

## THE LOSS OF POTASSIUM FROM FROG NERVES IN ANOXIA AND OTHER CONDITIONS

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It is known from the work of Cowan (1934) that unmyelinated nerves lose potassium in anoxia. Similar experiments on vertebrate myelinated nerves are not available except for a few preliminary experiments of Fenn (1934) which showed no definite change. The experiments to be reported here were made on frog nerves largely at the instigation of Dr. Lorente de Nó (1946), who was studying the relation between potassium loss, the membrane potential, and the capacity for recovery, and who sent us some of his experimental nerves for analysis. The results show definitely that frog nerves do lose potassium in anoxia.

### *Methods*

The sciatic nerves of two frogs were dissected, with care to avoid stretching, and were put into Ringer's solution. The right nerve of one frog and the left nerve of the other were used for anoxia while the other two nerves served as controls. The nerves were cleaned of vessels and other non-nervous tissue and were weighed on a torsion balance after absorbing the excess solution with filter paper. Each pair of nerves was then placed in 1 to 15 ml. of Ringer's solution at pH 7.4 in a glass vessel of 25 to 30 ml. capacity. The vessel was fitted with an inlet tube on the bottom provided with a glass stopcock and was closed at the top by a ground glass stopper fitted with an outlet tube and a second stopcock. Oxygen was then bubbled through the control vessel while nitrogen, purified over hot copper and conducted through a copper tube, was passed through the other vessel. After 15 minutes or more the stopcocks were closed and the vessels were left for varying periods of time up to 42 hours in the cold room at 4°C. The nerves were then removed, weighed again, ashed in platinum crucibles at about 500°C., and analyzed for potassium by a slightly modified Shohl and Bennett method. In some of the later experiments a flame photometer was used. Concentrations of potassium are reported on the basis of the initial wet weight. In general all the nerves gained slightly in weight during immersion in the solution but there was no significant difference in this respect between the experimental and the control nerves.

In four preliminary control experiments the nerves were dissected as usual and weighed directly into crucibles for analysis without previous treatment in solution. The percentage differences in the potassium contents in the four pairs were 6.9, 2.3, 6.7, and 6.7. In general, therefore, an average difference between matched pairs of nerves may be expected to be about 5.6 per cent.

The results of fifteen experiments with and without oxygen are shown in Table I. In every case the nerve in nitrogen contained less potassium at the end of the experiment than the control in oxygen. The percentage differences in the various experiments are quite irregular and there is no obviously greater anoxic loss at the longer as compared to the shorter times. On the average, the nerve in nitrogen contained at the end of the experiment 76 per cent as

TABLE I  
*Increased Loss of Potassium from Nerves in Nitrogen*  
Potassium content in milliequivalents per 100 gm. initial weight.

| Time                | K content of nerve     |                        | Increased weight |                 |
|---------------------|------------------------|------------------------|------------------|-----------------|
|                     | O <sub>2</sub>         | N <sub>2</sub>         | O <sub>2</sub>   | N <sub>2</sub>  |
| <i>hrs.</i>         | <i>m. eq. per cent</i> | <i>m. eq. per cent</i> | <i>per cent</i>  | <i>per cent</i> |
| 1                   | 4.43                   | 3.45                   | 0                | +2.8            |
| 2.5                 | 4.17                   | 3.05                   |                  |                 |
| 3.15*               | 3.32                   | 3.21                   | +1.29            | +6.58           |
| 3.2                 | 4.08                   | 2.93                   | +3.58            | +12.2           |
| 5.0*                | 2.67                   | 2.32                   | +4.53            | +6.47           |
| 5.0*                | 2.70                   | 2.27                   | +0.74            |                 |
| 6.7                 | 4.38                   | 2.86                   |                  |                 |
| 7.3                 | 3.63                   | 2.89                   | +2.22            | +6.12           |
| 9.0                 | 5.82                   | 3.45                   | +9.10            | +15.0           |
| 12.0                | 3.31                   | 2.82                   | +9.89            | +6.80           |
| 12.0                | 3.09                   | 2.28                   | +2.24            | +7.70           |
| 15.0                | 2.68                   | 2.26                   | -5.30            | +2.84           |
| 15.8                | 3.12                   | 2.50                   | -0.78            | +1.59           |
| 25.0                | 4.60                   | 3.60                   |                  |                 |
| 42.0                | 4.05                   | 3.37                   | +6.20            | +10.1           |
| Average † . . . . . | 3.74                   | 2.88                   | +2.81 ±1.2       | +7.11 ±1.1      |
| Per cent . . . . .  | 100                    | 76                     |                  |                 |

\* Threads tied around both ends of the nerve. These figures are not included in the averages.

† With standard deviation of the mean.

much potassium as the control in oxygen. The anoxic nerves gained 7.1 per cent weight as compared to the significantly lower value of 2.8 per cent in the controls but this does not explain the loss of potassium which was calculated on an initial weight basis.

In fourteen experiments an effort was made to find out whether the anoxic loss of potassium was reversible. For this purpose both nerves of each pair were incubated for several hours in Ringer's solution equilibrated with nitrogen. One nerve of each pair was analyzed after removal from the nitrogen while the other nerve was allowed to recover for a period in the oxygen before analysis. These experiments are summarized in Table II. In the first eight experi-

ments both nerves were left for an equal period in nitrogen after which the recovery nerve was left for a further period in oxygen so that the total period of soaking in solution was longer than for the anoxic nerves. In six of these eight experiments the recovery nerve contained more K than the anoxic control, in spite of its longer period in solution but in two experiments the nerves apparently continued to lose K in small amounts during recovery. In the last six experiments of Table II the anoxic nerves remained in nitrogen during the

TABLE II  
*Aerobic Recovery of Potassium Content of Nerves*  
Potassium content in milliequivalents per 100 gm. initial wet weight.

| Anoxic nerves (a)               |                 | Recovery nerves (b) |                |                 |                 | ΔK       | Increase in weight |     |
|---------------------------------|-----------------|---------------------|----------------|-----------------|-----------------|----------|--------------------|-----|
| Time in N <sub>2</sub>          | K content       | Time in             |                | K content       | m. eq. per cent |          | (a)                | (b) |
|                                 |                 | N <sub>2</sub>      | O <sub>2</sub> |                 |                 |          |                    |     |
| hrs.                            | m. eq. per cent | hrs.                | hrs.           | m. eq. per cent | m. eq. per cent | per cent | per cent           |     |
| 3.0                             | 4.40            | 3                   | 3              | 4.9             | +0.5            | +6.25    | 0                  |     |
| 4.0                             | 4.18            | 4                   | 3.7            | 4.32            | +0.14           | +5.8     | +9.8               |     |
| 2.7                             | 3.80            | 2.7                 | 3.0            | 4.0             | +0.20           | +3.8     | +2.9               |     |
| 2.7                             | 3.53            | 2.7                 | 1.8            | 3.98            | +0.45           | +6.6     | +4.8               |     |
| 3.3                             | 2.29            | 3.3                 | 1.9            | 3.75            | +1.47           | +13.5    | +20.0              |     |
| 3.5                             | 3.99            | 3.5                 | 2.5            | 3.48            | -0.44           | +7.0     | +9.5               |     |
| 3.0                             | 2.91            | 3.0                 | 1.7            | 2.64            | -0.37           | +7.5     | +9.2               |     |
| 6.0                             | 4.56            | 6.0                 | 2.0            | 4.95            | +0.39           | +6.4     | +7.5               |     |
| 4.7                             | 2.50            | 2.9                 | 1.8            | 3.58            | +1.08           | +8.3     | +1.9               |     |
| 9.0                             | 3.94            | 7.0                 | 2.0            | 3.62            | -0.32           | +17.0    | +10.2              |     |
| 5.0                             | 4.09            | 3.0                 | 2.0            | 4.57            | +0.46           | +11.5    | +4.2               |     |
| 16.2                            | 3.14            | 4.2                 | 12.0           | 3.78            | +0.64           | +3.2     | +11.1              |     |
| 7.7                             | 3.65            | 5.5                 | 2.2            | 4.06            | +0.41           | +1.0     | +12.5              |     |
| 4.2                             | 3.60            | 2.2                 | 2.0            | 3.80            | +0.20           | +14.6    | +12.7              |     |
| Mean.....                       |                 |                     |                |                 | +0.34           | +8.7     | +6.9               |     |
| Standard deviation of mean..... |                 |                     |                |                 | ±0.14           | ±1.09    | ±1.4               |     |

recovery period of their mates in oxygen so that the total period of immersion was the same for both nerves. In all but one of these experiments the recovery nerve contained more K after immersion than the anoxic controls. On the average, the recovery nerves contained 10 per cent more K than their controls, the actual difference being  $0.34 \pm 0.14$  m. eq. per 100 gm. (standard deviation of mean). The recovery nerves gained slightly less water than their controls but the difference was scarcely significant and could not explain the potassium figures. The results in Table II seem to show, therefore, that the potassium lost in nitrogen tends to return to the nerve during recovery.

In Table III are included the results of seven experiments in which the

two members of a pair of nerves were left for different periods of time either in nitrogen or in oxygen in order to measure the time course of the change in potassium content which occurred. In all but one case the longer period of immersion resulted in a smaller potassium content, indicating a progressive outward diffusion. The one exception is probably due to experimental error or random differences between nerves since the rate of loss of K would be expected to diminish after the 1st hour but could hardly reverse to negative values. Here again the average gain in weight is slightly greater for the longer period but the difference is not significant.

The question was raised whether this loss of potassium might be taking place entirely through the cut ends of the nerves rather than through the uninjured surfaces. The effect of the anoxia might then be on the fluidity of the protoplasm or some other mechanical condition rather than on the permeability of the nerve or the state of the physicochemical membrane equilibrium. To test this hypothesis three of the experiments in Table I (marked with an asterisk) were done with both ends of the nerve tied off. In spite of this change the loss of potassium was still accelerated by anoxia as before. Since the contents of the nerves in these particular experiments seemed rather low, we tried some experiments, summarized in Table IV, in which the ends of one pair of nerves were tied off with silk threads while the controls were left untied. In all we have completed twelve such experiments which are listed in Table IV. Varying numbers of ligatures were used and they were tied on both ends, one end only, or around the middle of the nerve. No special differences were noted in these varying cases but on the average the ligatures reduced the K content 0.29 m. eq. per 100 gm. of nerve with a standard deviation of the mean of  $\pm 0.11$ . In four of the twelve cases the nerve with a ligature contained slightly more K than the control but this difference was never greater than 4 per cent, the average loss being 8 per cent. We are grateful to Dr. F. O. Schmitt for the suggestion that this increased loss of K may represent the elimination by the ligature of the injury current at the cut end which flows in such a direction as to favor the uptake of K. Some actual injuring of the nerve due to compression and displacement of water near the ligature may also be considered.

In papers by Guttman and Medawar (1942) and by Weiss (1944) it was shown that a nerve *in situ* tends to swell or enlarge above a ligature and in this condition the permeability to potassium might be increased. It seemed conceivable that a process of this sort due to peripheral movement of fluid through the nerve might be taking place even in isolated nerves and might help to explain the results shown in Table IV. To test this possibility we tried nine experiments in which the sciatic nerve on one side of the body was tied *in situ* with a single ligature either on the central end or in the midhigh region as indicated in the data in Table V. The nerve on the other side of the body was left as a control. After about 7 hours both nerves were isolated and analyzed

TABLE III  
*Potassium Content of Paired Nerves (a and b) after Varying Times in O<sub>2</sub> and N<sub>2</sub>*  
 Potassium content in milliequivalents per 100 gm. initial wet weight.

| Gas            | (a)  |                 | (b)  |                 | ΔK              | Increase in weight |          |
|----------------|------|-----------------|------|-----------------|-----------------|--------------------|----------|
|                | Time | K content       | Time | K content       |                 | (a)                | (b)      |
|                | hrs. | m. eq. per cent | hrs. | m. eq. per cent | m. eq. per cent | per cent           | per cent |
| O <sub>2</sub> | 0    | 4.58            | 1    | 4.5             | -0.08           | 0                  | -0.1     |
| O <sub>2</sub> | 0    | 4.8             | 5.7  | 4.3             | -0.5            | 0                  | 0        |
| O <sub>2</sub> | 0    | 5.04            | 2    | 4.4             | -0.64           |                    |          |
| N <sub>2</sub> | 0    | 4.42            | 1    | 4.01            | -0.40           |                    |          |
| N <sub>2</sub> | 0    | 5.15            | 2    | 4.5             | -0.65           |                    |          |
| N <sub>2</sub> | 3.7  | 2.4             | 6.7  | 3.4             | +1.0            | +3.2               | +2.1     |
| N <sub>2</sub> | 6.7  | 3.1             | 16.8 | 2.8             | -0.3            | +7.0               | +13.2    |

Two pairs of nerves from two frogs were used for each experiment.

TABLE IV  
*Potassium Content of Nerves after Soaking in Ringer's Solution with and without Ligatures*  
 Potassium content in milliequivalents per 100 gm. initial wet weight.

| Time of immersion                    | K content    |                 | ΔK (b-a)        | Increase in weight |          | Condition      |
|--------------------------------------|--------------|-----------------|-----------------|--------------------|----------|----------------|
|                                      | Not tied (a) | Tied (b)        |                 | Not tied (a)       | Tied (b) |                |
|                                      | hrs.         | m. eq. per cent | m. eq. per cent | m. eq. per cent    | per cent | per cent       |
| (2 or more ligatures)                |              |                 |                 |                    |          |                |
| 5                                    | 3.34         | 2.95            | -0.39           | -2.1               | 0        |                |
| 5.45                                 | 3.44         | 2.62            | -0.72           |                    |          |                |
| 5.45                                 | 3.20         | 2.62            | -0.58           | +5.5               | +5.5     | N <sub>2</sub> |
| 6.0                                  | 2.52         | 2.29            | -0.23           | +4.7               | +7.6     | N <sub>2</sub> |
| 6.0                                  | 3.91         | 3.47            | -0.43           | -3.6               | +4.4     | X              |
| (One ligature)                       |              |                 |                 |                    |          |                |
| 7.0                                  | 3.25         | 3.26            | +0.01           | +1.8               | +3.6     | M              |
| 6.3                                  | 3.36         | 3.37            | +0.01           | -8.5               | -1.0     | M              |
| 4.3                                  | 4.29         | 3.41            | -0.79           | 0                  | 0        | M              |
| 7.5                                  | 3.00         | 3.12            | +0.12           | 0                  | -1.6     | M              |
| 6.5                                  | 4.34         | 4.26            | -0.08           |                    |          | C              |
| 5.5                                  | 3.30         | 2.90            | -0.40           | +2.5               | +3.3     | C              |
| 5.0                                  | 2.78         | 2.80            | +0.02           | -5.4               | +5.6     | C              |
| Mean . . . . .                       |              |                 | -0.29           | -0.51              | +2.74    |                |
| Standard deviation of mean . . . . . |              |                 | ±0.11           | ±1.40              | ±1.10    |                |

N<sub>2</sub>, solution equilibrated with nitrogen; others in oxygen.

M and C indicate location of ligature on central end (C) or middle (M).

X, two ligatures on (a) vs. many on (b).

for potassium. On the average the nerve with the ligature contained less potassium than the control, the difference being 0.15 m. eq. per 100 gm. with a standard deviation of the mean of 0.06. Although this difference is small (about 5 per cent) and the statistical reliability is not great, these experiments seem to confirm those with isolated nerves and indicate that the injury current is not the only factor involved in the changes which were observed. Furthermore, the fact that a ligature on the central end of the nerve has this effect eliminates the possibility that it is the result of an increase in pressure above the ligature

TABLE V  
*Effect of Ligatures on Nerves in Vivo on Their Potassium Content*  
Potassium content in milliequivalents per 100 gm. final wet weight.

| Time after tying                   | Location of ligature | K content              |                        | $\Delta K$             |
|------------------------------------|----------------------|------------------------|------------------------|------------------------|
|                                    |                      | Not tied               | Tied                   |                        |
| <i>hrs.</i>                        |                      | <i>m. eq. per cent</i> | <i>m. eq. per cent</i> | <i>m. eq. per cent</i> |
| 4.7                                | C                    | 3.89                   | 3.56                   | -0.33                  |
| 6.5                                | C                    | 4.34                   | 4.26                   | -0.08                  |
| 6                                  | C                    | 3.96                   | 3.70                   | -0.26                  |
| 6                                  | C                    | 2.88                   | 2.88                   | 0                      |
| 6                                  | M                    | 4.80                   | 4.34                   | -0.46                  |
| 7.3                                | M                    | 3.26                   | 3.25                   | -0.01                  |
| 7.3                                | M                    | 3.00                   | 3.12                   | +0.12                  |
| 7.0                                | M                    | 3.79                   | 3.42                   | -0.37                  |
| 7.0                                | M                    | 4.17                   | 4.17                   | 0                      |
| Mean.....                          |                      |                        |                        | -0.15                  |
| Standard deviation<br>of mean..... |                      |                        |                        | $\pm 0.06$             |

The ligatures were tied on the central end (C) or the midhigh region (M).

due to peripheral movement of fluid from the central nervous system. Some local mechanical trauma seems to be the only possibility.

#### *Effect of Acetylcholine on Potassium*

It was shown by Houssay, Marenzi, and Gerschman (1936) that the injection of acetylcholine into an animal causes a liberation of K into the blood stream so long as the liver is intact.

Other similar experiments have been quoted by Fenn (1940) together with converse experiments in which acetylcholine injection liberates K. In isolated preparations of the spinal cord of the frog immersed in Ringer's solution Dulière and Loewi (1939) have shown that acetylcholine plus eserine liberates 23 per cent of the potassium in 30 minutes. Conversely Shallek (1945) has found that immersion of isolated lobster nerves in isotonic potassium solutions caused 40 per cent of the acetylcholine to shift from the bound to the free form.

The experiments in Table VI were designed to confirm the loss of K from isolated nerves immersed in solutions of acetylcholine and to determine whether the acetylcholine liberated equivalent amounts of K. For this reason very strong solutions of acetylcholine were used which were 5, 50, or 100 per cent isotonic. Except in the first 3 experiments in Table VI in which the acid acetylcholine was not previously neutralized it is evident that the loss of K averaged

TABLE VI  
*Effect of Acetylcholine Bromide on the Potassium Content of Frog Nerve after Immersion*  
Potassium content in milliequivalents per 100 gm. initial wet weight.

| Time                            | Concentration of acetylcholine bromide | K content              |                        | $\Delta K$             | Increase in weight |                       |
|---------------------------------|--|------------------------|------------------------|------------------------|--------------------|-----------------------|
|                                 |  | Control                | Acetylcholine bromide  |                        | Control            | Acetylcholine bromide |
| <i>hrs.</i>                     | <i>per cent</i>                        | <i>m. eq. per cent</i> | <i>m. eq. per cent</i> | <i>m. eq. per cent</i> | <i>per cent</i>    | <i>per cent</i>       |
| 2.2                             | 2.5                                    | 3.50                   | 2.80                   | -0.70                  | +2.3               | +2.3                  |
| 3.5                             | 2.5                                    | 3.79                   | 2.95                   | -0.84                  | +2.1               | +7.8                  |
| 3.5                             | 0.13*                                  | 3.41                   | 2.40                   | -1.01                  | +2.1               | 0                     |
| 5.3                             | 0.13                                   | 4.03                   | 4.09                   | +0.06                  | +12.0              | +16.0                 |
| 4.2                             | 0.13                                   | 4.37                   | 4.20                   | -0.17                  | +8.8               | +7.6                  |
| 2.0                             | 0.13                                   | 4.64                   | 4.17                   | -0.47                  | +3.6               | 0                     |
| 1.6                             | 1.25                                   | 5.20                   | 4.78                   | -0.42                  | +4.1               | +1.8                  |
| 1.8                             | 1.25                                   | 4.73                   | 4.40                   | -0.33                  | 0                  | -3.6                  |
| 2.5                             | 1.25                                   | 4.10                   | 4.10                   | 0                      | +5.5               | -8.0                  |
| 2.5                             | 1.25                                   | 5.08                   | 4.30                   | -0.78                  | +14.0              | +2.8                  |
| 4.0                             | 1.25                                   | 3.70                   | 3.30                   | -0.40                  | -1.4               | -0.7                  |
| 4.0                             | 1.25                                   | 3.85                   | 3.80                   | -0.05                  | +7.8               | +4.2                  |
| Average.....                    |  |                        |                        | -0.28                  | +6.0               | +2.4                  |
| Standard deviation of mean..... |  |                        |                        | $\pm 0.05$             | $\pm 1.6$          | $\pm 2.2$             |

All solutions contained eserine, 1 part in 10,000. Except in the first 3 experiments the acetylcholine bromide was neutralized with NaOH prior to use. The larger losses of K in these 3 experiments must be attributed to the acidity; they are not included in the averages. In the last 6 experiments the solutions were made isotonic with NaCl solution to avoid precipitation of Ca. Ringer's solution was used in other cases except in 2.5 per cent acetylcholine which is already isotonic. Control nerves were in Ringer's solution or isotonic NaCl.

only 6.4 per cent of the amount present in the nerve and seemed to be no greater in the stronger solution. It must be concluded that the acetylcholine does not penetrate the nerve freely or that it liberates K by some process other than equivalent cation exchange. The experiments do not indicate, however, whether a similar loss of K would occur in physiological concentrations of acetylcholine.

#### *Effect of CO<sub>2</sub>*

In muscle it was shown by Fenn and Cobb (1935) that the effect of CO<sub>2</sub> on the loss of potassium depended upon the solution in which the muscle was im-

mersed. In Ringer's solution the loss of K from muscles was increased by 20 per cent CO<sub>2</sub> but in frog blood it was decreased. We have attempted to confirm this finding on frog nerve. In a single experiment in Ringer's solution equilibration of one of the solutions with 25 per cent CO<sub>2</sub> in O<sub>2</sub> instead of pure O<sub>2</sub> decreased the K content of the nerve after immersion for 3 hours from 4.13 to 3.70 m.eq. per cent.

For similar experiments in blood a needle was inserted into the aorta and as much blood as possible was withdrawn with heparin as an anticoagulant. The

TABLE VII  
*The Effect of CO<sub>2</sub> on the K Content of Nerves after Immersion in Blood*  
Potassium content in milliequivalents per 100 gm. initial wet weight.

| CO <sub>2</sub>               | Time        | K content of nerve     |                        | K difference           | Increase in weight     |                 |
|-------------------------------|-------------|------------------------|------------------------|------------------------|------------------------|-----------------|
|                               |             | O <sub>2</sub>         | CO <sub>2</sub>        |                        | Control O <sub>2</sub> | CO <sub>2</sub> |
| <i>per cent</i>               | <i>hrs.</i> | <i>m. eq. per cent</i> | <i>m. eq. per cent</i> | <i>m. eq. per cent</i> | <i>per cent</i>        | <i>per cent</i> |
| 20                            | 4.5         | 4.33                   | 4.32                   | -0.01                  | +1.4                   | +1.3            |
| 21                            | 8.3         | 4.75                   | 5.10                   | +0.65                  | +1.7                   | +8.8            |
| 21                            | 6.0         | 2.00                   | 2.13                   | +0.13                  | -2.8                   | +1.3            |
| 22                            | 6.2         | 4.17                   | 4.40                   | +0.23                  | +4.0                   | +3.0            |
| 35                            | 5.3         | 4.02                   | 4.05                   | +0.03                  | -9.1                   | -8.1            |
| 14                            | 5.0         | 4.37                   | 4.53                   | +0.16                  | -5.1                   | 0               |
| 9                             | 5.0         | 4.58                   | 5.09                   | +0.51                  |                        | (-4.0)          |
| 9                             | 3.2         | 3.55                   | 3.43                   | -0.12                  | -1.0                   | +8.5            |
| 9                             | 4.2         | 2.92                   | 3.50                   | +0.58                  | -10.4                  | -13.5           |
| 9                             | 3.3         | 3.02                   | 3.63                   | +0.61                  | -7.6                   | -4.0            |
| Average.....                  |             |                        |                        | +0.27                  | -3.2                   | -0.3            |
| Standard deviation of mean... |             |                        |                        | ±0.09                  | ±1.6                   | ±2.3            |

The last five experiments are from Fenn and Cobb (1935). Of these, the last three differed in that the control nerves were analyzed before the experimental period. Had they been left longer they presumably would have contained even less potassium.

results are shown in Table VII together with five similar experiments on nerve previously published (Fenn and Cobb, 1935). In seven of the ten experiments the nerves in CO<sub>2</sub> contained slightly more K at the end of immersion than the control nerves. The average difference for all the experiments was  $0.27 \pm 0.09$  (standard deviation of mean). Thus the losses which occurred were very small and the gains were relatively large so that on the average it seems probable that there was a gain (or less loss) of K in the presence of CO<sub>2</sub>, in agreement with the muscle experiments. One confirmatory experiment was tried in which both pairs of nerves were equilibrated in 15 per cent CO<sub>2</sub> but one was in blood and the other in Ringers' solution. After 3 hours' soaking, the potassium content was 4.15 m.eq. per cent in blood and only 2.66 m.eq. per cent in Ringer's.



Our interpretation of these experiments was that the K tends to move toward the solution where the increase in H ion was greater. Since the nerve is intermediate in its buffer capacity between Ringer's solution and blood (*cf.* Fenn, 1928) this seems to provide a satisfactory explanation.

## SUMMARY

1. Frog nerves immersed in Ringer's solution lose on the average 23 per cent more potassium if the solution is equilibrated with pure nitrogen than if equilibrated with oxygen.
2. Tying off the ends of the nerves during immersion increased rather than diminished the loss of potassium.
3. There was some evidence that nerves tended to regain potassium if they were returned to oxygen after a period of anoxia.
4. Addition of acetylcholine to the solution increases the loss of potassium.
5. Equilibration of the solution and nerves with 20 per cent CO<sub>2</sub> in O<sub>2</sub> increases the loss of K from nerves in Ringer's solution but decreases it in frog blood.

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