J. Physiol. (1941) 99, 401–414 612.744

VOLUME OF INTERFIBRE SPACES IN FROG MUSCLE AND THE CALCULATION OF CONCENTRATIONS IN THE FIBRE WATER

By P. J. BOYLE, E. J. CONWAY, F. KANE and H. L. O'REILLY

From the Department of Biochemistry, University College, Dublin

(*Received* 10 *August* 1940)

The methods hitherto used for determining the volume of the interfibre spaces in muscle have been mostly of three kinds; firstly, the histological method, from which exact results cannot be expected; secondly, that depending on the ratio of muscle chloride to the chloride in the external fluid, the muscle membrane being assumed to be impermeable to anions; and thirdly, measurements based on the fall of conductivity after washing in isotonic glucose or sucrose solution, electrolytes being considered to diffuse only from the interfibre spaces.

It is true that for muscle *in vivo* the chloride ratio may give a measure of the interfibre spaces which is in excess of the real value by only about 1–3% of the total muscle volume. For frog's sartorius the mean figure so obtained is 14–15 ml./100 g. muscle using the data of Fenn, Cobb & Marsh [1934] for the plasma chloride, and of Conway & Kane [1934] and of Fenn *et al.* [1934] for the muscle chloride. For mammalian muscle mean figures of 14 ml. have been given [e.g. Winter, 1934] or of 12 ml. as obtained here, whereas the inulin method gives only 7 ml. [Conway & Fitzgerald, 1940] and the use of radio-sodium, 8·5 ml. [Hahn, Hevesey & Rebbe, 1939]. This latter figure was raised to 11 ml. on excluding the fat and other extraneous tissue.

The use of the chloride ratio for muscle immersed in Ringer's solution for over 15 min. gives very erroneous values for the interfibre volume. The mean figure obtained is then 26 ml./100 g., whereas the true interfibre volume is in the region of 9–13 ml./100 g., as shown by the methods described here. The high figure for the chloride ratio with immersed muscle has led to the use of the term 'chloride spaces' with a different

meaning than pure interfibre volume [e.g. Ghaffar, 1935; Eggleton, Eggleton & Hamilton, 1937], the difference being referred to chloride adsorbed on the surface of the fibres [Eggleton *et al.* 1937].

With regard to the conductivity measurements, Schulze's calculations [as corrected by Fenn, 1936] amount to 13·9 ml./100 g. This is likewise somewhat too high, since electrolytes other than those in the interfibre spaces or adsorbed on the fibres are lost while soaking in isotonic sugar.

We have used here four different methods, three of which may be described as direct and one indirect. In the first method inulin was used instead of chloride to measure the interfibre spaces. Inulin penetrates into the tissue spaces and the ratio of muscle inulin to external inulin will apparently give for these a maximum value. In the second method magnesium was used, since it only very slowly penetrates into the excised muscle fibres. The magnesium already inside is quite indiffusible over short periods and loses only about 10% of its value after soaking for 24 hr. in magnesium-free Ringer solution at 2–3° C. [Conway & Cruess-Callaghan, 1935]. In the third method the diffusion of muscle sodium into isotonic glucose was studied and the amount in the interfibre spaces measured from the diffusion curve. Lastly, the amount within the fibres of normal muscle was calculated on the basis of a Donnan equilibrium—a procedure which will be shown later to be fully justified. This figure, amounting to only 1·1% of the external, is subtracted from the total muscle chloride. We have also made some studies of the total circulatory space in the fresh excised sartorius of the frog, showing that its mean value is approximately 2·3 ml./100 g. tissue.

Analytical methods

Sodium. This was estimated by a modification of the method described by Salit [1932] modified for use with the Pulfrich photometer. 1 ml. samples of the ash extract were used instead of the 2 ml. described and the other reagents added in corresponding volume. In the final comparison 10 ml. were taken, 0·25 ml. of the potassium ferrocyanide solution added, and, after standing 5 min., the reading taken in the Pulfrich photometer. The following equation was used (with Filter S 50):

$$\text{Mg. sodium in 1 ml. solution} = 0{\cdot}115\,(E - e),$$

where E is the extinction with 0·5 cm. stratum layer, and e that of the blank. This relation was found to hold best for solutions having extinctions between 0·3 and 1·4. With extinctions greater than 1·4, diluting before developing the colour was carried out, as described by Salit.

Potassium. This was determined by the method of Shohl & Bennett [1928] with preliminary ashing as described by Fenn & Cobb [1934]. The final coloured solution of the potassium iodoplatinate was examined spectrophotometrically, using a 0·5 cm. stratum layer and Filter S 50 in the Pulfrich photometer. The potassium in the sample solution of the ash was calculated from the following equation (applying to standard solutions treated exactly as the muscle):

$$\text{Mg. potassium} = 0{\cdot}32\,(E - e),$$

where E is the extinction of the potassium iodoplatinate and e the extinction of a blank, prepared under the same conditions.

Chloride was measured by a micro-diffusion method [Conway, 1935].

Inulin. This was determined after varying times in the 1·3% sodium sulphate solution in which the muscles were suspended after previous soaking in an inulin-Barkan fluid. The inulin was oxidized completely by potassium permanganate and sulphuric acid and the carbon dioxide measured by a micro-diffusion method [Conway, 1939]. In the procedure, 2 ml. of the solution were taken in the outer chamber, some solid permanganate added and 1 ml. of 70% sulphuric acid. The inner chamber contained 1·3 ml. of $N/40$ $Ba(OH)_2$ and 1 ml. was removed at the end into a small tube, a drop of thymolphthalein solution in alcohol added and the mixture titrated with $N/100$ HCl.

The inulin-Barkan solution in which the muscles were soaked initially was diluted 200 times and the inulin determined in a similar way. It was necessary to express concentrations only in terms of this fluid.

To measure the oxidizable non-inulin material diffusing from muscle, control curves were obtained with companion tissues similarly treated, but with inulin absent from the original soaking fluid.

Experimental methods and results

The inulin method

Twenty sartorii, of about 50 mg. mean weight, were soaked for 2 hr. at room temperature in 50 ml. of an inulin-Barkan solution with oxygen (97%) and carbon dioxide (3%) bubbling. The solution contained 2% inulin and 0·65% NaCl, the remaining constituents being the same as described by Barkan, Broemser & Hahn [1921]. The muscles were then removed, dried quickly, weighed and transferred to 30 ml. of a 1·3% sodium sulphate solution. In this way they were stirred by bubbling with oxygen for some hours. Throughout, at 4, 9, 16, 25, 49 and 100 min., 10 ml. samples were removed and replaced by 10 ml. of fresh sulphate

solution. With each of these samples three or four analyses of 2 ml. were carried out as described under 'Analytical Methods'. Control groups of muscles were treated in a similar way, but without inulin in the soaking fluid. With these the oxidizable non-inulin substances diffusing into the sulphate was determined.

Four groups of such experiments were carried out, the mean weight change on soaking being a loss of 1·6%. The results are summarized in the curve through the mean values in Fig. 1. It will be seen that about 2 hr. are sufficient for the outward diffusion, and by inference for the diffusion inwards.

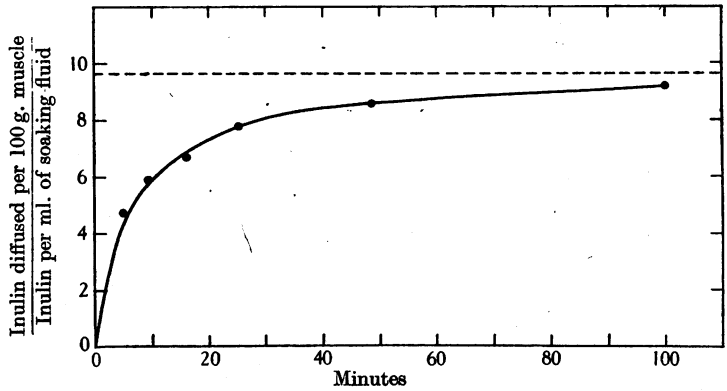

Fig. 1. Volume of the interfibre spaces in the sartorius muscle as measured by inulin diffusion into sodium sulphate (1·3%) after preliminary soaking for 2 hr. in an inulin-Ringer solution. Room temperature.

The ratio of muscle inulin (as judged from the inulin recovered after 100 min.) to the external inulin indicates a mean value for the interspaces of 9·4 ml./100 g. The slightly higher value of 9·6 ml./100 g. could be taken if recoveries after a hypothetically very long time were considered.

The four individual experiments gave 10·3, 8·5, 7·5 and 13·0 ml./100 g. respectively. The oxidizable material diffused from the control muscles amounted to approximately $1{\cdot}07 \times 10^{-3}$ m.equiv. carbon dioxide per g. per min. In 60 min., when the inulin diffusion from the soaked muscles was almost complete, this non-inulin oxidizable material accounted for about one-third of the total carbon dioxide found on oxidation.

The magnesium method

Chloride or sulphate ions diffuse almost completely into or out of the excised sartorius muscle immersed for 15 min. in Ringer or modified Ringer solutions. From a comparison of the diffusion coefficients of

magnesium chloride or sulphate with sodium chloride or sulphate the diffusion of the magnesium into or out of the interfibre spaces should likewise be almost complete in less than 30 min. At the same time the membrane in excised muscle is not quite impermeable to magnesium ions, but the penetration is comparatively very slow. The magnesium already contained in the muscle (24·7 mg./100 g.) is practically indiffusible and presumably organically bound within the fibre [Conway & Cruess-Callaghan, 1937]. When the sartorius muscle is immersed therefore in modified Ringer fluid containing 200 mg. magnesium/100 ml. (replacing an equivalent amount of sodium) the interfibre volume should be clearly

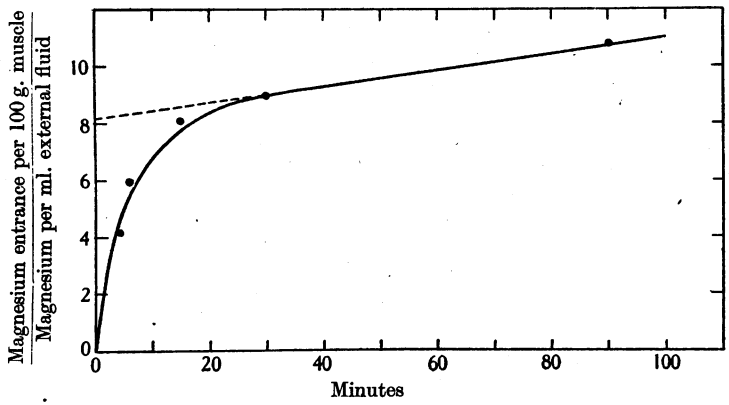

Fig. 2. Volume of interfibre spaces as measured by magnesium entrance from a magnesium-Ringer solution, containing 200 mg. magnesium/100 ml. and 0·26% NaCl. Room temperature.

determinable from the curve of magnesium increase expressed as a percentage of the external substance. Fig. 2 gives a summary of the results obtained for the magnesium increase after immersion. Each dot represents the mean change of magnesium for two or three experiments in each of which four muscles from the same number of frogs were used. The magnesium originally present was determined from companion muscles directly analysed. The ordinates give the increase per 100 g. original tissue divided by the magnesium content in 1 ml. of external fluid. From the line of slow increase after 30 min. produced backwards to cut the ordinate from the origin the interfibre spaces have a volume of 8·2 ml./100 g. tissue. This is similar to the inulin figure of 9·6 ml./100 g., and the difference may be attributed largely to the mean increase in weight of 5·8% in the magnesium immersions compared with a loss of 1·6% with inulin. That such weight changes can affect the interspace

volume is shown by the fact that using the magnesium method in a further series of experiments in which the external fluid (of higher tonicity than before) caused a fall in weight of 12·5%, the interfibre space was found to be 12·6 instead of 8·2 ml./100 g. We could conclude therefore that a relative *fall* in weight corresponding to 18·3 ml./100 g. of original tissue appears to be associated with an *increase* in the interfibre spaces of 4·4 ml./100 g. of fresh muscle. If we were to use this relation in a proportionate way the space would be 9·2 ml. with the inulin method and 9·6 ml. with the magnesium method, when no change in weight occurred on immersion.

The following methods enable us to assess the total sodium chloride outside the fibre, and to calculate the chloride inside the membrane.

The sodium and chloride method

In a series of experiments observations were made on the diffusion of sodium, potassium and chloride from sartorii immersed in glucose solution (3·2–3·8%). Urano [1908] seems to be the first to have made systematic observations on the inorganic cation and anion changes in muscle after immersion in isotonic sugar. It was shown that the muscle is free of sodium and chloride after several hours immersion, whereas potassium, though lessened, is still largely retained. Fahr [1908], confirming these findings, drew the same conclusions as Urano that not only chloride but also sodium is contained between the fibres and not within them. Later, Mond & Netter [1932], perfusing a frog's hind limbs with isotonic glucose for short periods (4–6 min.), showed that the sodium came out in two phases and that only part of it could be considered to exist free between the fibres, concluding from further experiments that the sodium was bound in some way in the fibre membrane itself. That part of the sodium behaves differently from the chloride is at once evident from exact analyses of sodium and chloride mean values in frog's plasma and muscle [e.g. Fenn *et al.* 1934]. The mean ratio of muscle chloride (g./100 g.) to plasma chloride (g./100 g.) is 0·14, whereas the corresponding ratio for sodium is 0·22. Leaving for subsequent consideration the question of the localization of the extra sodium, our object here is to demonstrate the total sodium chloride external to the fibre by an exact construction of the time curve of emergence of sodium and chloride from the excised sartorius into isotonic glucose. In the procedure twenty sartorii were immersed in 20 ml. glucose solution (3·2–3·8%), stirred with oxygen and 2 ml. samples of the fluid removed at intervals up to 2 hr., being replaced each time by 2 ml. of fresh glucose solution. At the end the remaining

sodium in the muscle was determined. A similar procedure was used for potassium, except that 5 ml. was removed instead of 2 ml. as for sodium.

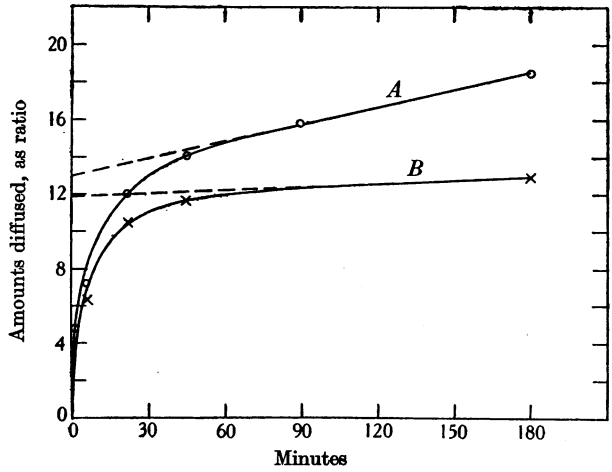

Fig. 3. Sodium (*A*) and chloride (*B*) diffusion from twenty sartorii muscles immersed in isotonic glucose, expressed per 100 g. muscle divided by the mean quantities in 1 g. plasma.

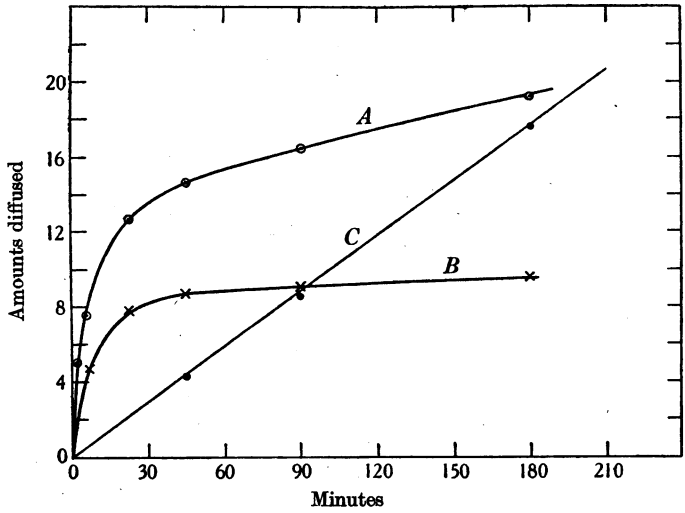

Fig. 4. Sodium (*A*), chloride (*B*) and potassium (*C*) diffusion from twenty sartorii immersed in isotonic glucose, expressed as m.equiv./kg. of original muscle.

For chloride a different procedure was adopted. Duplicate experiments were carried out in which four muscles were immersed in glucose solution for a certain interval, then removed and analysed for chloride. Fig. 3

gives a summary of the sodium and chloride results expressed as g./100 g. muscle divided by the mean concentrations in plasma (0·264 g./100 g. for chloride and 0·238 g. sodium/100 g. plasma [Fenn, 1936]).

Fig. 4 shows the actual quantities of sodium, potassium and chloride diffused and expressed as m.equiv./kg. of the original tissue. It will be seen that sodium comes out rapidly at first, after which it diffuses slowly and rather linearly so that an approximation to the amount in the first stage is given by extending backwards the line of the secondary increase to zero time. This gives 13%, and a similar extension of the chloride line gives approximately 12%. About 12·5 ml. would therefore represent a maximum value for the interfibre space from such results. There is, however, a total of 14% to be accounted for with chloride, leaving 1–2% as possibly present within the fibre. In the following method it will be seen that this is all the chloride that need be theoretically expected if the membrane is permeable both to chloride and potassium.

Indirect method from Donnan equilibrium calculations

That such calculations are relevant to this problem will be fully demonstrated in a later paper. They are based on the equivalence of the products of the potassium and chloride ion concentrations inside and outside the fibres. From the mean quantities of potassium, chloride and water in the muscle and plasma of the frog we have for the equivalence of the products

$$\frac{84{\cdot}6-2{\cdot}5s}{0{\cdot}80-0{\cdot}95s}\times\frac{10{\cdot}5-74{\cdot}3s}{0{\cdot}80-0{\cdot}95s}=\frac{74{\cdot}3\times 2{\cdot}5}{0{\cdot}954},$$

where 84·6 and 2·5 are the mean concentrations of potassium in muscle and plasma (m.equiv./kg.), 10·5 and 74·3 being the corresponding chloride values; s is the interspace plasma in kg., and 0·954 the water in 1 kg. of plasma. (The sources of these data are given in a subsequent communication.)

From the equation s may be reckoned as 0·127, or in 100 g. muscle there are apparently 12·7 g. plasma and in 1 kg. of muscle $0{\cdot}127\times 74{\cdot}3$ or 9·4 m.equiv. chloride. The total mean value for the muscle chloride is 10·5, so that only 1·1 m.equiv./kg. muscle (or 1·5/kg. fibre water) need be present within the fibre. No weight therefore need be attached to the presumed lack of chloride within the fibres depending on the apparent equivalence of chloride in whole muscle and that in the interfibre spaces, since the theoretical amount is too small for quantitative recognition by the methods used, and in particular the histological or histochemical methods.

The circulatory space in excised sartorii

In this determination the total haemoglobin in the sartorii was extracted and measured photometrically after conversion to alkali haematin and compared with blood suitably diluted and treated with alkali. The mean value for the circulatory space so obtained is naturally too high, as muscle haemoglobin is extracted as well as blood haemoglobin, but is of interest as a maximum figure. At the same time we have made some measurements of the extent to which the muscle haemoglobin interferes with the estimate by repeating the procedure on leg muscle subsequent to 4 hr. perfusion with Ringer fluid.

Procedure. Four experiments were carried out in each of which twelve sartorii from six killed and pithed frogs were taken. The surfaces of the sartorii were dried between filter paper, then ground to a fine paste with 3 ml. water, the mixture centrifuged and the supernatant fluid collected. The residue of ground muscle was mixed with 3 ml. Ringer fluid, returned to the mortar, ground, centrifuged and the fluid collected. This was repeated until four such extractions were made. The efficiency of the extraction was tested by the examination of further extracts with Ringer fluid or with 10% KOH, using the colorimetric procedure referred to subsequently. It was found that after the fourth extraction less than 5% of the total haemoglobin originally present could have remained, also the residual ground muscle appeared quite colourless. 5 ml. of the collected fluid were taken and 1 ml. of 25% KOH added and mixed.

Blood collected—and oxalated—from 2–6 frogs corresponding with each muscle experiment was diluted 500 times, using the same average diluting fluid as for the muscle extraction, including alkali, to bring to 4·2% KOH. The addition of the alkali to the muscle extract had the advantage of lessening turbidity, which was further diminished by centrifuging at 3500 rev./min. for 30 min. It also served to maintain the haemoglobin in solution as alkali haematin, the change from the reddish yellow of the diluted blood to greenish brown being immediate on adding the strong alkali, no further colour change being apparent on warming.

The extinction coefficients of the extract and diluted blood were then determined from 750 to 434 mμ and the region of marked change between 494 and 434 mμ selected for comparison. The limitation of the extinction measurements to this region helped to eliminate the effect of increasing light scatter with diminishing wave-length arising from the slight turbidity of the extract. This effect, which tends to increase the measure-

ment of the circulatory space, can be taken as comparatively small, since the turbidity gave only an apparent extinction of 0·1 at wavelength 729 mμ (the corresponding diluted blood value being practically zero).

Results. The results are summarized in Table I. The gross values obtained for the vascular space range from 2·1 to 3·2 ml./100 g. muscle with a mean of 2·8 ml./100 g. From this we may subtract 0·5 ml./100 g.

TABLE I

No. of exp.	Total wt. of sartorii g.	No. of blood samples	Total dilution of muscle in extract ml./g.	Differences between extinction coeffs. for mμ 434 and mμ 464		Circulatory space in muscle ml./100 g. gross values
				Extract	Diluted blood	
1	0·81	6	18·7	0·25	0·47	2·1
2	0·70	2	21·6	0·27	0·43	2·7
3	0·79	2	19·2	0·29	0·43	2·6
4	1·00	2	15·4	0·39	0·38	3·2
Results on leg muscles after 2 hr. perfusion with Ringer fluid						
5	1·03	—	15·0	0·10	(0·43)	0·7
6	1·04	—	14·8	0·04	(0·43)	0·4

In experiments 1–4, twelve sartorii from six frogs were taken for each experiment. Experiments 5 and 6 give results on single frogs, the bracketed figure for the blood value being the average for previous experiments.

determined on leg muscles from three frogs after perfusion with Ringer fluid for 2–4 hr. and representing the error arising from the muscle haemoglobin as well as that from increasing scatter, due to slight turbidity of the extract, on passing from 464 to 434 mμ. The mean circulatory space of excised sartorii appears therefore as 2·3 ml./100 g. muscle.

DISCUSSION

The interfibre spaces of excised and immersed sartorii as given by inulin and magnesium experiments are 9·6 and 8·2 ml./100 g. respectively and may be taken as 9–10 ml./100 g. when there is no weight change on immersion. The spaces as measured from the diffusion of sodium and chloride into isotonic glucose are 13 and 12·0, and the figure from Donnan calculations applied to chloride is 12·7. These figures indicate a small but real difference between the space occupied by inulin and magnesium on the one hand and chloride on the other. This corresponds to 3 ml. plasma chloride, but may be somewhat less, when we consider that for the four inulin experiments the values ranged from 7·0 to 13·2. The difference is possibly attributable to the circulatory space, assuming that inulin or magnesium does not penetrate appreciably into the capillaries within the

time considered. The experiments of Lavietes, Bourdillon and Klinghoffer (1936) may be cited in support of a delayed equilibrium (over 1 hr.) of such substances as sulphate, and hence magnesium and inulin. As against this there is the fact that the glomerular capillaries and capsule allow even inulin through as fast apparently as water. Though a distinction must be drawn between movement of a solute as part of the whole solution and diffusion across gradients, yet the passage of inulin appears to be of the freest kind through the renal capillaries and the capsular membrane; but the latter retains plasma proteins whereas the capillaries in general do not.

There are also the experiments of McCance [1938], which show that inulin penetrates rapidly into the extra-cellular spaces, no constant difference being determinable between observations made 30 min., and those made 150 min. after injection. Preliminary experiments made here with inulin injections into rabbits show also that inulin equilibria across the general capillary circulation is reached in less than 30 min. Yet it is possible, but unlikely, that such considerations may not apply to the muscle capillaries of the frog, and inulin or magnesium equilibria may be long delayed. It may, however, be safely assumed that these substances will not pass across the membrane of red corpuscles trapped in the circulatory space of the muscle, and this could account for about one-third of the difference between chloride and inulin, leaving the equivalent of about 2 ml. space to be explained. An adsorption explanation of such chloride is apparently outruled by a consideration of the consequent high charge densities on the fibre surface, but what may be considered as a probable cause of the small discrepancy is given subsequent to the following considerations.

While the difference between the interfibre space values determined with inulin diffusion into immersed sartorii and chloride diffusion from fresh excised muscle into isotonic glucose is comparatively small, it is very marked if we use the chloride in the sartorius immersed for some time in Ringer fluid, and consider this as existing external to the fibres. Such chloride (per 100 g. muscle) has been shown to reach 26% of the external concentration within 15 min. [Conway & Kane, 1934], or the original ratio is almost doubled. Here the extra chloride must have passed into the fibres and a quantitative explanation of this movement is given in a subsequent paper.

Considering this chloride entrance, the conditions producing it are probably initiated on the death of the animal or after the excision of the muscle when the circulatory space drops from a probable figure of 7% to one of 2%. During this collapse of the circulation some of the blood

chloride and sodium may enter the fibres and a larger amount be present therein than the expected value from Donnan calculations. This will result in the calculation of the interfibre space being too high. Yet this should not affect the conclusions drawn from Fig. 4, since the chloride free in the interfibre spaces will diffuse out rapidly and the amount be approximately determinable from the diffusion curve. However, if we consider the curves of diffusion in Fig. 4 the dotted straight line projections on to the ordinate are probably not quite correct, and should be somewhat curvilinear, giving slightly lower values for zero time.

Calculations with respect to Donnan equilibria

In such calculations we assumed certain points about the permeability of the muscle membrane which are demonstrated in a subsequent paper. Concerning the free water for solution, it may be said here, that as judged from the equilibrium concentration of urea, this is practically identical with the total water present, or about 80 ml./100 g. [Eggleton, 1930; Conway & Kane, 1934]. A similar result (77 ml./100 g.) was obtained by Hill & Kupalov [1930] from measurements of the vapour pressure. If we interpret the 'osmotically active water' as the free water for solution behind the external membrane of the muscle fibres, then we must take the measure of this as the total free water minus the water in the interspaces. The actual demonstration of the volume of this water by changes in muscle volume on varying the concentration of the external solution depends on the exact permeability of the membrane, and the equilibria established or approached under the conditions studied. It is only by a realization of the facts presented in a later paper that the exact nature of this problem can be appreciated. It will be shown that the 'osmotically active water' must be considered as not appreciably different from the total water minus the interspace water, and this we may term the 'fibre water'.

Calculation of concentrations in the 'fibre water'

When a substance is present only within the fibres the concentration may be obtained by dividing the amount per kg. muscle by the amount of 'fibre water'. Since the interspace fluid, from the above considerations with respect to inulin and chloride, has a mean value of 0·10–0·13 l./kg. muscle and the total free water is either 0·80 or 0·77, we could consider 0·64–0·70 l./kg. as the free water for solution within the fibres. Taking a mean of 0·67, we may interpret this as corresponding to 0·13 l. interfibre water and 0·80 total free water, or alternatively as 0·10 interfibre water and 0·77 total free water (the latter figure in accordance with

the estimate of Hill & Kupalov, 1930). Although such a mean figure may be in error by 0·03 l./kg. this is scarcely significant for the determination of concentrations in the 'fibre water' with respect at least to investigation of potassium accumulation and the like, as described later. For a substance, therefore, present within the fibres only and not in the external solution we may write

$$C_f = C_m/0{\cdot}67,$$

where C_f may be regarded as millimols/l. of 'fibre water' and C_m as millimols/kg. muscle. If the substance is present also in the external fluid this becomes

$$C_f = (C_m - 0{\cdot}13\, C_0)/0{\cdot}67,$$

taking 0·13 l./kg. as the interfibre water, C_0 being the millimols/l. of external solution.

Summary

1. The mean inulin 'space' in frogs' sartorii immersed in inulin-Ringer solution was found to be 9·6 ml./100 g. muscle. Individual experiments gave values ranging from 7·5 to 13 ml.

2. The interfibre space for free magnesium diffusion in immersed sartorii was found to be 8·2 ml./100 g. (or calculated as 9·2 ml. when there is no change of muscle volume after immersion).

3. From a study of the diffusion of sodium chloride from muscle in isotonic glucose the sodium chloride outside the fibres represents a mean of 12–13% of the external plasma value (the total chloride being 14%). The figure, however, may be as low as 10% for reasons given in the Discussion.

4. The circulatory space in excised sartorii has a mean value of 2·3 ml./100 g.

5. With the fibre membrane permeable to potassium and chloride (as shown in a later paper) the theoretical chloride within the fibre is only 1·1% of the external value, and the experimental evidence, based either on the balance of the chloride quantities, or on the shape of the chloride diffusion curve into glucose appears to agree with this figure.

One of us is indebted to the Irish Medical Research Council for a grant in aid.

REFERENCES

Barkan, Gg., Broemser, Ph. & Hahn, A. [1921]. *Z. Biol.* **74**, 1.
Conway, E. J. [1935]. *Biochem. J.* **29**, 2221.
Conway, E. J. [1939]. *Micro-diffusion Analysis and Volumetric Error.* London: Crosby Lockwood.
Conway, E. J. & Cruess-Callaghan, G. [1937]. *Biochem. J.* **31**, 828.
Conway, E. J. & Fitzgerald, O. [1940]. Unpublished data.
Conway, E. J. & Kane, F. [1934]. *Biochem. J.* **28**, 1769.
Eggleton, P. [1930]. *J. Physiol.* **70**, 294.
Eggleton, M. G., Eggleton, P. & Hamilton, A. M. [1937]. *J. Physiol.* **90**, 167.
Fahr, G. [1908]. *Z. Biol.* **52**, 72.
Fenn, W. O. [1936]. *Physiol. Rev.* **16**, 450.
Fenn, W. O. & Cobb, D. M. [1934]. *J. gen. Physiol.* **17**, 629.
Fenn, W. O., Cobb, D. M. & Marsh, B. S. [1934]. *Amer. J. Physiol.* **110**, 261.
Ghaffar, A. [1935]. *Quart. J. Physiol.* **25**, 229.
Hahn, L. A., Hevesey, G. Ch. & Rebbe, O. H. [1939]. *Biochem. J.* **33**, 1549.
Hill, A. V. & Kupalov, P. S. [1930]. *Proc. Roy. Soc.* B, **106**, 445.
Lavietes, P. H., Bourdillon, J. & Klinghoffer, K. A. [1936]. *J. clin. Invest.* **15**, 261.
McCance, R. A. [1938]. *J. Physiol.* **92**, 208.
Mond, R. & Netter, H. [1932]. *Pflüg. Arch.* **230**, 42.
Salit, P. W. [1932]. *J. biol. Chem.* **96**, 659.
Shohl, A. T. & Bennett, H. B. [1928]. *J. biol. Chem.* **78**, 643.
Urano, F. [1908]. *Z. Biol.* **52**, 483.
Winter, K. A. [1934]. *Z. ges. exp. Med.* **94**, 663.