THE PENETRATION OF AMMONIA INTO FROG MUSCLE

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These experiments were undertaken in order to discover to what extent ammonium behaves like potassium in skeletal muscle, and a preliminary report has already been made of the results obtained (Fenn, Haege, and Sheridan, 1942).

The general plan was simply to replace a part of the NaCl in Ringer's solution by NH_4Cl and to compare the composition of a muscle after immersion in this solution with the composition of its mate immersed under similar conditions in Ringer's solution. A few experiments were tried with other frog tissues, especially liver. The results have shown some tendency for the NH_3 to become concentrated in the muscles like K, but the inside-to-outside concentration ratios are very different for the two cations.

The ammonia enters mostly in exchange for K and Na. It does not take chloride in with it because there is no evidence of swelling of the muscle. This confirms the idea that the muscle membrane is permeable to cations but not to anions.

Methods

We have used mostly the small hind leg muscles of *Rana pipiens* including the sartorious, semitendinosus, ileofibularis, peroneus, and tibialis. Sometimes larger muscles such as the gastrocnemius have been used, but these have usually been cut longitudinally into 2 or 3 smaller pieces. About 0.7 to 1.0 gm. of muscle was soaked in 1.5 to 2.5 ml. of solution in small weighing bottles with ground glass and greased stoppers and equilibrated with O_2. These bottles were then rotated slowly around an axis inclined about 30° from the vertical at 19°C. or 5°C. for periods of time varying from 15 minutes to 5 days.

The control solution contained 0.65 per cent NaCl, 0.01 per cent KCl, 0.02 per cent $CaCl_2$, and $\frac{M}{150}$ phosphate buffers at pH 7.0. This was compared with the same solution with 1/5 of the NaCl replaced by an equivalent amount of NH_4Cl. The latter solution contained 22.8 m. eq. of NH_3 and had the same pH as the control solution within 0.1 of a pH unit. The two solutions will be referred to as the R and the N solution respectively.

Potassium was determined by a somewhat modified Shohl and Bennett

method (Fenn *et al.*, 1938). NH_3 was steam-distilled *in vacuo* by a simplification of the method recommended by Parnas and Heller (1924). The ammonia was caught in dilute H_2SO_4 (or boric acid) and titrated. By properly adjusting the pressure in the condenser to equal the vapor pressure of the solution, it was possible to run the distillation without losing any bubbles through the H_2SO_4 and without heating the solution.

The NH_3 content of muscles was determined in the same way after snipping up the muscles with scissors in a chilled alkaline borate solution and then transferring them to the distillation flask. Small muscles can be dropped into the borate solution whole. In some experiments the muscles were ground up in sand in the borate solution, as recommended by Parnas and Mozolowski (1927), but this permitted a loss of 10 per cent of the NH_3. In some of the earlier experiments, we made the mistake of adding strong alkali to the distillation flask, thereby forming some ammonia from the amide nitrogen present (*cf.* Schmidt, 1938). Since, however, this error was equal in both the control and the experimental muscle, the difference in NH_3 content was presumably unaffected and, indeed, the results obtained by this method (which will not be reported in detail because of this error) were nevertheless in good agreement with figures obtained by the correct procedure. The amount of NH_3 formed by the strong alkali amounted on the average to 8 m. eq. per kilo. of muscle.

1. Changes in the Weight of Muscles and Liver during Immersion

Weight changes in all our experiments are summarized in Table I. For purposes of comparison, the muscles are divided into four groups of more or less equal size. In each of these groups, the average weight of the muscles before and after immersion is given, and, in the last two columns, the percentage change in weight. The most essential point is that the presence of NH_4Cl in the solution does not alter significantly the movements of water. If anything, the muscles in the N solution lose more water or gain less than their mates in the R solution. It is evident, therefore, that the muscles are not like erythrocytes which swell in NH_4Cl because both anion and cation can penetrate, thus increasing the osmotic pressure inside (Jacobs, 1927). As a tentative conclusion, it may be said, therefore, that the muscle is impermeable to chloride. The same conclusion applies to liver, although there is a somewhat greater loss of water in the R than in the N solution. It is interesting to note that the small muscles, each weighing 100 or 200 mg., lost less weight than the larger muscles, weighing more nearly 1 gm. In series 4, the muscles were immersed in 100 ml. of solution instead of 1.5 to 2.5 ml., and the immersion was continued for 1 to 5 days rather than 5 hours as in the other series. In this greater interval, or perhaps because of the larger volume of solution, there was some swelling rather than shrinking of the muscles.

From these results, it is evident that the muscles were not seriously injured by the substitution of part of the Na by NH_4. They did not swell or go into rigor. The rheobase excitability was measured in many cases. In ten cases, the average rheobase voltage was 3 times as high in the muscle soaked in NH_4Cl solution as in the control. In spite of the NH_4Cl, however, these muscles did contract, although the threshold was somewhat higher. This confirms a loss of excitability previously reported in preliminary experiments (Fenn and Cobb, 1933).

TABLE I

Average Weight of Muscles and Liver before and after Immersion in Ringer's (R) and NH_4Cl-Ringer's (N) Solution

Series	Tissue	No. of muscles	Time	R		N		Per cent change	
				Before	After	Before	After	R	N
			hrs.	*mg.*	*mg.*	*mg.*	*mg.*		
1	Large muscles	20	5	1102	1086	1100	1073	−1.5	−2.5
2	Large muscles	21	5	1323	1290	1289	1261	−2.5	−2.2
3	Small muscles	13	5	846	840	852	846	−0.71	−0.70
4	1–5 day muscles	16	24–120	601	635	621	643	+5.7	+3.5
5	Liver	13	5	667	622	681	661	−6.8	−2.9

In series 1 and 2, the weights refer to single muscles, while, in series 3, the weights given are the sums of the weights of 4 or 5 small muscles.

2. *The Volume of Distribution of NH_3 in Muscle and Liver*

The volume of distribution is defined as that volume in which the NH_3 (as NH_4^+ or otherwise) would have to be distributed inside the tissue in a concentration equal to that in the ambient solution in order to account for all the NH_3 found in the tissue. When the tissue is immersed in a small volume of solution containing a known amount of NH_4Cl, the final distribution of this salt between the muscle and the solution is fully determined if the amount present in either the solution or the muscle is known by analysis. Thus, if the muscle is analyzed, the amount in the solution can be obtained by difference, and *vice versa*. In some experiments, we analyzed the solution and, in others, the muscle, and, in still others, we analyzed both tissues and solutions. A still simpler method which we followed in some cases was to immerse the muscles in a volume of solution so large that it did not change in concentration during the immersion. We usually used a smaller volume of solution, however, because we were interested in analyzing the solution for potassium escaping from the muscle, and we wished to make the change in concentration as large as possible.

The volume of distribution is calculated as follows. Let V equal the initial volume of the solution in milliliters and m the initial wet weight of the muscle

in grams. The initial concentration of the NH_4Cl in m. eq. per ml. is C_0, and the concentrations after immersion in the R and N solutions are C_r and C_n. The initial concentration of NH_4Cl in the control solution at the start is zero, but some NH_3 diffuses out of the control muscle during the immersion so that C_r has a definite value. Since the two muscles are nearly equal in weight, it is assumed that the preformed NH_3 in the experimental muscle will raise the concentration in the N solution to the same extent. If the two muscles differ appreciably in weight, a correction is made to take account of that fact. That fraction of the concentration of NH_3 in the N solution after soaking which is due to added NH_3 is therefore $C_n - C_r$. This concentration is less than C_0 because some NH_3 has diffused into some part of the muscle. The calculation is somewhat complicated by the change in weight of the muscle during immersion, but these changes are never very large so that a somewhat simplified calculation is justified. To meet this difficulty, it is assumed that if any water entered or left the muscle during immersion, that water will contain NH_3 in a concentration equal to C_n, and the calculation is carried out as if that amount of water were returned to its original position with its contained NH_3. The value will, therefore, be unchanged by immersion, and we may write

$$\frac{C_0}{C_n - C_r} = \frac{V + x}{V} \tag{1}$$

where x is the volume of muscle substance in milliliters in which NH_3 has diffused in a concentration equal to that in the solution. The volume of distribution in per cent is then given by the equation

$$\frac{100x}{m} = \frac{100V}{m}\left(\frac{C_0}{C_n - C_r} - 1\right) \tag{2}$$

The volume of distribution can also be calculated from the concentrations of NH_3 found in the muscles after the period of immersion. Let these concentrations be c_r and c_n. Then the concentration in the muscle due to the added NH_3 will be $c_n - c_r$, and the concentration left in the solution will be $(VC_0 - m(c_n - c_r))/V$. The volume of distribution will then be given by the equation

$$\frac{100(C_n - C_r)V}{VC_0 - m(c_n - c_r)} = \text{volume of distribution} \tag{3}$$

In this case, c_n and c_r were calculated per gram of initial weight of the muscles, so that if the muscles gained weight during the immersion, the calculated volume of distribution will be too large, and *vice versa*.

The results of a large number of experiments calculated by these two equations are summarized in Table II. The protocols of the individual experiments are not given chiefly because, with the exception of series 5, all the analyses

were made by the addition of excessive amounts of alkali to the distillation flask, so that there was some formation of NH_3 from amide nitrogen. It does not appear, however, that this error was very large when the solutions were being analyzed, nor does it appear that the values of $C_n - C_r$ or $c_n - c_r$ were changed by the addition of an equal amount to both muscles or both solutions.

In series 1, the muscles were soaked in Ringer's solution overnight in the cold room before use. They were then weighed and immersed in the R and N solutions for 3 to 5 hours at 19°C. The average of 22 experiments gives a volume of distribution of 112 per cent. Series 2 was similar except that the muscles were removed from the solutions at the end of the experiments, blotted,

TABLE II

Volume of Distribution of NH_3 in Muscle and Liver

Series	Tissues	No. of experiments	Average weight of muscles	Average volume of solution	NH_3 in solution mM per liter		Average volume of distribution	P.E. of mean
					R	N		
			gm.	*ml.*			*per cent*	
1	Muscle	22	1.24	2.07	1.41	15.36	112	3.4
2	Muscle	18	1.30	2.28	2.12	16.07	111	3.8
3	Liver	8	0.736	1.88	3.65	16.25	216	19.3
4	Liver	5	0.592	2.0	3.09	17.4	208	26
2	Muscle	18	1.30	2.28	9.87*	23.25*	88	3.9
4	Liver	5	0.592	2.0	12.7*	28.6*	80	21
5	Muscle	7	0.813	100	0.87*	15.75*	67	2.2

The weights given are the initial weights of the muscles in the N solutions. Concentrations refer to analyses made after immersion of the tissues. Concentrations in the tissues are calculated per gram initial wet weight of the tissues. Analyses were made both in the tissues and in the solutions. The numbering of the series in this table is not synonymous with that in Table I.

* NH_3 in tissue in millimols per kilo.

weighed, and analyzed for NH_3. The solution analyses, calculated by equation 1 gave a volume of distribution of 111 per cent, while the tissue analyses calculated by equation 2 gave a lower value of 88 per cent. The exact reason for the discrepancy between these two values is not known, but some of it is due to loss of NH_3 from the muscles during the process of grinding them in the borate solution. In two control experiments in which NH_4Cl was "ground" in borate in this way, the loss of NH_3 in the process amounted to 10.7 and 11.0 per cent respectively. In series 5, however, in which the muscle NH_3 was distilled from alkaline borate only, and a large volume of solution was used a still lower value of 67 per cent was obtained for the volume of distribution. This low value may perhaps be accounted for by the fact that in five of the seven experiments of series 5, the muscles were immersed at 5°C. instead of 19°C.

Series 3 and 4 of Table II represent similar experiments with pieces of liver in thin slices. In this case, the volume of distribution was 208 per cent and 216 per cent by solution analysis, and only 80 per cent by tissue analysis. This means that more NH_3 disappeared from the solution than could be accounted for by the NH_3 found in the tissues. This difference undoubtedly is real and is due to the formation of urea in the liver (Krebs and Henseleit, 1932). This was verified in one experiment by direct analysis of the liver for urea. Similar analyses were made in six of the muscle experiments. In these experiments, the R solutions contained urea after immersion in concentrations 1.7, 2.1, 3.7, 4.7, and 3.4 m. eq. per kilo respectively, while the corresponding concentrations in the N solutions were 0, 3.3, 2.2, 1.3, 3.5, and 4.6 m. eq. per kilo. On the

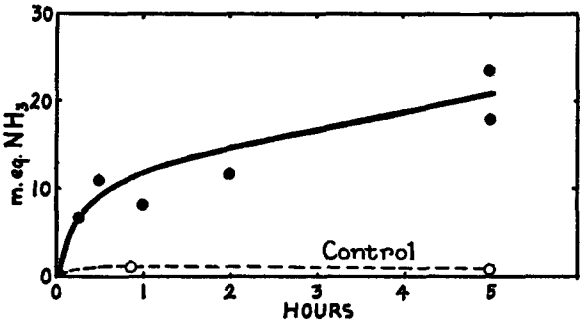

FIG. 1. Ammonium contents of muscles after immersion for various lengths of time in 100 ml. samples of R and N solutions at 5°C. and equilibration with pure oxygen and continuous rotation to insure gentle agitation. All the points marked by circles represent muscles obtained from the same frog, each sample weighing 600 to 800 mg.

average, therefore, the concentrations were 3.1 and 2.5 m. eq. per kilo respectively in the R and in the N solutions, and this difference is not significant. This confirms the finding of Krebs and Henseleit that urea is not manufactured by muscles from NH_3 and indicates that low values of the volume of distribution obtained by analysis of muscle for NH_3 cannot be explained by urea formation.

3. Time Course of the Penetration of NH_3

The previous experiments were carried out mostly after about 5 hours of immersion. At this time, the volume of distribution is about 100 per cent. Using the improved method of distilling from borate only, an investigation was carried out of the amount of NH_3 taken up at shorter and longer times. We first studied shorter times, and the results of these experiments are shown in Fig. 1. Ordinates represent mM of NH_3 per kilo of muscle. The NH_3 in the control muscle is uniformly about 1 mM per kilo and does not increase with

time. In the experimental muscle, however, exposed to a solution containing NH_3 in a concentration of 22.8 mM per kilo, the concentration increases in about 5 minutes to about 17 mM per kilo and subsequently increases more slowly and linearly until it reaches a value about equal to that in the solution at 5 hours. This finding confirms, therefore, the volume of distribution of 100 per cent found in the earlier, more numerous but less precise experiments. In interpretation of the curve of Fig. 1, it might be supposed that the initial rapid increase in the first 15 minutes represents the saturation of the extracellular space, while the subsequent slower rise is due to penetration of the muscle fibers themselves.

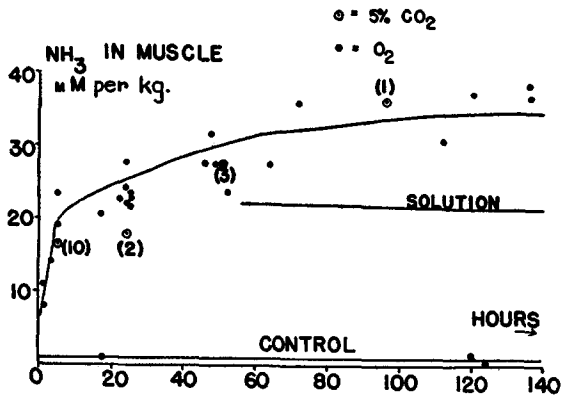

FIG. 2. Similar to Fig. 1, but extending over longer periods of immersion. Points marked by circles were obtained from muscles equilibrated with 5 per cent CO_2 and 95 per cent O_2. Figures on the graph represent the number of points which were averaged together to obtain the value plotted. The final concentration attained after 5 days is greater than the concentration in the solution represented by a horizontal line.

In Fig. 2 are plotted the results of other experiments in which muscles were immersed in NH_4Cl Ringer's solution for several days. This shows that the 100 per cent volume of distribution found at 5 hours did not represent a true equilibrium value because the NH_3 concentration continues to increase slowly until the volume of distribution reaches a maximum value of about 150 per cent at the end of 4 or 5 days. During this time, there is evidently some accumulation of NH_3 against the concentration gradient. Similar results were obtained in *Valonia* by Cooper and Osterhout in 1930.

4. The Effect of NH_4Cl on the Loss of Potassium from Tissues

In a previous preliminary investigation (Fenn and Cobb, 1933) it was found that NH_4Cl accelerated the loss of K from frog muscle, but more quantitative

data were desirable. For this purpose, the tissues were accordingly suspended in small volumes of solutions, samples of which were withdrawn at intervals for potassium analyses. At the end of the experiment, the muscles themselves were analyzed for potassium. By adding to the final content the amounts of potassium found in the solution, the original amount present could be calculated. In this way, the data of Fig. 3 were obtained. The potassium content of the muscles immersed in the NH_4Cl-Ringer solution fell more rapidly than that of the control muscles in Ringer's solution. This was especially true in the 1st hour. At the end of the experiment, the control muscle contained 13.6 m. eq. per kilo more K than the experimental muscle, while the NH_3 content calcu-

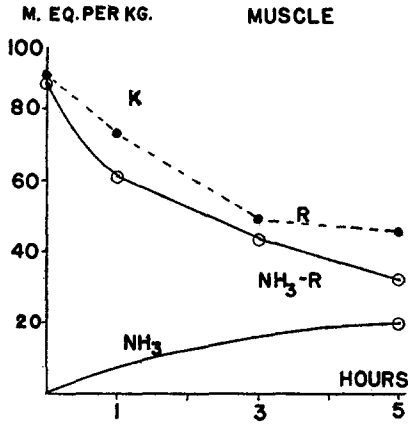

FIG. 3. The loss of potassium from muscles immersed in Ringer's solution is greater if the solution contains some NH_4Cl. The original solutions contained 1.49 m. eq. of K per liter. Samples (0.437 ml.) removed at 1 hour contained 3.47 and 3.84, and those removed at 3 hours contained 5.93 and 6.54 m. eq. of K per liter in the R and N solutions respectively. The final concentrations after 5 hours and other data are given in Table III (last striated muscle experiment).

lated for a volume of distribution of 100 per cent would be 19.8 m. eq. per kilo. (The NH_3 contained in 2 ml. of solution at an initial concentration of 22.8 m. eq. per kilo diffuses into a muscle weighing 0.222 gm. until the concentrations in solution and muscle are equal. Some similar experiments for other tissues are plotted in Fig. 4.) In every case, it is apparent that the loss of potassium was greater in the tissue immersed in the solution containing NH_4Cl. No actual analyses for NH_3 were made in any of these particular experiments, so that the graphs for NH_3 must be regarded as diagrammatic. They are, however, in accord with NH_3 analyses made in other similar experiments already described.

A summary of the data from experiments of this type is given in Table III.

The amounts of potassium which diffuse from the muscle into the solution because of the NH_4Cl can be calculated from the potassium contents of both the tissues and the solutions. The error of both these determinations is large because the errors of several potassium analyses are included. On the average, however, it can be seen that 9.75 m. eq. per kilo were lost from the tissues (assuming equal concentrations in the matched muscles at the start) while 9.25 m. eq. per kilo were found in the solutions. Large discrepancies were

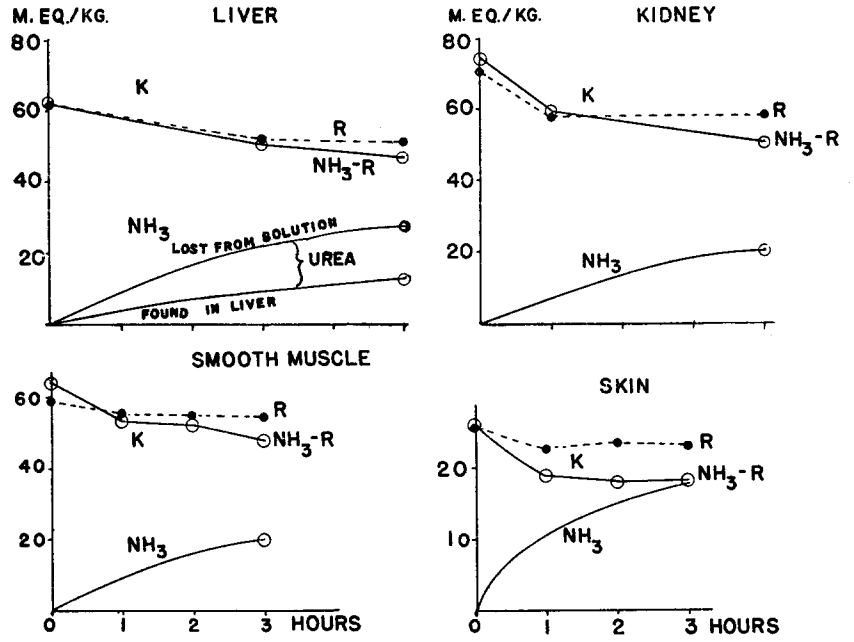

FIG. 4. Loss of potassium from tissues immersed in Ringer's solution with and without NH_4Cl substitution. Data concerning these experiments are given in Table III (see the second liver experiment). Further description in text.

found in some individual experiments. For comparison with this figure, there was a calculated amount of NH_3 diffusing in the opposite direction equal to 17.3 m. eq. per kilo. The NH_3 might, therefore, enter by exchange with other cations if potassium were not the sole cation involved. In *Valonia* also the penetration of NH_3 is accompanied by a nearly equivalent loss of K (Cooper and Osterhout, 1930).

The relation between the amount of K lost and the amount of ammonia gained is shown also in the data of Table IV. In these experiments, the tissue was analyzed directly for K while the solutions were analyzed for NH_3. The change in K reported in the table represents the difference between the K

TABLE III

Loss of K from Tissues after Soaking in Ringer's Solution with (N) and without (R) NH_4Cl

The weight of the piece of tissue suspended in the N solution is given in column 4 and the volume of the solution is contained in column 3. The weight of the control tissue was very similar in all cases. The final weights of both tissues are given in columns 5 and 6 in per cent of their respective initial weights. With a special pipette calibrated to contain 0.437 cc., samples were removed at intervals of a few hours for analysis. At the end of the experiment, the tissues were analyzed, and the results are shown in columns 7 and 8, the difference being given in column 9. At the same time the remaining solutions were analyzed, the results being listed in columns 10 and 11. Column 12 is not the difference between columns 10 and 11, but represents the total amount of extra K found in the N solutions, including the amounts removed in intermediate sampling during the experiment. This amount is represented as a concentration by dividing it by the initial volumes given in column 3. Column 13 equals (column 12) × (column 3) and divided by (column 4). Column 13 represents the amount of K lost from the tissue as calculated from the extra amount found in the solution (column 12) and should be equal to column 9. Column 14 equals 22 m. eq. per kilo × (column 3) and divided by (column 3 plus column 4). In the case of liver, the weight is multiplied by 2.11 for this formula to allow for a volume of distribution of 211 per cent.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Tissue	Time	Volume of solution	Initial weight	Final weight in per cent of initial		K in tissue m.eq. per kg. initial weight			K in solution Final concentration in m.eq. per l.			K m.eq. per kg. of Tissue	NH_3 m. eq. per kg. of Tissue
				R	N	R	N	ΔK	R	N	ΔK		
	hrs.	*ml.*	*gm.*										
Muscle	5.0	1.5	0.336	92.1	95.8	76.0	56.3	19.7	4.5	6.3	1.8	8.0	18.0
Muscle	5.0	1.5	0.198	97.2	101.4	65.8	51.8	14.0	4.56	6.82	2.26	17.1	19.4
Muscle	5.0	2.0	0.298	117.4	132.2	67.3	61.2	6.1	3.13	5.02	1.89	12.7	19.1
Muscle	5.0	2.0	0.222	119	117	45.8	32.2	13.6	6.61	8.75	1.36	12.2	19.8
Liver	5.0	1.0	0.752	110	108.4	56.9	45.6	11.3	8.6	9.7	1.1	1.5	8.5
Liver	5.0	2.0	0.638	119	124	51.1	46.6	4.5	4.7	7.1	1.58	4.95	13.2
Liver	3.0	2.0	0.239			70.3	61.2	9.1	1.77	3.38	1.1	9.3	17.6
Kidney	2.0	1.5	0.197			58.5	51.0	7.5	2.12	3.13	0.75	11.5	20.6
Smooth muscle	3.0	1.5	0.266			54.5	47.8	6.7	2.32	4.37	1.68	9.5	18.7
Skin	3.0	1.5	0.355			23.2	18.2	5.0	2.03	3.44	1.52	6.4	17.8
Average—for skeletal muscle								13.4				12.4	18.7
Grand average								9.75				9.25	17.3

TABLE IV

Potassium Loss and Ammonium Gain of Muscles in 5 Hours

Control K, *m.eq. per kg*	42.1	42.5	49.5	53.4	73.8	53.0	48.9	38.2	53.3	60.7	48.0	62.0	60.9	Average
Δ K, *m.eq. per kg.*	7.3	1.9	−10.2	−9.0	−19.9	−3.9	−8.9	2.2	1.4	−13.4	0.9	−8.1	−17.7	−6.0
Δ NH_3, *m.eq. per kg*	22.8*	22.8*	22.8*	22.8*	19.4	15.4	15.5	14.0	14.8	15.4	19.7	13.7	20.7	16.5

A minus sign means a loss from the muscle. No sign means a gain.

*Calculated, assuming a volume of distribution = 100 per cent.

contents of the control and experimental muscles at the end of the experiment. In ten of these thirteen experiments, the potassium loss was greater in the presence of NH_4Cl. The other three cases were doubtless due to experimental error. On the average, the gain in NH_3, averaging 16.5 m. eq. per kilo, was considerably greater than the loss of K which averaged only 6 m. eq. per kilo. It should be noted, however, that in these experiments the muscles were soaked overnight in Ringer's solution before the experiment. During this time, a considerable amount of K was lost. When the experiment was performed with-

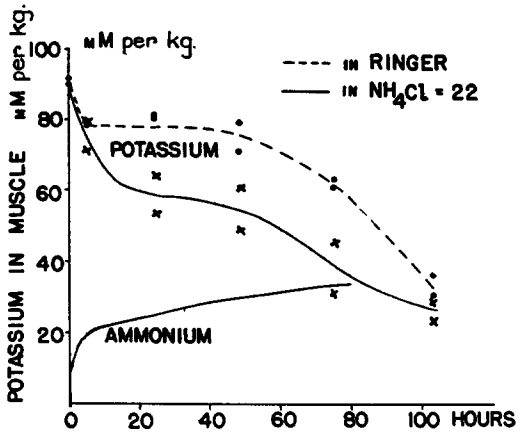

FIG. 5. Loss of potassium from muscles immersed in Ringer's solution with and without substituted NH_4Cl. The curve for penetration of NH_3 from Fig. 2 is included for comparison. These muscles were immersed for varying periods of time in large volumes of solutions (100 cc.) at about 5°C. In addition to these experiments, four additional pairs of muscles were analyzed after immersion under similar conditions for 24 and 48 hours. In these eight pairs of analyses, the mean difference in potassium content was 19.2 m. eq. per kilo. Six other similar muscles were analyzed for NH_3 after immersion for 24 (four muscles) and 48 (two muscles) hours, and were found to have an average NH_3 content of 24.2 m. eq. per kilo.

out this preliminary soaking, as in the experiments of Table III, the loss of K due to the NH_3 was 12.4 to 13.4 as shown instead of 6 m. eq. per kilo. This value is more nearly equal to the NH_3 gain. It is possible that during the soaking overnight some of the muscle K exchanged for Na, and, later, some of this Na exchanged in turn with NH_4. It seems likely, therefore, that all the NH_3 which enters does so in exchange for an equivalent amount of some other cation.

Other similar experiments were run over longer periods of time in order to see whether, at a point of more complete equilibrium, the equality between K loss and NH_3 gain might be more striking. The results of these experiments are to be seen in Fig. 5, where the potassium contents of the control and ex-

perimental muscles are plotted against time. The concentration of NH_3 in the muscle, as given in Fig. 2, is also included for comparison. According to the chart, it requires about 20 hours for the difference in potassium content of the two muscles to reach its maximum. At this point, the experimental muscle in NH_4Cl has lost about 20 m. eq. per kilo more K than the control muscle and this difference is about equal to the concentration of the NH_4Cl in the Ringer's solution which was 22 m. eq. per kilo. At 20 hours, however, the NH_3 content is already 24 m. eq. per kilo or slightly greater than the concentration in the solution, and this concentration continues to increase to about 35 at the end of 140 hours. While, therefore, there is a close relationship between the loss of K and the gain in NH_3, the two quantities are never exactly equal, and it seems likely that other cations are involved in the equilibrium.

The question of the reversibility of the loss of potassium caused by the penetration of NH_3 is an important one for which we have no answer. In one experiment, we endeavored to obtain information on the point by exposing matched muscles to the NH_4Cl-Ringer's solution overnight. In the morning, one muscle was analyzed for potassium, while the other was replaced in Ringer's solution without NH_3. When the second muscle was analyzed some hours later, its potassium content was still lower than that of its mate. The result shows only that, in Ringer's solution when NH_3 diffused out of the muscle, the potassium was not replenished at a rate which was fast enough to exceed the continuous loss due to disintegration of the muscle. These exchanges are apparently so slow in muscle that reversibility is difficult to demonstrate. According to Conway, O'Brien, and Boyle (1941) potassium of yeast can be completely replaced by NH_3, and this exchange can be reversed, but even in this small cell the exchange is not rapid.

5. Experiments with Radioactive Potassium

Evidence already presented has indicated that the NH_3 enters partly at least in exchange for K, and it has been shown that the loss of K is greater in the muscle immersed in the NH_3 solution. This conclusion is confirmed by another experiment in which artificially radioactive potassium (K^{42}) was used. A solution of radioactive K was injected into the dorsal lymph sac of a frog the day before the experiment. After this period of time, it can be assumed that K^{42} has completely mixed with K^{39} throughout the muscles of the body (Noonan, Fenn, and Haege, 1941). Muscles were then dissected as usual and were immersed in R and N solutions according to the usual technique. At intervals samples of the solutions were transferred to the cup surrounding the ionization chamber of a Geiger-Müller counter for a count of the β rays. The radioactivity of the solution presumably depends under these conditions (1) on the net amount of radioactive K lost from the muscles and (2) on the amount exchanged between the K^{42} in the muscle and the K^{39} of the solution. The

latter factor would presumably be the same for both muscles, but the net loss would be greater in the NH_3 muscle because of exchange of K for NH_4. The graphs of Fig. 6 are in accord with this expectation for the β ray count was always higher in the presence of NH_4Cl.

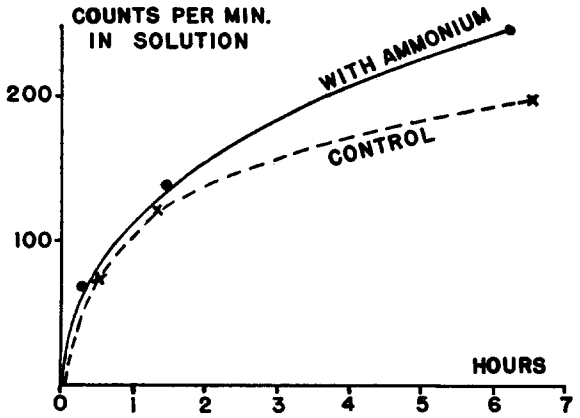

FIG. 6. Matched muscles taken from frogs previously injected with radioactive K^{42} were immersed in R and N solutions. The results show that K escapes more rapidly when it can exchange with NH_4^+ and the β-ray count is always higher in the Ringer + NH_4Cl solution.

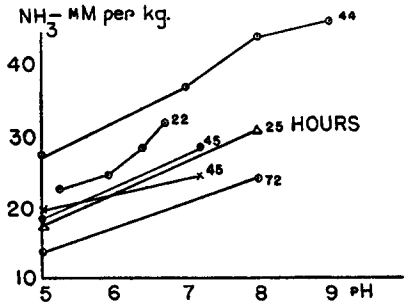

FIG. 7. NH_3 content of muscles immersed in NH_4Cl-Ringer's solution at varying pH. Alkalinity favors accumulation of NH_3 in the muscle. Figures indicate the number of hours of immersion. Each graph represents one experiment on muscles of one frog. The solution contained 22 m. eq. of NH_4Cl per l.

6. The Effect of pH

The effect of varying the pH of the solution is shown in Fig. 7. Each point represents the difference in NH_3 content between the control and the experimental muscle after immersion for varying lengths of time in the R and N

solutions. The duration of the immersion varied from 22 to 72 hours as indicated in the figure. Each graph represents an experiment with muscles from a single frog, each point being the result of analyses of a single pair of those muscles. In all cases, the NH_3 content is higher in the more alkaline solution. This is in accord with the theory of Osterhout (1930), according to which the NH_3 penetrates as undissociated NH_4OH, the concentration of which is proportional to the product of the concentrations of OH^- and NH_4^+ ions. It is also in accord with the idea that NH_4^+ enters in exchange for H^+ because the H^+ would come out more readily into a more alkaline solution. Similar results were reported by Cooper and Osterhout in 1930, for *Valonia.*

In this connection, it may be recalled that the loss of K from muscles immersed in Ringer's solution is greater in the more acid solutions (Fenn and Cobb, 1933). In the presence of NH_4Cl, however, this result might be expected to be changed because, in the alkaline solutions, the NH_3 penetrated better and released K or exchanged with K. To the extent, then, that the loss of K depends upon the penetration of NH_3, its loss will be increased by alkalinity in the solution. In one experiment, two sets of matched muscles were put into NH_4Cl-Ringer's solution, one set of muscles at pH 6.0 and the other at pH 7.7. Both the muscles and the solutions were analyzed for K after the period of immersion. The results showed no consistent difference between the amounts of K lost by the muscles in the two solutions. In the absence of NH_4Cl, the loss would undoubtedly have been greater at pH = 6.0.

7. Miscellaneous Factors

A number of other variations in the experiment were also tried, and the results may be conveniently summarized in Table V. The equilibration of the solutions with a gas mixture containing 95 per cent oxygen and 5 per cent CO_2, instead of pure oxygen, decreased slightly the penetration of NH_3, and this is in accord with the pH effect shown in Fig. 6. Equilibration with pure nitrogen instead of pure oxygen also decreased the penetration of NH_3, probably because of the increased acidity due to the lactic acid formed anaerobically. The degree of stirring of the muscles and the solutions was found to have some importance, for, if the muscles were left without any agitation, the penetration decreased from 30.8 to 25.3 mM per kilo. This result was duplicated in several experiments. An increase in the concentration of Ca, Mg, K, or Na in the Ringer solution also caused a decrease in the amount of NH_3 which accumulated in the muscles in the course of 5 hours. This suggests that NH_4 could exchange with any other cation. The four experiments in which the Na content was varied from 1.3 to 10.2 (Table Vh) were accomplished by replacing NaCl by isotonic sucrose solution. Both solutions contained the usual amount of NH_4Cl and phosphate buffer. The results are presumably due to the Na rather than to any specific effect of the sucrose.

From Table V, i, it is evident that an increase in the temperature of the

TABLE V

The Effect of Various Factors on the Penetration of Ammonia into Muscles Immersed in Ringer's Solution Containing 0.022 N NH_4Cl

	Solution		NH_3 in muscle		Time
	A	*B*	*A* m × 10^{-3}	*B* m × 10^{-3}	
					hrs.
a.	O_2	5 per cent CO_2	19.3	18.0	23
			17.8	16.4	5
			23.7	19.0	5
b.	O_2	N_2	20.3	14.0	24
			34.9	31.7	20
c.	Rest	Stir	25.3	30.8	24
d.	Ca=0	Ca=4	37.3	28.9	44
			31.5	28.2	21
e.	Mg=0	Mg=4	29.4	26.1	46
			37.0	35.8	47
f.	K=1.3	K=21.3	22.8	17.2	22
			27.4	21.1	24
g.	K=2	K=8	14.9	11.2	24
			12.6	11.8	24
h.	Na=1.3 plus sucrose	Na=10.2	32.8	26.2	24
			29.1	29.0	27
			41.1	37.45	45
			39.85	38.70	41
i.	5°C.	23°C.	11.3	12.7	1
			14.9	17.5	3
			19.0	24.8	5
			23.9	28.4	18
j.	Small muscles	Large muscles	19.83	18.78	5 to 48
k.	NH_3=11.2	NH_3=22.4	13.0	29.0	17
			19.8	31.6	41
			19 1	36.3	96
l.	—	$CHCl_3$	22.0	21.0	21
			29.3	28.7	42
m.	—	Boil	23.2	21.0	24

All concentrations are in m. eq. per kg. unless otherwise noted.
Figures in j are averages of eight experiments.

solutions from 5°C. to 23°C. also accelerated the diffusion process and increased the 5 hour concentration of NH_3 found in the muscles.

The data cannot be considered adequate for the calculation of a diffusion coefficient, but the indications certainly are that the Q_{10} is small enough to be a diffusion process. A limitation of the process by diffusion is also suggested by the lower concentration found in large muscles compared to small ones. The figures given are the averages of 8 separate determinations, in 6 of which the small muscles contained the most NH_3. The average weights of the large and small muscles were respectively 841 and 153 gm. Periods of immersion were 5 hours 1 day, and 2 days in 4, 2, and 2 comparisons respectively. Evidently even the largest muscles are nearly at equilibrium in these times because the difference in the NH_3 content is small for so large a difference in size of muscle.

In several experiments (Table V, k), matched muscles were immersed in solutions containing 11.2 and 22.4 m. eq. per kilo of NH_4Cl respectively. The NH_4Cl was introduced as usual in exchange for an equivalent amount of NaCl. Thus the concentration of NH_3 was twice as great in the stronger solution, and the concentration of the NH_3 in the muscles was found to be, on the average, 1.9 times as great. This shows that the amount diffusing is closely proportional to the concentration outside.

Finally, we tried some experiments with dead muscles, killed either by boiling or by chloroform (Table V, l and m). The addition of a drop of chloroform to the flask containing muscles in 0.022N NH_4Cl-Ringer's caused a slight decrease in the amount of NH_3 taken up, but, in one of the two experiments which ran for 42 hours, the ultimate concentration of the NH_3 in the chloroformed muscle was, nevertheless, considerably greater than that of the solution, thus indicating that the dead muscle could still exert some concentrating effect on the NH_3. The most remarkable feature of this experiment, however, was the result obtained when a similar pair of muscles were analyzed for K at the end of the experiment after immersion in similar solutions. In this case, the chloroformed muscle contained only 60 per cent as much K as its mate in the same NH_3-Ringer's, but without chloroform. The ability to hold K was apparently lost without eliminating altogether the ability to concentrate NH_3. In another experiment, both muscles were put into NH_3-Ringer's, but one of the muscles was heated to 53°C for 5 minutes until it was well contracted. This served to reduce slightly the amount of NH_3 taken up, and the concentration of the NH_3 in the dead muscle was 21 mM per kilo of muscle or about 26 mM per kilo of muscle water. This is slightly greater than the concentration in the solution but the difference can probably be attributed to endogenous NH_3.

8. Exchange of Sodium and Chloride

To obtain information concerning the total electrolyte balance in these muscles, some experiments were tried in which the muscles were analyzed

for chloride and for sodium. Chloride was determined by titration with KSCN after complete ashing in HNO_3, according to the method of Van Slyke as modified by Manery *et al.* (1938). Sodium was determined gravimetrically after complete dry ashing in a furnace, according to the method of Butler and

TABLE VI

Effect of NH_4Cl on the sodium content of muscles. Muscles were soaked in Ringer's solution (R) or in the same with 0.022N NH_4Cl substituted for an equivalent amount of NaCl(N). Immerse in 50 cc. of solution at 4°C. for 30 hours. The first three sets of muscles were taken from one frog, and the last three from another.

Muscles		Sodium content after immersion (m. eq. per kg. initial weight)	
No. of muscles	Weight of muscles	R	N
	mg.		
2	1077	41.4	35.5
6	628	57.8	53.9
3	872	53.3	49.9
2	1269	44.3	30.0
6	979	69.5	53.0
3	734	57.9	64.8
Average (per kg. initial weight)		54.0	47.9
Average (per kg. final weight)		52.9	45.0

TABLE VII

Effect of NH_4Cl on the chloride content of muscles. Muscles soaked in Ringer's solution (R) or in the same with 0.022N NH_4Cl substituted for an equivalent amount of NaCl. Immerse at 4°C. for 18 hours.

No. of muscles	Weight of muscles	Chloride after soaking per kgm. initial weight		Initial chloride
		R	N	
	mg.	*m. eq.*	*m. eq.*	*m. eq. per kg.*
1	920	29.2	32.4	9.2
1	519	32.4	34.9	11.2
1	645	43.2	51.7	10.5
4	660	63.4	68.9	11.4
2	509	36.7	38.9	14.6
1	595	48.5	61.4	10.9
Average (per kg. initial weight)		42.2	48.0	11.3
Average (per kg. final weight)		42.1	46.6	

Tuthill (1931). The muscles were analyzed after soaking overnight in the cold room. The results show that the chloride content was increased from 42.2 to 48.0 by the NH_3 (Table VI), while the sodium was decreased from the control value of 54.0 to 47.9 m.eq. per kilo (Table VII). The chloride contents of both muscles are, of course, much increased by soaking as compared to the initial value of 11.3 m. eq. per kilo (obtained by analyzing other similar muscles from another frog which were dissected quickly without any contact with Ringer's solution during the process). The same was true for the sodium contents, but no initial sodium contents were determined in this series of experiments. It is interesting to note, also, that samples which consisted of four to six small muscles such as the sartorius, semitendinosus, ileofibularis, and tibialis took up more of both chloride and sodium than the larger muscles. This is possibly due to a greater relative amount of connective tissue in the smaller muscles. The chloride content, after immersion, is so large that it hardly seems possible to account for it all in the extracellular spaces, and one must suppose that some of it is inside the cells. The sodium analyses suggest that some of the NH_3 enters in exchange for Na, which entered or otherwise would have entered in combination with the chloride.

9. Effect of NH_4Cl on Muscle pH and Bicarbonate Content[1]

If the ammonia enters the muscle as NH_3 or NH_4OH, then it might be expected to make the muscle more alkaline inside. If, however, it enters as NH_4 in exchange for K, then no great change in pH would be expected (Osterhout, 1930). In order to elucidate this point, pairs of muscles were soaked in the R and N solutions as before, and, after soaking, they were transferred to bottles of differential volumeters in an atmosphere of 5 per cent CO_2 in O_2 and analyzed for HCO_3 by tipping citric acid on them from a side arm after temperature equilibrium had been established. The CO_2 evolved was measured and calculated as volume per cent or milliliters of CO_2 per 100 gm. of muscle. The muscles were originally soaked at 5°C or 19°C for varying periods of time and with constant gentle agitation. In most of the experiments, the muscles were blotted gently when they were removed from the solution, weighed on a torsion balance, and placed on the dry bottom of a flask. In another series of experiments, however, they were immersed in the respirometer bottle in 0.5 cc. of unbuffered Ringer's solution.

The results of these two experiments are shown in Tables VIII and IX. When the muscles were in the dry bottles (Table VIII), the control and experimental muscles contained, on the average, 8.3 and 8.4 volumes per cent of CO_2 respectively, but, when further exchange with a solution was permitted, the CO_2 evolved was 15.2 and 24.0 volumes per cent respectively (Table IX).

[1] Experiments of J. B. F.

TABLE VIII

Effect of NH_4Cl on the CO_2 Content of Muscles Measured in Dry Respirometers

Time	Temp.	CO_2 content	
		R	N
hrs.	*°C.*	*cc./100 gm.*	*cc./100 gm.*
4	5?	8.9	16.5
4	5?	22.7	20.0
4	5?	21.3	19.9
5	5	1.5	3.3
5	5	8.3	7.6
5	5	8.8	8.5
18.5	5	4.0	5.2
18.5	5	2.6	1.8
24	5	6.2	6.1
24	5	5.8	6.1
43	5	4.6	4.6
1.2	19	7.2	11.4
1.1	19	11.9	11.5
1.2	19	11.6	2.9
3.8	19	3.8	6.7
5.1	19	4.4	6.4
5.8	19	4.8	3.6
5.0	22	10.6	8.6
Average.....		8.3	8.4

TABLE IX

Effect of NH_4Cl on the CO_2 Content of Muscles Measured in Respirometers Containing Unbuffered Ringer's Solution

Experiment No.	Time	Temperature	CO_2 content	
			R	N
	hrs.	*°C.*	*cc./100 gm.*	*cc./100 gm.*
1	22	5	12.0	30.0
2	22	5	19.0	32.6
3	1.2	19	15.8	26.7
4	3.9	19	13.3	15.9
5	17	19	8.1	15.5
6	21	19	10.3	17.7
7	0.5	5	17.5	23.4
8	3.5	5	18.2	22.5
9	6.0	19	19.1	22.8
10	5.7	19	18.8	33.1
Average.....			15.2	24.0

The original unbuffered Ringer's solution by itself combined with no CO_2, so the increased amounts evolved must have been due to base which escaped from the muscle into the solution. The muscles previously soaked in the Ringer's solution containing NH_4Cl invariably contributed more base to the solution than the control muscle.

There is no doubt that some of the evolved CO_2 in the second series was contained in the solution because we have removed the muscle before dumping the acid in some experiments and have found that the Ringer's solution remaining has acquired the ability to combine with CO_2 by its contact with the muscle. In one experiment, we soaked pairs of muscles in the R and N solutions for 46 hours and then placed them in separate tubes containing unbuffered Ringer's solution through which a stream of oxygen was passing. The pH of the two solutions was measured at intervals by a glass electrode. The initial pH of both solutions was 5.2; and, 10 minutes later, it was 6.55 for the control muscles and 6.77 for the NH_3-soaked muscles. After 35 minutes, the maximum alkalinity was reached in both cases at 6.72 and 6.97 respectively. Thereafter, both solutions became progressively more acid, although the difference between them remained substantially the same.

The experiments in dry respirometers gave much more variable and less reliable results than those in which Ringer's solution was used. This was presumably due to uncertainties in the vapor pressure inside the flasks. Nevertheless, the results give no indication that the penetration of NH_3 made the muscle, as a whole, any more alkaline. This is perhaps, not surprising since the pH outside was the same whether NH_4Cl was present or not.

Even in the absence of NH_4Cl, the total amount of CO_2 liberated by the addition of acid is increased from 8.3 to 15.2 volumes per cent when the muscle is immersed in a solution. This is presumably due to a dilution effect. The solution, without buffers, is quite acid when it comes into equilibrium with 5 per cent CO_2, so that base tends to diffuse out of the muscle, thus equalizing the pH between the inside and the outside. In effect, the buffers of the muscle release base to form more bicarbonate to replace that lost in the solution.[2] This process would occur more rapidly if some of the base inside the muscle were NH_4 which could diffuse more rapidly than the K. If this explanation is correct, then the experiments justify the conclusion that the pH of the muscle is not increased by penetration of NH_3, and this is consistent with the idea that the NH_4 which forms inside the muscle displaces an equivalent amount of either K or Na.

Assuming a bicarbonate content of 8.4 volumes per cent after immersion

[2] The H_2CO_3 concentration is the same in the solution as in the muscle. By adding the solution to the muscle the ratio HCO_3/H_2CO_3 of the whole system is decreased. To keep the pH constant more $NaHCO_3$ is formed from the base borrowed from proteins.

in the N solution, it is possible to estimate the pH inside the muscle. When equilibrated with 5 per cent CO_2, the solution had a pH of 6.38 and contained by calculation (at 23°C.) 4.02 volumes per cent of H_2CO_3. The calculated HCO_3 was then 6.53 volumes per cent which may be assumed to represent the composition of the solution in the chloride space of the muscle representing perhaps 15 per cent of the muscle. The HCO_3 content of the fiber water would then be $\frac{8.4 - (6.53 \times 0.15)}{1.00 - 0.15 - 0.20} = \frac{5.55}{0.65} = 8.5$ per cent volumes per cent, and the pH would be $6.17 + \log \frac{8.5}{4.02} = 6.49$. If the pH values inside and outside are 6.49 and 6.38 respectively, then the corresponding H ion concentrations are 3.24 and 1.95×10^{-7} respectively, and the H_i/H_o ratio is 1.66. If now the concentration of NH_3 in the muscles after 5 hours is taken to be 22.8 m. eq. per kilo (100 per cent volume of distribution), then the amount in the extracellular space will be 3.4 m. eq., and the concentration in the fiber water will be $(22.8 - 3.4)/(1.0 - 0.15 - 0.20)$ or 29.9 m.eq. per kilo. This is 1.31 times the concentration in the solution. Thus $A_i/A_o = 1.31$ (A = ammonia) while $H_i/H_o = 1.66$. Although these calculations are based upon many doubtful assumptions (such as the correction for the extracellular space), this approximate agreement must be regarded as offering some support to the theory that the pH will determine the amount of NH_3 which can accumulate inside. If this theory is correct, then the additional NH_3 which accumulates in the muscles between 5 hours and 5 days' time (Fig. 2) must be due to a progressive increase in the acidity inside the muscle.

10. Injection of NH_4Cl into Cats

In three experiments, we have injected NH_4Cl intravenously into anesthetized cats with the idea that, under these conditions also, the NH_4 would exchange with K from the muscles and cause an increase in the concentration of K in the blood plasma. At varying intervals after the injection, the plasma was sampled and analyzed for K. On account of the rapid diffusibility of NH_3 it may be assumed that this disappears rapidly into the tissues in exchange for K. This K, in turn, might behave like an equivalent amount intravenously injected. As an approximation, therefore, one might calculate the volume of distribution of this hypothetically exchanged K in percentage of the body weight by the formula (mM NH_4Cl injected) $\times$ 100 divided by (K in mM per liter of plasma $\times$ body weight in kilos).

In Table X are listed details of these three experiments with the corresponding values of the volumes of distribution calculated in this way. It will be seen that after 14 minutes the volume of distribution is 212, and this large value may be attributed to some formation of urea. According to the assumptions made, the other two values obtained after 3 and 4 minutes are not

unreasonable, since it has been shown that injected potassium is distributed in all of the body water or about 70 per cent of the body weight (Fenn, 1939, and others). In any event, this experiment shows that potassium is mobilized when NH_4Cl is injected. Subsequently, the urea content of the blood increases as Kaprowski and Uninski (1939) have shown and the excess K presumably disappears.

TABLE X

Exchange of K and NH_4 in cats. The increase in the concentration of K in the blood plasma after intravenous injection of NH_4Cl under dial anesthesia.

Experiment No.	Body weight	NH_4Cl injected	Time after injection	ΔK in plasma	Vol. of distribution
	kg.	*mols*	*min.*	*mM per l.*	*per cent*
1	2.9	1	3	+0.41	84
2	3.0	3	14	+0.47	212
3	3.5	2	4	+1.56	37

DISCUSSION

Within 5 hours after immersing a frog muscle in a Ringer solution containing NH_4Cl in place of some of the NaCl the NH_3 has reached a concentration inside the muscle which is equal to that in the surrounding solution. After several days, the inside concentration may be 1.5 times as great as that outside. Allowing for 20 per cent dry weight in the muscle, the concentration in the muscle water may be calculated to be respectively 1.25 to 1.9 times as concentrated as in the solution. Allowing further for an extracellular space, the concentration inside the muscle fibers themselves would be still higher. The corresponding inside to outside ratio for the potassium concentration is more like 40, depending upon the concentration of the potassium in the Ringer solution.

If the distribution of electrolytes in muscle is determined by a membrane equilibrium, as for example in the theory of Boyle and Conway (1941), then the ratios A_i/A_o (for ammonia), K_i/K_o(for potassium), and H_i/H_o (for hydrogen ions) should all be equal. Boyle and Conway have argued that the K and H ratios are indeed equal under normal conditions in muscle, but this requires that the pH inside the muscle should be 5.6. Unfortunately, the measurements of the bicarbonate content of muscle equilibrated with a known CO_2 tension without previous soaking in any solution have shown that the pH is 6.8 to 6.9 (Fenn and Maurer 1935). Boyle and Conway have chosen to ignore these measurements and have selected, instead, a colorimetric determination made by Rous (1925) in muscles which might well have been injured in the process. We are unable to concede, therefore, that the inside to outside K and H ratios are equal in normal muscle.

Recently, Conway, O'Brien, and Boyle (1941) have published a brief report

of some experiments on the penetration of NH_3 into muscle from very dilute solutions. Their results agree very well with our own in showing A_i/A_o ratios varying from 1.28 to 1.5. The authors reconcile this with their theory by pointing out that the potassium diffuses out until its inside-to-outside ratio is also only a little above unity. They believe, therefore, that the NH_3 has changed the membrane in such a way that it requires a lower inside-to-outside cation ratio than normal. This argument, however, is rather weak when it is realized that eventually the inside-to-outside K ratio must necessarily equal 1.0 when the muscle is completely dead, so that, at some time, the NH_3 and K ratios must be equal whether there is a true membrane equilibrium or not. Since, however, the K and H ratios are not equal normally, there seems no reason to suppose that the K and A ratios will be equal either. It is noteworthy, however, that the NH_3 and H ratios are not far from equal in our experiments. According to the experiments of Netter (1934), the A_i/A_o ratio in perfused frog muscle is 4.5, and this is taken as a measure of the H_i/H_o ratio. Even this somewhat larger ratio is not equal to the K_i/K_o ratio.

Since the K content of muscle changes very slowly, it is possible to devise experiments which will make the K_i/K_o ratio almost anything which is required for a theory by simply changing the value of K_o. Having tried many experiments of this type, we are convinced that this ratio has no theoretical value under experimental conditions in excised muscles. It is still more difficult to make the facts fit the theory if Na is also regarded as a penetrating ion. In that case, some "pump" theory like that of Dean (1941) is required to explain the facts.

We have not been able to do a complete electrolyte balance on the same muscle, but we have shown in different muscles that both K and Na decrease in the muscle as NH_3 increases, and the magnitudes are such that the combined losses of K and Na are nearly equal to the gain in NH_3. It appears likely, therefore, as a first approximation, that the NH_3 enters in exchange for K and Na. It is possible, of course, that the ammonium enters as NH_3 and then dissociates into NH_4 ions. If this occurs, as Osterhout has pointed out, the pH should first increase as the NH_3 accumulates, but it should decrease again as soon as the K and Na diffuse out. A change of this sort was actually demonstrated in *Valonia*. The evidence which we have presented for muscles indicates that any increase in alkalinity due to penetration of NH_3 was minimized by an equal loss of K or Na, so that the bicarbonate content of the muscles at constant CO_2 tension was not increased.

The theory of the penetration of NH_3 into cells has been clearly presented by Jacobs (1927), who postulates that it is the products of hydrolysis of the salt, the NH_3, and the HA which actually penetrate. The muscle cell, according to this scheme, behaves as if only the NH_3 were able to penetrate. It is reasonable to expect, therefore, that penetration of ammonia into muscle cells would be quite independent of the nature of the anion, and this seems to

be the case. The acetate was used in place of the chloride in one experiment without changing the speed of penetration. Substitution of bicarbonate buffers for phosphate buffers and equilibration with 5 per cent CO_2 in place of pure oxygen did not change the result. The absence of swelling of the muscle indicates that the anion does not penetrate along with the NH_3 of the NH_4 ion. In this respect, the muscle behaves like the eggs of *Arbacia* (Jacobs and Stewart, 1936). In erythrocytes, which are permeable to chloride, there is a rapid swelling which may be sufficient to cause hemolysis (Jacobs, 1927). It is impossible to tell from the results whether the ammonia penetrated in ionized or non-ionized form because the end result would be the same either way. Presumably the concentration of NH_3 is the same both inside and outside, in which case the total amount present (as NH_3, NH_4OH, and NH_4) depends upon the pH. The ability of dead muscles to concentrate NH_4^+ presumably indicates that the activity coefficient is diminished inside the muscles.

SUMMARY

1. A study was made of the electrolyte changes which occur when frog muscles are immersed in a Ringer solution with 1/5 of the Na replaced by NH_4Cl. Analyses were made in the solution and in the muscles for K and NH_3, and the muscles were also analyzed for Cl, HCO_3, and Na. Control muscles were immersed in normal Ringer's solution and similarly analyzed.

2. The amount of ammonia taken up was about equal to the K and Na lost. There was also a small increase in chloride content. The bicarbonate content was the same in both experimental and control muscles, indicating no change in the muscle pH due to the NH_3 which penetrated. An increased loss of K due to the penetration of NH_3 was also demonstrated by the use of radioactive K.

3. After 5 hours, the concentration of ammonia per gram of muscle is about the same as the concentration in the solution. After 4 or 5 days, the concentration in the muscle is about 1.5 times that in the solution. The inside to outside NH_3 ratio is about equal to the corresponding H ion ratio, but is much less than the K ratio.

4. The rate of penetration of the NH_3 is increased by a rise of temperature, by stirring the solution, and by decrease in the concentration of Na, K, Ca, or Mg in the solution; it is decreased by increasing the size of the muscles or by killing them with chloroform or boiling.

5. Liver, smooth muscle, skin, and kidney, in a few experiments, behaved much like muscle except that there was a formation of urea in the case of liver.

6. The injection of NH_4Cl into anesthetized cats causes an increase in the level of K in the blood plasma.

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