in approximate calculations, so that we may write for (1) and (2) :

$$V = \frac{2Kc}{c^2 - 4kd} \quad . \quad . \quad . \quad . \quad . \quad (6)$$

$$\text{and} \quad k_1 = c/2, \quad . \quad . \quad . \quad . \quad . \quad (7)$$

From equations 6 and 7 it appears that as potassium is substituted for sodium in the external solution, the potassium concentration in the cell will remain unchanged, but potassium will enter and the cell volume increase. As the external concentration is increased, the potassium concentration inside will increase ; but if the external potassium is also raised, the volume will stand higher than otherwise and potassium will have entered the cell.

When excised muscle is immersed in Ringer or Barkan fluid, potassium is lost until the external potassium is 29 milliequiv./litre. The raised equilibrium value appears associated with widening of the cation and anion pores, and it is from this raised value we shall consider the effect of potassium changes. The interspace fluid is taken as 9 ml./100 gm. tissue (from magnesium and inulin methods), though small differences with total volume changes will not materially affect the calculations.

The following table illustrates the kind of results obtained ; each figure being the mean of four or five experiments. Concentrations are expressed as milliequiv./litre of external fluid or 'fibre' water (which latter is normally 71 ml./100 gm. muscle).

Potassium conc. in ext. fluid	Total conc. of ext. fluid	Potassium conc. of 'fibre' water		Potassium entry. Milliequiv./litre of 'fibre' water
		Exper.	Theor.	
2·5	240 (plasma)	119	120	
33·5	248	115	124	12·5
103	256	121	128	83·8
103	384	196	192	33·2
103	534	263	267	8·8

For equation 3 we have the following data, taking chloride as an example of the permeable anion.

External conc.		Inside conc.		Products	
K	Cl	K	Cl	K × Cl	K × Cl
29	106	119	26·6	3074	3165
80·4	132	145	73·0	10613	10585

Original entrance of potassium and relation of potassium interchanges to carbohydrate metabolism. Here we shall consider briefly an apparently easy and effective means of increasing the potassium in cells without change in external potassium or in total concentration outside. If, in the above scheme, impermeable anions are formed from permeable, V the volume of the cell will increase from equation 6, and potassium and more diffusible anion will enter the cell in accordance with equations 3, 2 or 7. No intermediation of hydrogen ion with its excessively low gradients will be necessary. A permeable anion that could play this part pre-eminently is phosphate, and when we examine the nature of the impermeable non-colloidal anions within the fibre we find that they are mostly if not quite formed of phosphorylated compounds important for the carbohydrate cycle. If such compounds decrease in concentration during rapid carbohydrate oxidation, potassium should leave the cell, and when reformed the reverse should occur. A relation between carbohydrate metabolism and potassium interchanges has, in fact, been already noted (for example, by Verzar) and is here rationally interpreted.

With the above membrane the potential changes with varying external solutions can also be readily understood, previous explanations assuming chloride impermeability being demonstrably incorrect.

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Action Potentials Recorded from Inside a Nerve Fibre

NERVOUS messages are invariably associated with an electrical change known as the action potential. This potential is generally believed to arise at a membrane which is situated between the axoplasm and the external medium. If this theory is correct, it should be possible to record the action potential between an electrode inside a nerve fibre and the conducting fluid outside it. Most nerve fibres are too small for this to be tested directly, but we have recently succeeded in inserting micro-electrodes into the giant axons of squids (*Loligo forbesi*)[1].

The following method was used. A 500 μ axon was partially dissected from the first stellar nerve and cut half through with sharp scissors. A fine cannula was pushed through the cut and tied into the axon with a thread of silk. The cannula was mounted with the axon hanging from it in sea water. The upper part of the axon was illuminated from behind and could be observed from the front and side by means of a system of mirrors and a microscope ; the lower part was insulated by oil and could be stimulated electrically. Action potentials were recorded by connecting one amplifier lead to the sea water outside the axon and the other to a micro-electrode which

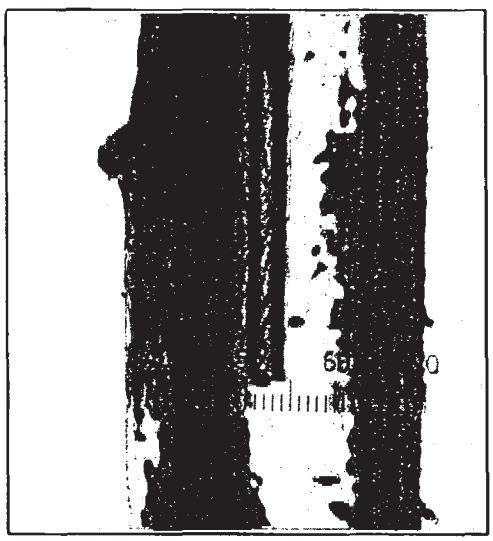

Fig. 1.

PHOTOMICROGRAPH OF ELECTRODE INSIDE GIANT AXON. 1 SCALE DIVISION = 33 μ.

was lowered through the cannula into the intact nerve beneath it. The micro-electrode consisted of a glass tube about 100 μ in diameter and 10–20 mm. in length; the end of the tube was filled with sea water, and electrical contact with this was made by a 20 μ silver wire which was coated with silver chloride at the tip. Fig. 1 is a photograph of an electrode

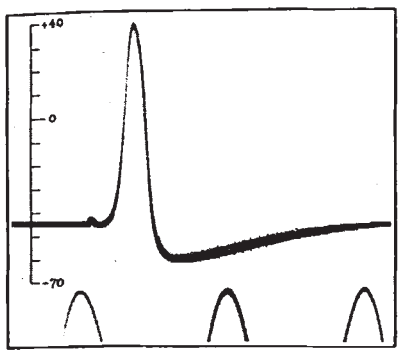

Fig. 2.

ACTION POTENTIAL RECORDED BETWEEN INSIDE AND OUTSIDE OF AXON. TIME MARKER, 500 CYCLES/SEC. THE VERTICAL SCALE INDICATES THE POTENTIAL OF THE INTERNAL ELECTRODE IN MILLIVOLTS, THE SEA WATER OUTSIDE BEING TAKEN AT ZERO POTENTIAL.

inside the living axon. The giant axon shows as a clear space and is surrounded by the small fibres and connective tissue which make up the rest of the nerve trunk. The silver wire can be seen inside the electrode and about 1 mm. from its tip. A small action potential was recorded from the upper end of the axon and this gradually increased as the electrode was lowered, until it reached a constant amplitude of 80–95 mv. at a distance of about 10 mm. from the cannula. In this region the axon appeared to be in a completely normal condition, for it survived and transmitted impulses for several hours. Experiments with external electrodes showed that the action potential was conducted for at least a centimetre past the tip of the micro-electrode.

These results are important for two reasons. In the first place they prove that the action potential arises at the surface, and in the second, they give the absolute magnitude of the action potential as about 90 mv. at 20° C. Previous measurements have always been made with external electrodes and give values which are reduced by the short-circuiting effect of the fluid outside the nerve fibre.

The potential difference recorded between the interior and exterior of the resting fibre is about 50 mv. The potential difference across the membrane may be greater than this, because there may be a junction potential between the axoplasm and the sea water in the tip of the electrode. This potential cannot be estimated, because the anions inside the nerve fibre have not been identified.

We wish to express our indebtedness to Mr. J. Z. Young, whose discovery of the giant axon in *Loligo* made this work possible.

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[1] Young, J. Z., *Proc. Roy. Soc.*, B, 121, 319 (1936).

Legume Nodule Metabolism and Nitrogen Fixation

THE extent of nitrogen fixation in legume nodules is governed primarily by the carbohydrate supply[1,2]. Quantitative information concerning the carbohydrate and nitrogen metabolism of nodules is, however, somewhat limited. Investigations of this subject, conducted during the past four years, are here reported upon briefly.

The experimental procedure involved the use of standard manometric techniques supplemented by chemical analyses. Nodules from twelve species of legumes were compared with normal root tissues of these legumes and of twelve species of non-legumes. These tissues were detached from young field-grown plants, washed, and used as quickly as feasible in Warburg respiration experiments, or otherwise as desired. The outstanding results of these studies are given below.

Variations in behaviour between samples of nodules of different species were not much greater than between samples of the same species, hence the selection of a species for an experiment was relatively unimportant. The same generalization applies also to root tissues.

The inner tissues of most nodules kept in air were under partially anaerobic conditions; this was likewise true for large nodules even in oxygen. This conclusion is based upon the relative rates of respiration and respiratory quotients of nodules and roots in different partial pressures of oxygen. In air, the average Q_{O_2} of whole legume nodules and small roots of legumes and non-legumes was about 2·2; the values for nodules immediately after crushing usually varied between 3 and 7. The use of pure oxygen increased the average Q_{O_2} of whole nodules to about 5·3, but changed that of small roots only slightly. Small root tissues are evidently well aerated, even in air. The *R. Q.* values of nodules increased with nodule size and with decrease in oxygen concentration. In air most nodules, even though small, showed *R. Q.* values considerably above 1·0, whereas in pure oxygen the values were usually near 1·0. Small legume and non-legume roots usually gave values appreciably below 1·0 even in air.

Small roots apparently contain less available carbohydrates than do nodules, since glucose additions increased the Q_{O_2} of the former about 40 per cent and of the latter 12 per cent. The smaller diameter of the roots, however, favoured sugar penetration, and this fact may account for a portion of the greater increase. Under anaerobic or partially anaerobic conditions both nodule and root tissues formed ethyl alcohol[3]; under aerobic conditions alcohol was oxidized completely to carbon dioxide and water.

The rates of respiration per unit of tissue nitrogen of legume and non-legume roots was 3–4 times as great as for nodules in air, and twice as great as for nodules in oxygen. This indicates that a considerable portion of the nitrogen in nodules is either in storage, translocatory or other biologically inactive compounds.

Repeated attempts to obtain nitrogen fixation by detached nodules of different species and ages and maintained under various conditions were, with one possible exception, unsuccessful. Nitrogen analyses were made either by the Kjeldahl method or by gas analysis.

These experimental results are in harmony with the generally accepted idea that the bacteria in nodules are mostly in a dormant condition. Under ordinary atmospheric conditions the Q_{O_2} of nodule