 mg. Mg/100 ml., the other magnesium-Ringer solutions contained 20 mg. Mg/100 ml. and 0.39 mg. Cl/100 ml.

Fig. 1. "Permeations" of chloride and magnesium after long period immersions in Ringer and magnesium-Ringer solutions at 2-3°. The magnesium-Ringer contained 20 or 40 mg. Mg/100 ml. The free magnesium already present in the muscle is taken as 1.4 mg./100 g. giving a mean "permeation" at zero time of 6 % with respect to an external fluid of 30 mg. Mg/100 ml.

and in one set by introducing 200 mg./100 ml., the sodium chloride being reduced to 0.2 %. The magnesium was introduced to see if it had any effect on the chloride equilibria compared with that for the ordinary Ringer solution. The results as given in Table III show that up to the fourth day of immersion there is no significant change from a mean chloride "permeation" of 41 %, though this is a little higher than the "permeation" after only 1 hr. under similar conditions, which is 36 %. After 4 days' immersion the barrier to full chloride "permeation" breaks down, the value after 6 days approaching 100 % (see also Fig. 1).

(4) *Short period (0-60 min.) magnesium and chloride muscle diffusion at room temperature*

In these experiments the tissues were kept continually stirred with an oxygen-carbon dioxide current. The results of short period chloride diffusions have been already described in a previous communication [Conway & Kane,

1934]. The external chloride then used was 0.74% NaCl. Here the effect of magnesium on the equilibrium was examined and the effect of varying the chloride so as to produce rather marked changes in the water content. In Fig. 2 the rate of chloride entrance and equilibrium is given mainly from previous data [Conway & Kane, 1934], the external chloride being 0.74% NaCl. The large circles represent the means of experiments carried out with the present series, with high external magnesium (120–200 mg./100 ml.) sodium chloride being suitably reduced to maintain the same or similar osmotic pressure. The equilibrium as will be seen, is reached in about 15 min. and the magnesium has no appreciable effect on the equilibrium value, just as it has no effect on the long period equilibria.

Fig. 2. "Permeations" of chloride and magnesium ions for the sartorii after immersions up to 90 min. The chloride curve is drawn from previous data [Conway & Kane, 1934]. The free magnesium in the muscle at zero time is taken as 1.4 mg./100 g., or a "permeation" of 0.9% with respect to the external 200 mg. Mg/100 ml. The chloride begins with a "permeation" of approximately 10% with respect to the external Ringer solution used (0.7% NaCl).

The short period magnesium diffusion is markedly different from the chloride, being very much slower. In 15, 30 and 60 min. the chloride "permeations" are 35, 36 and 36% (these figures differing slightly with change of tissue water as shown below). By contrast the magnesium entrance from an external 200 mg./100 ml. magnesium-Ringer solution and per 100 g. tissue water is only 10, 12 and 13% of the external magnesium (see Table IV and Fig. 2). The total free magnesium in the muscle can at most only exceed this by a few per cent, since even after a few days' immersion only about 1–5 mg. magnesium per 100 g. tissue have diffused out into magnesium-free Ringer and for a group of 4 experiments in which the companion tissues were also analysed, only 1.4 mg./100 g. diffused. This latter figure represents only 0.7% of the magnesium in an external 200 mg./100 ml. solution. It is obvious therefore that if the chloride existed only in the interspace water over three times the observed magnesium should have diffused inwards. If the magnesium be taken as entering the tissue substance the discrepancy becomes all the greater. In Fig. 2 the permeations of the ionized magnesium are given for 200 mg./100 ml. in the external solution, 1.4 mg. being considered already present in 100 g. of immersed tissue.

In one group of experiments the magnesium entered in somewhat greater proportions, the percentage of the external fluid in the tissue water being 21% after 60 min. In these experiments a mean 14% reduction of tissue volume occurred due to immersing the tissue in a hypertonic solution containing magnesium chloride and sodium chloride, the magnesium being 194 mg./100 ml. and the chloride ion 640 mg./100 ml. (see Table IV). The chloride "permeation"

Table IV. *Short period magnesium diffusions into and out of the frog's sartorii.*

External fluid	No. of expts.	Period of immersion min.	Weights of immersed tissues before and after. mg.	Magnesium content of companion tissues mg./100 g.	Increase of magnesium content of immersed tissues mg./100 g. original tissue	Increase of magnesium in tissue water as % of external magnesium
Mg-Ringer (Mg 200 mg./100 ml. added as sulphate)	2	3.7	242, 243	20.8	8.9	5.5
	2	7.5	175, 190	28.8	12.2	7.6
	3	15.0	228, 245	29.3	16.4	10.2
	2	20.0	203, 210	29.1	15.7	9.7
	3	30.0	221, 240	19.0	18.4	11.6
	2	75.0	209, 216	30.5	21.6	13.4
	2	280.0	235, —	30.1	67.4	41.7
Mg-Ringer (Mg. 194 mg./100 ml. added as chloride)	3	17.0	187, 167	27.9	27.4	17.7
	3	60.0	156, 134	27.0	35.6	22.9
Ringer solution without magnesium	4	14.0	294, 301	26.4	-1.0	
	6	30.0	292, —	21.3	-3.5	
	(4)	(1560.0)	(228, 217)	(24.7)	(-1.4)	

The bracketed figures at bottom of table give a summary of 4 long-period equilibria for comparison.

after 60 min. was 39% so that the magnesium after 1 hr. had entered to the extent of only 54% of the chloride present. These figures with marked water loss are not entered in Fig. 2.

(5) *Chloride equilibria and change of tissue water in the sartorius*

In Fig. 3 the chloride equilibria expressed as "permeations" are plotted against the changes of tissue water produced by solutions of varying tonicity. The 60 min. periods were taken for observation and the external chloride in the Ringer varied from about 0.5 to 0.9% NaCl (some of the solutions contained magnesium). Less than 0.5% NaCl with potassium and calcium in the usual Ringer concentrations seemed to damage the normal permeability of the tissue. The line through the points indicates that a given percentage increase in the tissue water causes a corresponding fall in the "permeation," and vice versa. The permeation expressed with respect to 100 g. of the original tissue water remains constant, and for chloride is 33.5%. The independence of the "permeation" to changes in the tissue water when expressed in 100 g.

of the original tissue water is easily interpreted if we suppose the chloride interchanging largely with diffusible anions within the tissue cells, since the total quantity of these would presumably be unaffected by the water changes.

Fig. 3. Percentage change in tissue water of sartorii against chloride "permeation" after 60 min. immersion.

DISCUSSION

The main points of interest in the present communication are firstly the markedly different rates of penetration of the excised muscle of the frog by magnesium and chloride ions, then the curious features of the long period equilibrium at 2–3°, whereby the bound magnesium can be determined. The long period equilibria at low temperatures show conclusively that of the mean value of 26.8 mg. in 100 g. of the muscle only about 1–5 mg. are free as the magnesium cation. The muscle retains its bound magnesium up to 5 days' immersion at low temperatures, but even in 24 hr. free magnesium cation enters from outside to a full 100 % equilibrium with the external concentration.

When the fresh muscle is immersed in magnesium-Ringer solution containing 200 mg. magnesium per 100 ml. this enters at a much slower rate than chloride. After 60 min. only 13 % of the 200 mg. have entered per 100 g. tissue water, the total free magnesium present representing 14 %. By contrast chloride enters to a "permeation" value of 36 %, this figure varying somewhat with the changes of tissue water, and is 33.5 % when no change of this water occurs. The contrast of these figures shows that some differential barrier must exist to the magnesium entrance and this is of course the tissue membrane.

The anions such as chloride which enter the tissue substance exchange there no doubt with other diffusible anions. This anion exchange in the excised tissue is accompanied by a slower but more complete cation interchange in what we may regard as a slowly dying tissue. The barrier to complete "permeation" of the anions is maintained for several days at a rather constant figure of about 40 %. This we could explain on the ground that the greater part of the anion content of the tissue substance is colloidal, though short of further data under similar conditions for the other cations a further theoretical interpretation is scarcely warranted.

In connexion with anion penetration, notice may be taken of Fenn's suggestion [1936] that lactic acid may come out of the excised muscle entirely as the free lactic acid. This, we think, is impossible. In the excised muscle lactic acid formed by previous stimulation, must exist in by far the greater proportion within the tissue elements. Considering that the dissociation constant of lactic acid is $10^{-3.85}$ (at 25°), at a *pH* of 7.2 the concentration of undissociated lactic acid to the total present is approximately 1 : 2200, and even at *pH* 6.85, the proportion is 1 : 1000. Lactic acid without reference to its ionization diffuses out of muscle after stimulation at a rate expressed by 6.0×10^{-5} (cm.²/min.) [Eggleton *et al.* 1928] (this holds at least up to 100 mg./100 g.). Such a coefficient is one-tenth that of the *in vitro* diffusion, but if the substance is diffusing from a concentration about 1/2000 part of the total present, obviously its calculated diffusion rate would represent an impossible figure. The lactic acid diffusion from excised muscle forms in itself a clear demonstration of anion penetration through the tissue elements.

Once the lactic acid has diffused from the excised muscle into an external Ringer fluid, its equilibrium concentration with that in the tissue [Ghaffar, 1935] would appear to follow the general anion relationship [Conway & Kane, 1934]. From this we may suppose that in the interspace water before diffusion it tends to a higher concentration than within the cells, though it would be unprofitable to consider how much of the total acid is here accommodated, without a more exact knowledge of the true volume of this interspace water. From the present and previous findings [Conway & Kane, 1934] this

water would appear to be locked off into compartments by the tissue elements in contact, and is probably of the order of 10% or less of the whole tissue water.

SUMMARY

1. The magnesium content of the frog's sartorius—as determined by the hydroxyquinoline method is 26.8 ± 0.6 mg./100 g. (35 determinations). The average magnesium after 1–5 days' immersion in magnesium-free Ringer solution at $2-3^{\circ}$, is 22.3 ± 1.0 mg./100 g. original tissue (8 determinations). For 4 experiments where the companion tissues were directly analysed, the muscles only lost 1.4 mg./100 g. after 1 day's immersion.

The magnesium in frog plasma was found to be 2.9 mg./100 ml. (from 3 different 1 ml. samples of haemoglobin-free and mixed plasma). The corresponding urine concentration was 1.8 mg./100 ml. Values for other tissues are given in the text.

2. Over the long period of retention of the bound magnesium, ionized magnesium enters freely, reaching a "permeation" of approximately 100 in 24 hr., the total magnesium being then much higher than in the external fluid.

3. For short period immersions at room temperature magnesium enters the frog's sartorius at a much slower rate than chloride, the "permeation" of the free magnesium being only one-third to one-half that of the chloride after 90 min., although a full "permeation" for chloride is practically reached in 15 min. From this it may be concluded that other evidence apart, the chloride "space" cannot be merely the interspace between the tissue elements with free diffusion therein of external electrolytes.

4. When there is no change in the volume of the immersed sartorii the "permeation" of the chloride is 33.5. When volume changes occur the "permeation" value alters in an inverse proportion to the change in tissue water. Magnesium has no influence on the chloride "permeation".

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