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ELECTROLYTE CONTENT AND ACTION POTENTIAL OF THE GIANT NERVE FIBRES OF *LOLIGO*

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It is usually assumed that the polarization of the bounding membrane of nerve and muscle fibres, and the consequent manifestation of a resting or "injury" potential, may be ascribed to the differential permeability of the membrane to the various species of ions which are found in the intra- and extracellular fluids. To be more precise, it is suggested that the membrane is almost impermeable to anions, very slightly, if at all, permeable to the larger cations (including $Na^{\cdot}$), but freely permeable to $K^{\cdot}$ and $H^{\cdot}$. The action potential, on which the conducted impulse of the tissues probably depends [see Hodgkin, 1937, and others], is produced, then, by a momentary change in these properties of the membrane, which, during the passage of the impulse, is freely permeable to all ions. A full discussion of the theory, together with some new evidence, is given by Höber, Andersh, Höber & Nebel [1939].

Hitherto the electrical phenomena have been studied most closely in nerve, and the distribution of electrolytes in muscle, so that no really complete attempt has yet been made to discover whether the magnitudes of the resting and action potentials agree with that of the diffusion potential which might be expected from the distribution of electrolytes. Cowan [1934] estimated that the concentration of potassium was 13 times as great inside as outside the axons of the crab *Maia*. This would give a maximum possible injury potential of 64·6 mV., whereas the greatest potential which he observed was 42·1 mV. It now seems probable

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that his estimates both of the internal potassium concentration and of the injury potential are much too low. Fenn, Cobb, Hegnauer & Marsh [1934] have made careful estimates of the amount of potassium in whole nerves of frogs and Crustacea, and hence, on the assumption that all the chloride is outside the nerve fibres, of the internal potassium concentration. But the assumptions involved are so uncertain that it is not possible to accept with confidence the view of Erlanger & Gasser [1937], based on these analyses, that the internal potassium concentration is 65 times as great as the external, and able therefore to give a diffusion potential of 118 mV.[1]

In fact all previous attempts to establish a quantitative co-ordination between electrolyte content and action potential have met with the difficulty that, since they were made on whole nerve trunks, there was present a considerable volume of intercellular fluid, fibre sheaths and connective tissue elements interspersed among the fibres. Thus on the one hand the observed potentials were, on account of short-circuiting, less than those produced at the surface of individual fibres, and on the other hand there was introduced into the chemical analyses an error which is considerable and difficult to assess.

These difficulties may be eliminated by using the nerves of the squid, as it is possible to isolate a single giant fibre from all associated tissues (with the exception of a thin sheath), measure its potential, and then submit it to chemical analysis. This has been done for a number of fibres, and the results are presented below. It must be emphasized that the relation between action potential and potassium concentration gradient has been determined separately for each individual fibre; there has been no averaging out of results.

At the same time Hodgkin & Huxley [1939] have investigated the absolute magnitude of the action potential, especially as recorded by means of an electrode *inside* the axoplasm. The maximum potentials which they observed in this way make available for the first time for correlation with the electrolyte concentrations a reliable estimate of the value of the action potential.

Unfortunately the work was terminated by the outbreak of war, which rendered the capture of further squids impossible, so that the number of fibres dealt with is much smaller than might have been wished. Nevertheless, the results are sufficiently consistent to afford a basis for discussion.

[1] In any case this figure is erroneous. A concentration ratio of 65 could give rise to a diffusion potential of only 105 mV. at a temperature of 20° C.

While this work was in progress a paper by Bear & Schmitt [1939] was published, in which similar analyses of the giant fibres of a closely related species, *Loligo pealii*, were given, though without any attempt to correlate them with the action potential. On all essential points we find ourselves in agreement with their conclusions; such minor differences as exist are discussed below. The number of their analyses is far greater than that of ours, but the amount of variation in the figures for their different batches (each of which represents several fibres analysed together) would seem to indicate that experimental error is on the whole likely to be greater than errors of sampling. On the other hand it is probable that our analytical methods were the more satisfactory; the fact that Bear & Schmitt obtained average values for the K/Cl and Total base/Cl ratios in sea water of 0·0257 and 0·0296, whereas the true values are 0·0201 and 0·0312 [see Webb, 1939], suggests the possibility of systematic errors, particularly in the potassium determinations. Bear & Schmitt deduced the composition of the axoplasm by analysing first the whole fibre, and then the sheath from which the axoplasm had been extruded. Our method for correcting for the sheath is indirect. Neither method is wholly satisfactory; ours rests on assumptions which are plausible but cannot be directly proved, while that of Bear & Schmitt is open to suspicion on account of the figure (almost certainly too high) which they give for the concentration of chloride in the sheath. The volume of the sheath is, however, so small in comparison with that of the axoplasm that errors in making allowance for it are not likely to affect seriously the figures calculated for the axoplasm.

During this investigation special attention was paid to the state of the fibres before analysis, and the action potential of each fibre was tested along its whole length before it was dried for analysis. Each fibre was then analysed individually to allow of a comparison between the observed action potential and that which could be obtained as a diffusion potential from the observed potassium content.

Methods

The fibres were dissected out, and the action potentials recorded at Plymouth; the chemical analyses were carried out at Cambridge. The hindmost stellar nerve was removed from a freshly killed squid (*Loligo forbesi*), care being taken to cut all branches as long as possible and to avoid kinking of the nerve. The ends were tied with fine cotton before removal, and the nerve then stretched out by means of the threads in a Petri dish filled with off-shore sea water.

The giant fibre was carefully dissected away from the smaller fibres in the nerve by means of a dissecting microscope, fine scissors, watchmaker's forceps and a very fine, sharp cornea knife. The procedure adopted was to cut transversely across a bundle of the smaller fibres close to the end of the nerve, and then, holding this bundle of small fibres with the forceps, to separate it gently from the giant fibre, cutting downwards with the cornea knife against the bottom of the dish. On reaching a point at which the giant fibre branches great care is taken to clean the branch, which must be cut *long*, otherwise the main fibre is apt to be damaged by the cut. By carefully working up and down the length of the giant fibre in this way the small fibres can be wholly removed in 2–3 hr. The best results were obtained by using a standard technique, in which the fibre was cleaned first from its upper outer edge downwards, and then from its lower inner end upwards. The final stages of the cleaning are very laborious, and small pieces of connective tissue were still left adherent even after the best dissections, especially near the points of branching. In all cases these were reduced as much as possible, and their volume must be very small in relation to that of the fibre.

After dissection the fibres were tested on the oscillograph, using an arrangement of non-polarizable electrodes similar to that described by Hodgkin [1938]. In order to avoid contamination of the surface the fibres were not raised into oil but into air. The maximum action potential of 83·5 mV. observed with this procedure is little less than the "80–95 mV." obtained by Hodgkin & Huxley [1939] by means of an internal electrode. The figures given here for the action potential are therefore likely to be only slightly less than the maximum that could be obtained from the fibres.

Except for the first two fibres studied, only those parts which gave an action potential of 60 mV. or more were used. The first two, though showing no visible signs of injury, gave only local potential responses. In the other cases lengths up to 10 cm. were obtained which gave action potentials of over 60 mV. throughout their length, and had a wet weight of up to 23 mg.

After the oscillograph record had been taken the fibres were in some cases washed for 2 min. with two changes of a sucrose solution isotonic with the sea water; in other cases they were taken direct from sea water. The significance of these two series is explained later. In either case the fibre was carefully blotted twice on filter paper, lifted at one end, and the ligature at the other end cut off with dry and very fine scissors. The severed end was allowed to fall on to a small slip of Monax glass, formed

by blowing out a thin bulb from the end of a tube and breaking it into fragments. The fibre was then laid on this slide in loops, and finally the remaining ligature cut off, the slide placed in a small Monax tube, and the whole weighed immediately on a short-arm micro-balance. The fibres were then dried for 3 hr. at 105° C. and the tube weighed once more and finally sealed off for storage and transport. The slide and tube, which had been freed from soluble material by treatment with nitric acid followed by rinsing in distilled water and steaming, were weighed together on the micro-balance before the fibre was dissected, so that by this means the wet and dry weights of the fibre were obtained. The latter was also determined again at Cambridge by weighing the glass slip with the dried fibre attached, and reweighing after the fibre had been washed off for analysis. The two determinations of the dry weight agreed, in all cases but one, to within 0·02 mg.

The following experiment shows that a short immersion in isotonic sucrose solution, though it temporarily sends up the resting potential and abolishes the irritability, brings about no irreversible change in the properties of the fibre, and may therefore be presumed not to lead to the loss of any electrolytes.

Fibre excitable in central region only.

7.15. Action potential in sea water 63 mV.

7.20. Action potential in sea water 59 mV.
Resting potential in sea water 28 mV.

7.21. Sucrose solution dropped on fibre near the electrode, sucked off and replaced by a fresh drop. Threshold rises, and fibre becomes inexcitable in 1 min. Resting potential rises steadily to 48·5 mV.

7.25. Fibre replaced in sea water. With half a minute excitability is recovered.
Action potential 56 mV.
Resting potential 28 mV.

N.B. The absolute value of the resting potential is of no significance, as it was measured with respect to the inexcitable region, which was probably not wholly "dead".

Some fibres were used for the determination of chloride; others for determination of potassium and total base.

Chloride estimations were performed by the micro-diffusion method of Conway [1935], the iodine being estimated titrimetrically against 0·05 N sodium thiosulphate, delivered from a Rehberg micro-burette. The slip on which the fibre had been dried was soaked for half an hour in the outer compartment of the Conway unit in 1 ml. of water before the other reagents were added. Several blanks were carried out; their mean value was equivalent only to 0·4 μg. of Cl. Trials with standard sodium chloride solution showed that the analytical error seldom exceeds 2 %.

For the determination of potassium and total base the fibres were detached from the glass slips by warming them in a mixture of dilute sulphuric and nitric acids in a silica crucible of 5 ml. capacity. When most of the organic matter had been destroyed the glass slip was removed with wax-tipped forceps and washed with a fine jet of distilled water. The contents of the crucible were evaporated down, heated cautiously at 300° C. to drive off the excess sulphuric acid, and then ignited for half an hour at 800° C. in an electric furnace. At this temperature the bisulphates are completely decomposed, but there is no risk of volatilization of the sulphates. The crucible was then weighed on a micro-balance, and reweighed later after the sulphates had been washed out for the potassium determinations. From the difference in weight, which represents the weight as anhydrous sulphates of the total base present in the fibre, it is possible to calculate the total base in milli-equivalents with considerable accuracy, even without precise information as to the relative abundance of the different bases. Since the potassium is determined later the weight of potassium sulphate can be calculated. The difference between this and the total weight of sulphates may be presumed to be accounted for by sulphates of Na, Ca and Mg. One equivalent of each of these three bases yields 71·0, 68·1 and 60·2 g. of sulphate respectively. If a mean figure of 68 is taken as a basis for calculation it is extremely unlikely that the error arising from unexpectedly large quantities of Na or Mg will exceed 4 % in the estimation of Na + Ca + Mg as equivalents, and therefore should not exceed 2 % in the estimation of total base.

The determination of potassium was performed by the method of Robertson & Webb [1939] (precipitation as potassium silver cobaltinitrite, which is estimated by titration with ceric sulphate). The quantities and volumes of all reagents were halved, since the potassium to be estimated was only of the order of 0·15 mg. The final titration was carried out with 0·006*N* ferrous ammonium sulphate. A blank was carried out by washing out one of the Monax tubes, containing a glass slip, with a known quantity of standard potassium sulphate solution. The result was indistinguishable from a direct analysis of the same amount of potassium. Since at least two standards were analysed with each fibre the method may be considered to be free from systematic errors; fluctuating errors are usually less than 2 %.

Allowance for sheath and external film of fluid

The sheath by which the giant fibre is surrounded consists mainly of concentric collagenous lamellae; between these and the axoplasm lie the very thin metatropic layer and a "protoplasmic" layer in which nuclei are imbedded [Bear, Schmitt & Young, 1937]. It is reasonable to assume that the bulk of this sheath becomes rapidly permeated by whatever solution bathes it; in other words that the effective barrier to permeation lies at the surface of the axoplasm. This is the basis of our method for correcting the results for the volume of the sheath and of the thin film of fluid which persists on its surface even after blotting. Two fibres (nos. 1 and 2, Table I), which had come from sucrose solution, and two (nos. 4 and 7), which had come straight from sea water, were analysed for chloride. The former gave a mean value of 2·88 μg., and the latter of 4·77 μg. per mg. of total wet weight. The difference represents the chloride present in the sheath and surface film of the fibres from sea water. Since the sea water contained 19 g. Cl per litre we may calculate that for each mg. of total wet weight there is present 0·099 c.mm. of sheath plus external solution. From this the necessary corrections to the wet weight, dry weight, total base and chloride content of each of the fibres can be calculated. It is assumed that this 0·099 c.mm. of fluid is associated with 0·015 mg. of collagen, this being the approximate ratio between water content and dry weight in connective tissue.

The figures thus arrived at (axoplasm 88·5 %, sheath 11·5 % of the whole) are supported by histological data. Examination of a number of sections of giant fibres, fixed, embedded and stained in various ways, showed that the average thickness of the sheath is 6·5–7 % of the radius of the whole fibre (including sheath), and therefore that the relative volumes of the sheath and axoplasm are as 12·5 to 87·5. Fig. 2 in Bear *et al.* [1937], which is of a section cut very close to the ganglion, and selected in order to show the details of the sheath structure, is quite misleading with regard to the relative volumes of axoplasm and sheath over most of the course of the fibres. A better idea is given by Fig. 1 in Pumphrey & Young [1938].

Bear & Schmitt [1939] found that the axoplasm which they extruded represented only 82 % of the weight of the fibre. It is probable however that some axoplasm adheres to the inside of the sheath; it is also possible that they did not carry the dissection of the fibres to the maximum possible extent. There is therefore no need to assume any discrepancy between the two sets of figures in this respect.

Results

The individual analyses are set out in Table I, and summarized for comparison with those of Bear & Schmitt in Table II. It will be seen that we find a chloride concentration in the axoplasm equivalent to 20 % of that in sea water, and a potassium concentration 29 times as great as that in sea water. The corresponding figures of Bear & Schmitt are 25·5 % and 26 times. The only serious discrepancy lies in the figures for

Table I

No. of fibre ...	1	2	3	4	5	6	7	8	9
Taken from ...	Sucrose	Sucrose	Sucrose	Sea water	Sucrose	Sea water	Sea water	Sucrose	Sucrose
Wet weight (mg.)	8·078	8·222	8·814	11·105	8·650	17·677	7·425	11·722	23·072
Dry weight (mg.)	1·296	1·536	1·607	1·540	1·445	2·308	1·034	1·914	3·888
Weight of ash as sulphates (mg.)	—	—	0·226	—	0·201	0·625	—	0·323	0·759
Total K (μg.)	—	—	54·5	—	64·7	167·6	—	101	208
Total Cl (μg.)	21·4	25·5	—	45·9	—	—	40·1	—	—
Wet weight of axoplasm (mg.)	7·028	7·153	7·668	9·773	7·526	15·559	6·534	10·200	20·074
Dry weight of axoplasm (mg.)	0·971	1·207	1·254	1·318	1·099	1·955	0·886	1·445	2·965
Dry weight of axoplasm as % of wet weight	13·8	16·8	16·3	13·5	14·6	12·6	13·6	14·2	14·8
Weight of intracellular water (mg.)	6·057	5·946	6·414	8·466	6·427	13·604	5·648	8·755	17·109
Extracellular ash as sulphate (mg.)	—	—	0	—	0	0·072	—	0	0
Extracellular Cl (μg.)	0	0	—	20·9	—	—	14·0	—	—
Intracellular Cl (m.eq./kg. water)	100	121	—	83·2	—	—	130	—	—
Intracellular K (m.eq./kg. water)	—	—	218	—	258	314	—	295	311
Total base (m.eq./kg. water)	—	—	460	—	389	510	—	460	565
Maximum potential attainable by diffusion of $K^{\cdot}$ (mV.)	—	—	78	—	82	87·5	—	85·5	87
Maximum action potential observed (mV.)	—	—	63	83·5	73	83·5	73	82	80·5

total base; we find this to be 20 % lower in the axoplasm than in sea water, whereas Bear & Schmitt find it to be 15 % higher than in sea water, though slightly lower than in the blood. It is hard to say which of these is more likely to be correct; instances may be found in the literature of analyses of the tissues of marine molluscs which reveal a total base content far higher [M'Cance & Shackleton, 1937] or far lower [M'Cance &

Shipp, 1933; Krogh, 1938*a*] than that of the sea water in which the animals were living. Cowan [1934] and Schmitt, Bear & Silber [1939] found the concentration of total base in the whole nerves of Crustacea to be almost identical with that in the blood and in sea water. It is probably best to assume that the same is true in *Loligo*, and that a figure intermediate between ours and that of Bear & Schmitt is correct.

TABLE II. Figures are expressed in mM. or m.eq./kg. of water

	Webb & Young		Bear & Schmitt [1939]		
	Axo-plasm	Sea water	Axo-plasm	Blood	Sea water
Cl (mM.)	109	540	130	530	510
K (mM.)	279	9·7*	310	17	12
Total base (m.eq.)	477	597*	620	670	540
Sum of ions accounted for (mM.)	565†	1102*	715†	1130†	995†
Anion deficit (total base – Cl) (m.eq.)	368	57‡	490	140	30

* Calculated from the chlorinity in accordance with the established ionic ratios.

† These figures are subject to some uncertainty, as it is not known how much of the base other than K is univalent (Na) and how much bivalent (Ca + Mg). In calculating our own figure it has been assumed that the ratio of Na to Ca + Mg is the same in the axoplasm as in sea water, so that the mean valency of the bases other than K is 1·12. The figures of Bear & Schmitt (which were given as 750, 1200 and 1050 mM. for axoplasm, blood and sea water respectively, on the assumption that all the base is univalent) have been emended accordingly. This procedure is obviously justifiable for sea water, probably also for the blood, which, judging by the data available for other molluscs [see Krogh, 1939, p. 56], is not likely to differ much in composition from sea water, and with respect to the axoplasm seems to be more plausible than assuming complete absence of bivalent bases.

‡ Of this over 55 m.eq. is accounted for by SO_4^{--}, the remainder by HCO_3^-.

It will be noticed that Bear & Schmitt provide analyses of the blood for comparison with those of the axoplasm. We have not done this, since for our immediate objective—the determination of the physico-chemical basis of the action potential—it is irrelevant; the potential is measured when the nerve is bathed not with blood but with sea water. But for general questions of electrolyte equilibria at the cell surface it is of course with the blood that the axoplasm must be compared. A difficulty lies in the fact that Bear & Schmitt do not state whether they irrigated the nerves during dissection with blood or with sea water; if, as is probable, it was with the latter, it is uncertain to what extent the composition of the fibre may have altered by reaching a new equilibrium with its changed environment. In the case of our fibres, which had always been bathed in sea water for at least 2 hr. before being dried for analysis, it might be supposed that re-equilibration was fairly complete, but it is impossible to speak with any certainty. In any case it seems that except for the calculation of the potassium ratio the differences in composition between the blood and sea water are not sufficient to give rise to any serious errors.

Discussion

(1) *Action potential.* The last two lines of Table I show that there is good agreement between the action potentials observed and the diffusion potentials which would arise if the membrane separating the axoplasm from the sea water were permeable only to potassium ions and were rendered permeable to anions during the passage of the impulse. These have been calculated from the formula $E=\frac{RT}{F}\log_e\frac{[K^+]\text{ inside}}{[K^+]\text{ outside}}$, which at 20° C. becomes $E=58\log_{10}\frac{[K^+]\text{ inside}}{[K^+]\text{ outside}}$ mV. The observed potentials are on the average 7·5 mV. below the calculated ones. This may be ascribed to various causes, of which the most important is probably the short-circuiting between the electrodes, which were placed, of course, on the outside of the fibres. In no case was the observed potential greater than that calculated from the potassium concentration, but it must be remembered that, using internal electrodes, Hodgkin & Huxley [1939] found potentials as high as 95 mV., that is to say greater than could be explained by any of the potassium concentrations found in the series of fibres here analysed. It would appear, therefore, that diffusion of potassium ions is the principal factor responsible for the development of the action potential, but that there may be other as yet unknown agencies which raise it by about 10 mV.

The figures quoted for the action potential denote the difference between the resting potential and the summit of the spike. After passage of the impulse the potential returns to a figure 15 mV. or more above that of the resting potential (the "positive after-potential"), and only gradually returns to its initial value. The total span of the curve may be as great as 105 mV.; to obtain this as a diffusion potential would require a ratio of internal to external potassium concentrations of 65, which is out of the question. It is clear that the process responsible for the phase of after-positivity cannot be the migration of potassium ions across the cell membrane.

(2) *The resting potential.* Hodgkin & Huxley find that the resting potential is about 50 mV. This figure is of necessity an arbitrary one, as it differs from the true potential across the cell surface by the amount of the liquid junction potential between the axoplasm and the internal sea-water electrode. The sign of this correction will clearly be such as to make the real resting potential greater than the apparent one; for the greater mobility of K^+ (dominant in the axoplasm) in comparison with Na^+ (dominant in the sea-water electrode) and the greater mobility of the anions of sea water in comparison with those in the axoplasm (which

are largely unknown, but almost certainly less mobile than Cl′) will both tend to render the internal electrode positive with respect to the axoplasm. The magnitude of the correction is far more difficult to assess; ideally it should, of course, be such as to make the resting and action potentials identical, so that at the top of the curve the potential across the surface is zero. This would imply a liquid junction potential of 35–40 mV., which seems improbably high. Some more definite indication as to the nature of the missing anions in the axoplasm is necessary before any conclusion can be reached on this point.

If the resting potential arises entirely from diffusion of potassium ions it should be possible to abolish it, or even to reverse it, by raising the concentration of potassium in the surrounding solution. We have not had an opportunity of performing any experiments of this type, but Cowan [1934], working on the nerves of *Maia*, found that as the potassium content of the surrounding solution was raised the resting potential fell off, until at a concentration of 17 to 26 times the normal it vanished. This figure is approximately what would be expected from the internal potassium concentration which we and Bear & Schmitt [1939] have found in *Loligo*. Cowan's figure of 13 for the potassium concentration ratio is of course too low; since the nerve he used contains about 65% axoplasm [Young, 1936] the true ratio is presumably about 20. He found that even an external potassium concentration of 50 times the normal did not produce a reversal of potential. Cowan's explanation of this in terms of a Donnan equilibrium can hardly be adequate, since for the establishment of such an equilibrium it is necessary that the membrane should be permeable to at least one species of anion. It is more likely, as Höber *et al.* [1939] suggest, that the cytolytic effects of such high potassium concentrations render the membrane much more permeable, and thus prevent the realization of any such potential reversal.

(3) *Intracellular chlorides.* The agreement of our results with those of Bear & Schmitt leave little doubt that the axoplasm contains chlorides at a concentration of about 0·12 *M*. There is a considerable body of evidence for supposing that the cells (other than erythrocytes) of vertebrates, or at any rate of mammals, are chloride free [see Fenn, 1936, for discussion], but the indiscriminate extension of this principle has recently been contested by Krogh [1938*a*, 1938*b*] and by Conway and his school [Conway & Cruess-Callaghan, 1937; Conway & Boyle, 1939; Conway, Kane, Boyle & O'Reilly, 1939]. According to Krogh [1938*b*] "while it may be true that Cl is not normally present inside muscle fibres in mammals and perhaps even in frogs it is very doubtful if this holds for

tissues generally, even in mammals, and it is certain that it does not hold for a probably very large number of lower animals". It seems possible that the distinction lies not between higher and lower animals as such, but between those forms (vertebrates and non-marine invertebrates) in which the anionic concentration of the body fluids is sufficiently low (*ca.* 0·2 *M*) to be entirely replaced inside the cell by protein, phosphates, bicarbonates and organic anions, and marine invertebrates, in which the anionic concentration of the body fluids is so high (*ca.* 0·6 *M*) that inside the cell some chloride is required to make up the total. On the other hand, recent analyses of the muscle of *Loligo* [Manery, 1939] and the nerve of *Homarus* [Schmitt *et al.* 1939] suggest that in these tissues the concentration of intracellular chlorides is much less than that in the axoplasm of *Loligo*, and is perhaps little greater than that in the cells of vertebrates.

The claim of Conway and co-workers to have demonstrated the presence of chloride in the fibres of the frog's sartorius is of importance with reference to the properties of the membrane and the mechanism of potassium accumulation, but the normal concentration of intracellular chloride would appear to be so small (not more than 4 mM., and perhaps less than 2 mM./kg. water) that, in the drawing up of osmotic and electrolytic balance sheets, it may be ignored. Conway & Boyle claim that the product of potassium and chloride concentrations inside and outside the fibre are equal. It is clear from our figures that this rule does not apply in the case of our material.

(4) *Potassium concentrations in nerve and other cells.* The concentration of potassium in the axoplasm of *Loligo* seems to be as high as in any type of cell that has been investigated, though the number of reliable analyses is rather meagre. Fenn *et al.* [1934] found 158 mM./kg. of whole nerve in *Libinius* (a spider crab) and 203 mM. in *Homarus*, which probably implies a potassium concentration in the axoplasm of about 300 mM./kg. water, approximately equal to that in *Loligo*. Cowan's [1934] analyses of *Maia* nerve, corrected on the assumption that 65 % of the nerve is axoplasm [Young, 1936], yields a similar but rather lower figure of 230 mM./kg. of axoplasm water. In vertebrates, where the total concentration of the serum is only 240–350 mM., the potassium content of nerve is naturally rather lower. Fenn *et al.* [1934] found 3·0–4·8 m.eq./100 g. whole nerve in the frog, from which they calculate that the "potassium space" (axoplasm) contains 176 mM.[1] potassium/kg. water,

[1] Figures quoted from Fenn *et al.* [1934] and Fenn [1936], which are there expressed in terms of the total water of the tissue, have been recalculated and expressed in terms of intracellular water.

that is to say a concentration about 65 times as great as that in the plasma. Pichler's [1934] analyses of the whole central nervous system of frogs points to a similar figure for intracellular potassium. The analyses made by Alcock & Lynch [1911] of the splenic nerve of the horse do not permit of more than about 125 mM./kg. axoplasm water.

For non-nervous tissues the only figures of particular relevance are those of Manery [1939] and Bialaszewicz & Kupfer [1936]. The latter in their analyses of muscles found 190 mM. potassium/kg. in *Loligo*; this corresponds to perhaps 250 mM./kg. of fibre water. Manery's figures for the same material are, however, much lower (114 mM./kg. muscle, corresponding to about 160 mM./kg. of fibre water). Figures for vertebrate muscle are again lower (125 mM./kg. water in the "potassium space" of frog muscle, according to Fenn [1936]).

It should be mentioned that M'Cance & Shackleton [1937] reported very high potassium concentrations, up to 400 mM./kg. of total body water, in certain Gastropods, but did not localize the potassium in any particular tissue.

It has often been suggested that part of the potassium inside cells is "bound", that is to say non-ionized. If this were true of our material it would mean that the anion deficit would be less than appears, but on the other hand the osmotic deficit would be greater. Actually the evidence for bound potassium (discussed by Fenn [1936]) has never been very strong, and it seems best to assume that in the axoplasm it is entirely ionized. Pichler [1934] argues from the insolubility of much of the potassium of the frog's central nervous system in 96 % alcohol (in which KCl is easily soluble) that less than half of it is ionized. But, as has been shown above, the chloride content of vertebrate cells is so low that only a small fraction of the potassium can be present as KCl. Furthermore, the condition in the central nervous system may be different from that in peripheral nerve, and it also seems possible that treatment with 96 % alcohol may precipitate or fix the potassium in the form of compounds which do not exist in the living cell. No plausible chemical explanation of the condition of the alleged bound potassium has yet been given. The theory of Koch & Pike [1910] that most of the potassium is bound to the lipoids, particularly cephalin, can only apply to heavily myelinated fibres; in any case it has been criticized by Page [1937, p. 90], who points out that cephalin has no base-binding powers.

(5) *Anionic and osmotic deficits.* This aspect has been discussed by Bear & Schmitt [1939], and little more need be said. Our analyses imply an anion deficit of 368 m.eq. and an osmotic deficit of 735 mM.; theirs an

anion deficit of 490 m.eq. and an osmotic deficit of 280–415 mM. As is pointed out by Bear & Schmitt, bicarbonates, sulphates, phosphates, lactates, and proteins acting as acids are quite inadequate to balance the excess base, since together they could hardly amount to more than 120 m.eq. It follows, therefore, that some completely unknown anion or anions must be present at a concentration of at least 0·25*N*. From the figures of Bear & Schmitt it would appear that at least a fraction of these anions must be multivalent; on the other hand, if ours are accepted it would be possible for them all to be univalent and still leave an osmotic deficit of 170 mM. to be filled by non-electrolytes or ampholytes. It is worth recalling in this connexion that Kelly [1904] found taurine in concentrations up to 400 mM./kg. in the muscles of lamellibranchs, and that considerable quantities of betaine are present in the muscles of *Octopus* [Henze, 1911]. Schmitt *et al.* [1939] have brought forward some evidence which suggests that in *Homarus* nerves the missing anions may for the most part be dicarboxylic amino-acids.

The only other animal cells for which balance sheets of the internal and external electrolytes have been drawn up are the fibres of the frog's sartorius and the human erythrocyte. In the former [Fenn, 1936] there is no osmotic deficit, but there is an anion deficit of about 30 m.eq. after allowance has been made for proteins. Variation in the phosphate content, however, coupled with lack of precise information as to the *p*H of the interior of the fibre, renders the calculations rather precarious. In the case of the human erythrocyte there is neither an osmotic nor an anion deficit, since Farmer & Maizels [1939] have recently shown that the organic phosphates are more than adequate to cover the base not bound by chloride, bicarbonate or protein.

From various fragmentary analyses scattered through the literature [see Krogh, 1939] it would seem that considerable osmotic and anion deficits, particularly the latter, are often to be found in the tissues of marine invertebrates.

Summary

1. Action potentials have been recorded from a number of isolated and cleaned giant nerve fibres of *Loligo forbesi*, and the same fibres then subjected to chemical analysis.

2. The average concentrations of chloride, potassium and total base in the axoplasm are 109 mM., 279 mM. and 477 m.eq. respectively per kg. of water. There are large anionic and osmotic deficits. These analyses agree in most respects with those of Bear & Schmitt on the fibres of *L. pealii*.

3. The observed action potentials approximate closely to the diffusion potentials which would be produced if the bounding membrane of the axoplasm were permeable only to $K^{\cdot}$, except during the passage of the impulse.

4. The results are compared with the available data for the electrolyte content of other types of cell.

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