CCXXXIV. DIFFUSION RATES OF ANIONS AND UREA THROUGH TISSUES.

By EDWARD JOSEPH CONWAY AND FRANK KANE.

*From the Department of Physiology and Biochemistry,
University College, Dublin.*

(Received July 27th, 1934.)

FOR diffusion to or from a semi-infinite medium bounded by one plane the following formula may be shown to apply

$$\frac{\text{Amount diffused}}{\text{Surface}} = 2 (c_0 - c_1) \sqrt{\frac{kt}{\pi}} \quad \dots\dots(1)$$

where c_0 is a constant concentration in an outside phase and c_1 a uniform concentration in the medium at time t , k being the diffusion coefficient. The formula applies also to a fully bounded medium if t be not too great. It is in fact approximately true up to 70 % of the final value for media of the type of the frog's sartorius which are bounded by parallel planes, and gives values for k which are then theoretically within 5 % of the true figure.

In using equation (1) it is supposed that the equilibrium concentration in the medium is the same as the external concentration c_0 . If however the equilibrium concentration is not c_0 but ec_0 where e is a factor, then the above formula must be written

$$\frac{\text{Amount diffused}}{\text{Surface}} = 2 (ec_0 - c_1) \sqrt{\frac{kt}{\pi}} \quad \dots\dots(2).$$

The reason for this is apparent if we consider a film of extreme thinness immediately underneath the surface of the medium. The concentration in this film may be regarded as attaining almost instantaneously the concentration ec_0 and the subsequent diffusion therefore takes place as if from this film, the "diffusion head"—as one may express it—being $(ec_0 - c_1)$.

Neglect of this latter consideration leads to an apparently large difference between the diffusion constants of phosphate and lactate anions (at relatively low concentrations) through muscle [Eggleton *et al.*, 1928; Stella, 1928]. This was explained by Eggleton *et al.* as being due to the expansion of the muscle fibres after a long period in Ringer solution. The phosphate diffusion was examined by Stella after 360-minute periods and the lactic acid diffusion by Eggleton *et al.* after 7½–52 minutes. It was supposed that swelling of the fibres (which was presumed to develop late) would diminish the interspace water in which the diffusion was rapid and cause a fall in the diffusion constant. A fall in the diffusion constant for lactate was shown to occur in the fatigued muscle and was attributed to a similar diminution in the lymph interspaces from swelling of the muscle fibres. This swelling should presumably occur from a rise in osmotic pressure inside the fibres. No evidence however was brought for such diminution in the interspace water in Stella's experiments, and furthermore no such diminution even in fatigued muscle could be demonstrated by G. Eggleton [1933] in subsequent experiments. There is in fact no explanation necessary as the diffusion constant for phosphate from the combined data of

Stella [1928], G. Eggleton [1933] and Semeonoff [1931] turns out to be almost identical with that for lactate through muscle when equation (2) is used. Also it will be shown below that sulphate and chloride diffuse in a very similar manner to phosphate and all four anions give the same diffusion coefficient through muscle when equation (2) is used, but widely different values when equation (1) is used.

Where other tissues (kidney, liver) were investigated for sulphate diffusion (in the presence or absence of cyanide) the diffusion coefficient was also the same as in muscle, although it may be noted that the equilibrium values for the diffusion were often very different. This general diffusion coefficient for the anions is, as will be shown, 6.0×10^{-5} cm.²/min., the standard deviation of the individual estimate being 1.3×10^{-5} .

For comparison with the anion diffusion that of urea was also investigated. As shown below urea has a much slower diffusion rate than that of the anions considered, the value of the coefficient being 1.1×10^{-5} , although urea has a smaller molecular weight than three of these anions.

For convenience of calculation equation (2) may be written in the following form for tissue diffusion:

$$\text{Ratio of equilibrium value reached} \times \frac{\text{Weight in g.}}{\text{Surface in cm.}^2} \times 0.843 = \sqrt{kt}.$$

This is derived from equation (2). (In the derivation it is assumed that concentration per unit weight of tissue may be converted into concentration per unit volume by multiplying by 1.05.)

The weight to surface relationship may be read off from curves for the individual tissue, or derived from the following formulae for the sartorius or kidney of the frog:

$$\frac{\text{Weight}}{\text{Surface}} = \text{Wt.}^{\frac{1}{3}} \times 0.0844 \text{ (sartorius).}$$

$$\frac{\text{Weight}}{\text{Surface}} = \text{Wt.}^{\frac{1}{3}} \times 0.0858 \text{ (kidney).}$$

Methods of investigation.

The general procedure was the same as that described in the previous communication. The sartorii, kidneys or liver sections from the frogs were washed for 30 minutes as a rule in Locke's fluid and then immersed in sulphate-Locke, being stirred by a stream of oxygen. The rate of emergence of sulphate from the freshly excised kidney was also investigated. The analytical procedure for sulphate was the same as that previously described.

The chloride diffusion into the sartorius muscle was examined in the following manner. For each experiment four frogs were taken and a sartorius from each removed for analysis—the four sartorii so taken being analysed in bulk. The four twin sartorii were then immersed in Locke's fluid, containing however 0.7 % of sodium chloride, and stirred with oxygen for 2–5 minutes when they were removed, dried at once on filter-paper, the excess fluid being removed by gentle pressure between filter-papers, and then taken for analysis. The equilibrium values were obtained by immersion of other groups of muscles for 30- or 60-minute periods in the same Locke fluid, the frogs being taken from the same consignment. The chloride analyses were carried out (after weighing) by firstly grinding in an agate mortar with 1 ml. of water with the addition of a little of the purest quartz sand. The grinding was sufficient to reduce the muscle to a thin paste. 1 ml. of 3.0 % sodium tungstate was now added and stirred and subsequently 1 ml. of 0.2 *N* sulphuric acid. The same proportions of tungstate

and sulphuric acid were therefore used as in the well-known Folin method [Folin and Wu, 1919] of blood analysis. The mixture was transferred to a 15 ml. centrifuge-tube and centrifuged for a short time. 2 ml. of the clear fluid were taken for the chloride analysis which was carried out as follows. The chloride was converted entirely into chlorine at room temperature by a suitable acid-permanganate mixture in the outer chamber of the special absorbing unit previously described [Conway, 1933], the chlorine being absorbed in 20 % potassium iodide in the central chamber of this apparatus. The iodine liberated after full absorption of the chlorine ($1\frac{1}{2}$ hours) was titrated with standard thiosulphate using most conveniently the micro-burette described in a previous communication [Conway, 1934]. Suitable controls were carried out in all cases.

Sulphate diffusion into tissues.

After a preliminary washing of excised tissues in ordinary Locke for 30 minutes the values for sulphate found on analysis ranged from 0.4 mg. S per 100 g. in the kidney to 4.0 mg. per 100 g. S in the liver. Prolonged washing did not change this value for the kidney and it was considered—as noted in the last communication—that these values very probably do not represent free inorganic sulphate and have only an analytical significance. They were treated as blank values and the true concentration of free inorganic sulphate in the tissues after 30 minutes' washing was taken as zero. Subsequently to this preliminary washing the tissues were immersed in sulphate-Locke solution containing 50 mg. S—or 1.56 millimols—per 100 ml. Equilibrium with respect to sulphate is practically reached after 15 minutes as shown in the previous communication. Up to 5 minutes equation (2) may be used for calculating the diffusion coefficient, there being no appreciable fall from the value obtained at 2 minutes as may be seen from Table I (for chloride and sulphate).. For muscle

Table I.

Tissue	Substance	No. of obs.	Average weight in mg.	e	Diffusion head ($ec_0 - c_1$) ($\times 10^2$)	t mins.	Mean % of equil. value reached	Mean value of k ($\times 10^5$)
Kidney (in cyanide)	Sulphate	4	61	0.58	0.90	2.0	40	6.7
Sartorius	Chloride	4	67	0.30	2.73	2.0	35	5.6
Sartorius	Sulphate	4	93	0.25	0.39	2.5	38	5.4
Kidney	Sulphate	4	48	0.58	0.90	5.0	73	6.5
Sartorius	Sulphate	5	92	0.25	0.39	5.0	56	6.9
Sartorius	Chloride	2	70	0.30	2.73	5.0	56	6.0

In the table e is the equilibrium concentration (per unit volume) reached expressed as a ratio of the external concentration. c_0 and c_1 are the external concentration and the tissue concentration at zero time expressed as millimols per 100 ml. (For the calculation of the diffusion coefficient it is immaterial whether we express quantities as g. or millimols.)

the diffusion coefficient calculated after 5 minutes would seem greater than after 2.5 minutes but the difference is within the sampling error. Table II gives a summary of the data obtained for sulphate diffusion with the data for other anions. The mean value of 39 estimations of the diffusion coefficient of sulphate through tissues at room temperature (20°) is 6.3×10^{-5} , or 6.1×10^{-5} omitting two aberrant results, the standard deviation of the individual estimate being then 1.3×10^{-5} . The diffusion coefficient was found to be independent of the tissue investigated whether muscle, liver or kidney.

Table II.

Tissue	Substance	No. of obs.	Millimols		e	$(ec_0 - c_1)$ millimols per 100 ml.	k from equation (1) ($\times 10^5$)	k from equation (2) ($\times 10^5$)
			$\frac{\text{per}}{\sqrt{t}}$ ($\times 10^5$)	$(c_0 - c_1)$ per 100 ml.				
Sartorius	Chloride	6	23.5	12.1	0.30	2.73	0.27	5.7 (3.0-8.2)
Kidney (in cyanide)	Sulphate	8	8.2	1.56	0.58	0.90	2.22	6.6 (5.0-10.7)
Kidney	Sulphate	4	6.1	1.56	0.45	0.70	1.21	6.0 (5.8-6.7)
Liver	Sulphate	3	3.8	1.56	0.29	0.45	0.47	5.6 (5.0-6.6)
Liver (in cyanide)	Sulphate	2	3.3	1.56	0.25	0.39	0.36	5.7 (6.2-5.2)
Sartorius	Sulphate	9	3.3	1.56	0.25	0.39	0.39	6.3 (2.9-13.5)
Sartorius (in cyanide)	Sulphate	6	3.2	1.56	0.23	0.36	0.33	6.3 (5.5-7.5)
Leg muscle	Phosphate	8	0.66	—	0.29	0.075	0.51	6.0
Kidney	Sulphate	7	-0.082	-0.091	—	-0.091	6.4	6.4
Leg muscle	Phosphate	8	-3.43	—	0.29	-0.393	0.51	6.0
Leg muscle	Lactic acid	8	-8.9	-1.05	—	-1.05	5.6	5.6 (3.2-6.9)

The data for phosphate diffusion were taken from Stella [1928], the values at the ends of his curve being taken as illustrating the widest positive and negative diffusion for resting muscle investigated by him in that communication. The e value for phosphate, 0.29, has been discussed in the text. The data for the lactic acid diffusion were taken from the figures of Eggleton *et al.* [1928]. The k value in the penultimate column for phosphate is Stella's calculation.

Diffusion of sulphate from tissues.

This was investigated for the freshly excised kidney. A number of such kidneys were analysed for sulphate and others from the same batch of frogs were washed in Locke's fluid for varying periods and subsequently analysed. A summary of the data is given in Table III. The average value for sulphate

Table III.

Time mins.	Sulphate in kidney.	No. of analyses	Average weight of tissue analysed mg.	Average weight of kidney mg.
	Mean values as mg. S per 100 g.			
0	3.3 ± 0.3	17	155	42
2	1.9 ± 0.3	7	111	57
30	0.4	2	166	42
60	0.3	2	145	36

in the normal kidney is 3.3 ± 0.3 mg. S per 100 g. (mean of 17 observations). After 2 minutes' washing this is reduced to 1.9 mg. S per 100 g. (mean of 7 observations, average kidney weight being 57 mg.). After prolonged washing the value is 0.4 mg. S per 100 g. This as before mentioned is considered as a blank value. From the data in Table III the diffusion coefficient is calculated as 6.4×10^{-5} , or practically the same as that for the entrance of sulphate. This in itself provides an interesting example of the validity of equation (2) in calculating diffusion coefficients through tissues. For diffusion of sulphate from the tissues into Locke's fluid it is immaterial whether we use equation (1) or (2) since the external solution has practically zero sulphate concentration. We get

then with either formula 6.4×10^{-5} . For diffusion inwards on the other hand if we used equation (1) we should obtain 0.95×10^{-5} whereas with equation (2) we get 6.1×10^{-5} . From this illustration alone the correctness of equation (2) is apparent since the diffusion rates in either direction must be identical (except in the special case which does not arise here of a large internal storage).

Diffusion of chloride into muscle.

The manner in which this was carried out has been described above. The large number of data for sulphate diffusion were collected in the course of the previous investigation. In regard to chloride it was considered sufficient here to demonstrate its diffusion coefficient through muscle. Table IV gives a summary

Table IV.

NaCl at zero time g. per 100 g.	NaCl after immersion in Locke containing 0.74 % NaCl g. per 100 g.	Time of immersion in minutes	Average weight of single sartorii after immersion mg.
0.051	0.109	2	72
0.044	0.109	2	79
0.065	0.124	2	62
0.070	0.110	2	57
0.082	0.150	5	72
0.053	0.157	5	69
0.057	0.232	30	47
0.061	0.221	30	42
0.051	0.227	30	45
0.061	0.220	30	61
—	0.220	60	53
—	0.207	60	43

The analyses of the first two columns are for twin sartorii, two sartorii from two frogs being analysed after excision and the remaining two after immersion for varying periods in Locke solution containing 0.74 % NaCl at room temperature.

of the data obtained. The mean value of the six sets of determinations of the diffusion coefficient (for the 2- and 5-minute periods) is 5.7×10^{-5} . This value is thus the same as that for sulphate within the limits of sampling error. The distribution of the individual values is also similar. The value obtained from equation (1) is 0.27×10^{-5} .

It may be noted also that the chloride diffuses from the external Locke fluid into the muscle until it reaches an equilibrium figure 30 % of that of the external chloride. The sulphate diffused up to 25 %. The average value for the chloride in the frog's muscle as taken directly from the frog was found to be 0.060 %, expressed as sodium chloride. After 30 or 60 minutes' immersion in the Locke fluid containing 0.74 % total chloride the average value was 0.221 %.

Comparison of the rates of chloride and sulphate diffusion with those of lactic acid and phosphate.

The data for lactic acid diffusion were taken from the paper by Eggleton *et al.* [1928]. In their technique frog's legs were skinned, stimulated in nitrogen and then immersed for the diffusion period or periods in bicarbonate-buffered oxygen-free Ringer solution, the amount diffused being determined by analysing the fluid, the muscles themselves being finally analysed for lactic acid. It is immaterial whether we use equation (1) or (2) in determining the k value for lactic acid here as the external concentration is zero. The diffusion coefficient

falls after a lactic acid concentration of 1.08 mg. per ml. (In Table I of their paper the authors have given the percentage values of lactic acid in muscle instead of the mg. per ml. mentioned at the head of the column.) The authors have summarised the k value for low initial concentrations as "about 6.0×10^{-5} ." The diffusion coefficient of lactate is therefore practically identical with those for sulphate and chloride.

The values for phosphate diffusion have been taken from the data of Stella [1928]. He immersed frogs' legs in Ringer containing varying quantities of phosphate and measured the phosphate diffused in or out of the muscle after 6-hour periods. The diffused amounts were calculated from the analyses of the Ringer's fluid. When the concentration of phosphate in the Ringer fluid is such that no diffusion of phosphate occurs into or out of the muscle then the diffusion head ($ec_0 - c_1$) must be zero, c_0 being the concentration in the Ringer fluid and c_1 in the muscle, e being a factor already considered. If we consider any change from this particular phosphate concentration in the Ringer fluid, then we may write the diffusion head or effective diffusing concentration as ec' where c' is the change of this Ringer-phosphate concentration. The values of e from the figures given by Stella for prolonged immersion are as high as 0.55. G. Eggleton [1933] has pointed out that such are certainly too high owing to the diffusion of phosphate from the bone. From her data the e value would be 0.21. From Semeonoff's data [1931] on the isolated sartorius the e value is 0.38. Taking the mean of Eggleton's and Semeonoff's figures e is 0.29. This is close to the values obtained by us for sulphate and chloride, namely 0.25 and 0.30 respectively, and thus has this added probability of being the most representative figure. Using this value of e and Stella's data for phosphate diffusion in muscle, from equation (2) we get 6.0×10^{-5} as the corrected value for the diffusion coefficient. This value is again the same as those of the anions previously considered.

For the four anions, chloride, sulphate, lactate and phosphate, we have then the mean values of the diffusion coefficient through the tissues of the frog at room temperature, 5.7×10^{-5} , 6.1×10^{-5} , 5.6×10^{-5} and 6.0×10^{-5} , which values differ from each other no more than one might expect from the sampling error. It may also be noted that diffusion coefficients are proportional to the square of the amount of substance diffused, and any error or divergence in this is therefore of considerable influence in the calculated coefficient.

The diffusion of sulphate was examined for three different tissues, sartorius, liver and kidney, the mean values being 6.1×10^{-5} , 5.6×10^{-5} and 6.0×10^{-5} , which are again the same within the expected error of sampling. It is also immaterial whether the sulphate diffuses inwards to 58 % of the external concentration as with the kidney in cyanide or to 23 % as with the sartorius in cyanide; the diffusion coefficient remains the same, *viz.* 6.6×10^{-5} and 6.3×10^{-5} respectively. We are therefore concerned here with a general diffusion coefficient for anions which remains the same under a variety of conditions.

The equivalence of these k values for the different anions turns on the use of the factor e and equation (2)—the use in other words of the conception of the diffusion head as $(ec_0 - c_1)$ and not as $(c_0 - c_1)$. The validity of this conception is again apparent from Fig. 1 where the amount diffusing per cm.² divided by the $\sqrt{t}$ is plotted against the values of $(ec_0 - c_1)$. In introducing $\sqrt{t}$ we are in effect considering the amount diffused in the first unit of time reckoning from the beginning of the diffusion period. The amounts diffused then in this first unit of time and through unit surface lie closely along a straight line passing through the zero point—as shown in Fig. 1. The points in the figure are for three different tissues and four different anions. The quantities diffused plotted

against the values of $(c_0 - c_1)$, on the other hand, show no relationship whatever as may be gathered from an inspection of the penultimate column in Table II, which also gives the data from which Fig. 1 has been drawn.

Fig. 1. The diffused quantities of the four anions per unit surface and time when plotted against the diffusion head ($ec_0 - c_1$) lie closely on a line which passes through the zero point. The same values plotted simply against the difference between the internal and external concentrations ($c_0 - c_1$) show no relationship whatever.

○ Chloride through muscle.	○ Sulphate through muscle.
+ Sulphate through kidney.	● Phosphate through muscle.
x Sulphate through liver.	⊕ Lactate through muscle.

The diffusion of urea through the frog's muscle (sartorius) and kidney.

The method for analysing the urea has been described in detail in a previous communication [Conway, 1933]. For the present purposes it has been extended to the analysis of urea in the tissues. Certain discrepancies have been found between the results of the xanthidrol method and the urease method in the analysis of suspensions of kidney tissue [Rehberg and Clem, 1932; Kirk, 1933]. The experiments to be described show in the clearest manner that all the urea in the kidney as determined by the method used here exists in the same freely diffusible condition as in muscle and that all the urea in muscle is present in free solution, diffusing into and out of muscle at the same rate and having the expected equilibrium value in the tissue-water. We do not consider it essential therefore to discuss in detail in the present communication the alternative methods or their results, but to put forward what we consider to be the conclusive nature of the present findings.

Method. In the analytical method used the kidneys or sartorii, usually about 50–300 mg. in weight, were removed to a small agate mortar and ground with 3 ml. of water accurately measured, a little of the purest quartz sand (Merck's) being used to facilitate the grinding of the tissue quickly to a fine paste. The mixture was then centrifuged, 1 ml. taken for the urea analysis and 1 ml. for the ammonia analysis. It may be noted that in this centrifuging and removal of an aliquot part of the clear fluid we have at least one important difference in technique from the urease method as used by Rehberg, who analysed the tissue suspension without centrifuging. It may be readily shown that the diffusion of urea from the particles of the suspension is a matter only of some seconds and the kidney suspension was thoroughly ground for a few minutes. The 1 ml. of the clear fluid taken for analysis was transferred to the outer chamber of the absorbing unit previously described [Conway, 1933], the

inner chamber containing 1 ml. of 0.001 *N* sulphuric acid. 0.5 ml. of a glycerol-water extract of jack bean diluted with a phosphate buffer solution was added to the outer chamber and incubated for 15 minutes. 1 ml. of saturated potassium carbonate was added to the fluid in the outer chamber in the manner already described and the unit incubated at 37°, the acid in the inner chamber being titrated after 75 minutes, most conveniently with the special burette described in a previous communication [Conway, 1934]. The 1 ml. portion used for the ammonia analysis was treated in an exactly similar manner without the addition of the urease solution. Suitable controls and blanks were carried out in all experiments.

As the amount and concentration of the urease used appear to be of importance in relation to the question of the extra ammonia coming from the enzyme preparation itself, as indicated by preliminary experiments carried out in this laboratory, the constitution of the extract used in the experiments described above was 10 g. of jack bean meal to 50 ml. of glycerol and 17 ml. of water, the whole diluted 20 times with a dilute phosphate buffer solution, 0.5 ml. of this diluted solution being used to about 20–100 mg. of tissue contained in 1 ml. of a centrifuged suspension. With this method and urease solution all the urea analysed in kidney or muscle was of a freely diffusible kind and passed out of the tissue at exactly the same rate as free urea in solution diffused inwards.

Urea in the blood and tissues of normal frogs.

As shown by Marshall and Crane [1924] the concentration of urea in the normal frog's kidney and liver far exceeds that in the sartorius or that in the blood. (This was also demonstrated by Höber [1932] for the perfused kidney.) The absolute quantities of urea vary in different consignments of frogs. We are not concerned however with giving a very general average so much as with the relative quantities. For one consignment of frogs 12 corresponding analyses of the blood, sartorius, kidney and urine were carried out. Neglecting one of these sets in which the blood result is aberrant, we have for the series the following mean values in mg./100 ml.

Blood (whole)	...	...	...	45 ± 2.5
Sartorius	...	...	...	41 ± 2.8
Kidney	...	...	...	85 ± 3.6
Urine	...	...	...	140 ± 12.1

The *plus* and *minus* values give the probable errors of the means. The ratios of the means in the above order are 1.0 : 0.91 : 1.89 : 3.21. (Practically identical ratios are reached if for each set we divide the blood value into the others and take the means of these results.) The value for the sartorius is such as we may expect to be reached by passive diffusion equilibrium between the muscle-water and that of the blood.

Another group of five sets was carried out on corresponding samples of blood, sartorius, kidney, urine, liver and bile, giving the following series expressed as before in mg./100 ml.

Blood (whole)	...	...	53 (39–65)
Sartorius	...	...	48 (38–52)
Kidney	...	...	97 (76–100)
Urine	...	...	160 (106–228)
Liver	...	...	147 (118–170)
Bile (gall-bladder)	...	...	225 (57–440)

(The figures in brackets give the range.) The ratio of the blood-urea to that in the other tissues is here again approximately the same. The liver and bile are even richer than the kidney and urine in urea. As before the sartorius has a mean value 0.91 times that of the blood.

The diffusion of urea through the frog's muscle.

In this diffusion we must clearly distinguish between the rate at which urea diffuses and the final equilibrium value reached. The latter is such as we may expect from the urea diffusing freely into all the water of the tissues. This has been shown previously by P. Eggleton [1930] for Ringer-urea solutions in which sartorii muscles were immersed. He determined the urea as nitrogen using the companion sartorius for a correction in regard to other non-protein-nitrogen. Taking the water of the muscle as 80 % by weight the mean value of the urea concentration at equilibrium in this water from Eggleton's data is 1.02 ± 0.05 , expressed as a multiple of the external concentration. This corresponds with the results obtained by us in respect to the equilibria between normal blood-urea in the frog and that in the sartorius. We may take it then that the urea in muscle reaches a passive equilibrium with the urea in an external solution, the concentration in the tissue-water being the same as in the external fluid. (This same relation also holds we find for the normal frog's kidney when immersed in cyanide-Locke solution containing urea. It does not hold for the active isolated kidney in which the equilibrium concentration may greatly exceed that of the external fluid.)

Since the water in the muscle occupies about 84 % of the total volume, the analytical results at equilibrium will give an e factor of 0.84. In such a case we may use equation (2) with this e value and regard the diffusion head as $(ec_0 - c_1)$ where c_0 is the external concentration and c_1 the muscle concentration. Strictly however the diffusion head is rather $(c_0 - \frac{c_1}{e})$ when we consider the diffusion into the water of the tissue. At the same time we must regard the diffusing surface not as the whole measurable surface but rather as this value multiplied by e . We have then for the diffusion equation

$$\frac{\text{Amount diffused}}{\text{Surface} \times e} = 2 \left(c_0 - \frac{c_1}{e} \right) \sqrt{\frac{kt}{\pi}},$$

or

$$\frac{\text{Amount diffused}}{\text{Surface}} = 2 (ec_0 - c_1) \sqrt{\frac{kt}{\pi}},$$

which latter has again the form of equation (2). For all such cases therefore this equation is valid.

In the experiments for investigating the diffusion coefficient of urea one of the sartorii or one of the kidneys from one or more frogs was transferred to Locke's fluid (or to a cyanide-Locke fluid) and stirred with a stream of oxygen in the manner already described. Such tissues were analysed after varying periods of time. The companion sartorii or kidneys were analysed immediately after excision. In some experiments both sartorii or both kidneys were washed for a preliminary period in Locke's fluid and one was then transferred to a urea-Locke fluid, the other being analysed for urea. In such experiments the diffusion occurs from without inwards. The results are given in Table V. The diffusion of urea is a much slower process than that of lactate, phosphate or sulphate. As before mentioned equation (2) is applicable up to about 70 % of the equilibrium value. This however is not reached for urea until after 30 minutes or more with the tissues examined. Fig. 2 gives the values of the amount of urea diffusing

Table V.

No. of frogs used	Mean urea % at zero time (mg. per 100 g.)	Time of diffusion mins.	Mean urea % after diffusion (mg. per 100 g.)	Mean % of equilibrium reached	Mean weight in mg.	Amount diffused per cm. ² Diffusion head ($\times 10^3$)	k from equation (2) ($\times 10^5$)
Tissue: Kidney:							
7	138	60	21	75	39	2.18	—
2	145	30	45	66	56	2.11	1.16
3	100	20	39	61	40	1.77	1.23
3	86	10	61	29	55	0.96	0.72
6	87	3.6	68	22	45	0.68	1.00
Kidney (in cyanide):							
8	110	60	21	81	41	2.43	—
5	100	20	38	62	42	1.86	1.36
4	155	10	98	37	29	0.96	0.72
7	129	3.3	99	23	47	0.71	1.19
Kidney (in cyanide and 0.08 % urea after 30 mins. washing (in ordinary Locke solution)):							
4	27	30	53	61	65	2.00	1.02
Sartorius:							
5	66	15	42	36	83	1.33	0.83
4	52	30	22	57	113	2.34	1.38
Sartorius (in 0.2 % urea after preliminary washing):							
4	38	5	66	21	130	0.90	1.14
2	28	60	128	70	104	2.80	1.09

With the kidneys the individual tissue was analysed separately, a single kidney from one frog being analysed after excision and its companion after the diffusion period. With the sartorii, single sartorii from the number of frogs mentioned in the second column were analysed together, duplicate analyses being made and the companion tissues being similarly treated after the diffusion period.

In the penultimate column quantities are reckoned in millimols and the diffusion head in millimols per ml.

Fig. 2. See text. The diffusion of urea from muscle and kidney follows the square root relationship and the values for both tissues lie along the same line.

- Kidney freshly excised.
- Kidney washed and transferred to a cyanide solution.
- Muscle freshly excised in Locke.
- × Kidney freshly excised in cyanide.
- + Muscle washed and immersed in urea-Locke.

per cm.^2 surface of muscle and unit diffusing concentration (unit diffusion head) against $\sqrt{t}$. The square root relation clearly holds. Fig. 3 is of considerable interest. It shows the amount of urea diffusing per unit surface in the first unit of time (obtained by dividing by the square root of the observed time) against the diffusion head of urea. The results lie along a straight line passing through the zero point. This means that the diffusion into or out of the muscle proceeds at the same rate—an expected result in this case.

Fig. 3. The diffused quantities of urea per unit surface and time—in or out of the tissues—lie on a straight line when plotted against the diffusion head. This line passes exactly through the zero point, and both muscle and kidney values lie along it. The significance of these results is discussed in the text.

- Kidney freshly excised in Locke.
- × Kidney freshly excised in cyanide-Locke.
- ⊗ Kidney washed and transferred to a cyanide-urea-Locke solution.
- Muscle freshly excised in Locke.
- + Muscle washed and immersed in urea-Locke.

The diffusion of urea through the frog's kidney.

When however we consider similar values (amounts per unit surface *etc.*) for the freshly excised kidney of the frog in which the urea concentration is about double that of the muscle or blood, the figures lie along the same line as for muscle. So also do the values for the urea diffusion into or out of the kidney immersed in cyanide. The diffusion experiments on the kidney were carried out in a similar manner to those on muscle. Collecting the data for the diffusion coefficients we have:

Tissue	Diffusion coefficient ($\times 10^6$) (from equation (2))
Kidney (freshly excised in Locke)	1.03
Kidney (freshly excised in cyanide-Locke)	1.09
Kidney (washed for 30 mins. in Locke and transferred to a cyanide-urea-Locke solution)	1.02
Sartorius (freshly excised)	1.10
Sartorius (washed for 30 mins. in Locke and transferred to a urea-Locke solution)	1.11

These figures are summarised from Table V. Using the correct form of the diffusion equation (equation (2)) no significant difference exists between the diffusion coefficients of urea diffusing either from the freshly excised active or inactive kidney or into the inactive kidney or to and from the sartorius muscle. The urea in the freshly excised kidney is however about twice that in the blood or in the sartorius muscle. All this "excess" urea diffuses out from the kidney as

fast as it diffuses into the inactive kidney when the equilibrium value is that to be expected in a purely passive equilibration.

We may combine these findings with those in Fig. 3 where the amount of urea diffusing in the first unit of time and per cm.² of surface is plotted against the diffusion head. The figures lie around a straight line passing from positive to negative values through the zero point, there being no apparent difference between the muscle and renal points. We may take it then that the following points are proved by these experiments.

(1) The "excess" urea over the blood value in the frog's kidney as determined by the above method exists in a form as freely diffusible as the urea from an external fluid and as the urea in muscle.

(2) No appreciable amount of this excess urea as above determined is bound in the active kidney.

(3) The freely diffusing urea concentration in the frog's kidney freshly excised has here been shown to be about twice that of the blood value.

The significance of these results for tissue diffusion and renal theory need scarcely be emphasised. The experiments are simple and readily reproducible. It may be noted that the diffusion coefficients are proportional to the square of the amount diffused so that any variation in this quantity is very much increased in the calculation of the coefficient. The similarity of the figures obtained is hence all the more striking.

DISCUSSION.

At the outset we may define for convenience of description and in an empirical manner a term "permeation." By this is meant here that fraction of the total concentration in a tissue which would be reached if there were no barrier to free solution in the tissue water. For lactic acid formed within the muscle fibre, for example, this permeation is 100 %, but for sulphate entering muscle from an external sulphate-Locke solution it is 25/0.84 or 30 % (25 being the percentage equilibrium concentration of the tissue with respect to the external concentration and 0.84 the proportion of water in one volume of tissue). We may make a list of the following permeations with the corresponding diffusion coefficients.

	Permeation	Diffusion coefficient
Lactate (muscle)	100	5.6×10^{-5}
Sulphate (kidney in cyanide)	69	6.6×10^{-5}
Sulphate (kidney)	54	6.0×10^{-5}
Chloride (sartorius)	36	5.7×10^{-5}
Sulphate (liver)	35	5.6×10^{-5}
Phosphate (leg muscles)	35	6.0×10^{-5}
Sulphate (liver in cyanide)	30	5.7×10^{-5}
Sulphate (sartorius)	30	6.3×10^{-5}
Sulphate (sartorius in cyanide)	28	6.3×10^{-5}

The diffusion coefficient is thus independent of the degree of "permeation" (and it may be noted that the square of this value will enter into its calculation). From this it must follow that the interspace water has practically no influence on the observed diffusion rate. The diffusion rate of the anions through this interspace water is no doubt the same as through water itself, that is to say about ten times the observed rate through the tissues. With a permeation of 28 the interspace water must account for a considerable proportion of this and with a permeation of 100 for a comparatively small proportion. Yet for both these permeations the diffusion rate is nearly the same. We are forced therefore

to the conclusion that at least for these excised tissues the tissue elements govern the rate of diffusion and that all diffusion must be primarily conducted through them. The interspace water in such tissues would then exercise very little influence on the observed rate even if the velocity through such interspace water were practically infinite.

If the anion diffusion must pass through the tissue elements which are in contact how is the fall in the diffusion rate of lactic acid with rising concentration in stimulated muscle to be accounted for? This result was obtained by Eggleton *et al.* [1928] and explained by them as due to a diminution in the interspace water through which the diffusion was comparatively very rapid, the assumption being that there was a free passage of substance through the interspace water between the tissue elements and that these latter were not in contact. From the preceding considerations this view cannot be accepted. An explanation of the fall in the diffusion coefficient of lactate may be given as follows. If instead of the diffusion coefficient of lactic acid or anion we consider the amount diffused divided by $\sqrt{t}$, we have in effect the amount diffused in the first minute. If we plot this value against the lactate concentration in muscle we get a very striking relationship (see Fig. 4). The curve begins necessarily at

Fig. 4. This figure has been constructed from the data in the paper of Eggleton *et al.* [1928]. The dots show the concentration of lactic acid in stimulated muscle and the circles show the concentration in muscle dead at 37° (muscles R_1 and R_2 of their series).

the zero point, since with zero lactic acid in the muscle there must be zero diffusion. It then rises linearly till about 100 mg. per 100 g. lactic acid exists in the muscle. The amount diffusing then remains constant up to about 300 mg. of lactic acid. After this concentration in the stimulated muscle is reached there is a slight rise in the diffusion rate, the curve leading upwards for the high lactate values in dead muscle at 37°.

The reaching of a steady state may be explained when we consider that the cation impermeability makes the outer diffusion of anions dependent on a similar internal diffusion of anions from the external fluid. This latter must therefore impose a limitation at a certain stage. It may be possible that this

stage is reached at the beginning of the steady region of the lactic anion diffusion. At this stage it is to be noted that there also exists a large increase in free phosphate within the muscle and possibly of other diffusible anions. After 300 mg. lactic acid per 100 g. are reached in the internal concentration the cation impermeability begins, on this explanation, to break down, the process tending to completion with the dead muscles at 37°.

The passage of the anions through the cells makes the whole of the tissue-water available for solution, but owing to the operation of membrane equilibria we may get very much reduced total concentrations in the tissues compared with the external concentration. These membrane equilibria in the kidney seem to be very different from those in muscle. With the kidney in cyanide-Locke the permeation value for sulphate is 69 and with the sartorius in cyanide-Locke it is 28. However, for these two degrees of permeation, due no doubt to different degrees of membrane equilibria, the diffusion coefficients are 6.6×10^{-5} and 6.3×10^{-5} respectively—that is to say, the same within the limits of error for such determinations. The anion diffusion thus appears to be independent not only of the degree of permeation but also of the kind of tissue examined, and also it may be added of the chemical properties of the anion.

When we now consider the diffusion of urea we find that urea has a much smaller diffusion coefficient than the four anions considered. For diffusion into or out of muscle, into or out of the inactive (cyanide) kidney or out of the active kidney, the diffusion coefficient of urea is the same (within the limits of determination), namely 1.1×10^{-5} . Such an agreement in itself, as already mentioned, disproves the conception that all the “excess” urea in the frog’s kidney is in a bound condition. The permeation value of urea is 100. At first sight the low diffusion coefficient may be thought to be associated with this fact. That this cannot be so will appear when we have for lactic acid with the same permeation a diffusion rate 5–6 times faster and when the diffusion of sulphate into the inactive kidney is three times faster reckoned as a proportion of the external concentration (without a consideration of equilibrium values). The molecule of urea is also markedly smaller than three of these anions. We must therefore have a special facilitation of the passage of anions as compared with the passage of electrically neutral and even smaller and hence more rapidly moving molecules. This recalls the fact that the cations are on the other hand specially inhibited from diffusing through the cell membrane. It is possible that these are aspects of the same mechanism. It is well known that for large molecules only those which are lipid-soluble diffuse readily into the cell, from which it is concluded that the cell membrane is mainly of a lipid nature [*vide* Höber, 1932]. Small molecules however can readily enter the cell although they have little or no lipid solubility. This has been explained by a pore theory, the small molecules passing through the pores in the cell membrane. It is difficult to conceive of pores except in a solid membrane and that of the cell seems to be of a fluid nature since its function persists even with marked distension or distortion. Water droplets in a membrane of such fine dimensions may, however, serve all the purposes of pores in a larger membrane. Further, if these droplets were positively charged we should have at least a working picture of the discrimination towards the passage of the differently charged ions.

SUMMARY.

1. It has been shown, using a more correct form of the diffusion equation than that used by Eggleton *et al.* [1928], that the diffusion coefficient through the frog’s sartorius of the four anions, sulphate, chloride, phosphate and lactate,

is the same within the limits of sampling error. This value is 6.0×10^{-5} (cm.²/min.). The variation from this figure of a single estimate may be expressed as a standard deviation of 1.3×10^{-5} .

2. This diffusion coefficient, as exemplified by sulphate, is the same through the muscle, liver and kidney of the frog, and is independent of the degree of "permeation" of the anion.

3. The equilibrium concentration of chloride in isolated muscle expressed as a ratio of the external concentration is 0.30. This compares with 0.25 for sulphate and 0.29 for phosphate (this latter being the mean value of the results of Eggleton and Semeonoff who obtained 0.21 and 0.38 respectively).

4. With the urease method employed here [Conway, 1933] in an extensive series of experiments it has been shown that the "excess" urea analysed in the frog's kidney diffuses outwards at exactly the same rate as urea diffuses inwards from free solution, and furthermore that this rate of diffusion is identical with the diffusion outwards or inwards of urea in the frog's sartorius. It is clear therefore that the urea analysed in the frog's kidney by this method is in free solution. Such urea in the freshly excised kidney of the frog gave an average value of 85 mg. per 100 g., whereas the blood value was 45 mg. and the sartorius value 41 mg. per 100 g. The diffusion coefficient of urea through muscle or kidney is 1.1×10^{-5} .

REFERENCES.

- Conway (1933). *Biochem. J.* **27**, 430.
— (1934). *Biochem. J.* **28**, 283.
— and Byrne (1933). *Biochem. J.* **27**, 419.
Eggleton, G. (1933). *J. Physiol.* **79**, 31.
Eggleton, P. (1930). *J. Physiol.* **70**, 294.
Eggleton, Eggleton and Hill (1928). *Proc. Roy. Soc. Lond. B* **103**, 620.
Folin and Wu (1919). *J. Biol. Chem.* **38**, 81.
Höber (1932). *Pflüger's Arch.* **70**, 294.
— (1932). *Ann. Rev. Biochem.* **1**.
Kirk (1933). *J. Biol. Chem.* **102**, 683.
Marshall and Crane (1924). *Amer. J. Physiol.* **70**, 465.
Rehberg and Clem (1932). *Pflüger's Arch.* **230**, 698.
Semeonoff (1931). *Quart. J. Exp. Physiol.* **21**, 187.
Stella (1928). *J. Physiol.* **66**, 19.