

rent on faradic stimulation. The first few of these spikes grow in height, the later ones stay constant. The mechanical effect does not become apparent before a number of action currents have been set up in the muscle, but continues to grow long after the action currents have reached their maximum.

From isolated single nerve fibre preparations, which on stimulation evoked the slow contraction, monophasic action currents were led off during stimulation with constant current. A repetitive discharge was found, but here the spikes were all of the same height, therefore the increase of the muscle action current must be due to facilitating processes in the muscle itself. The frequency of the nerve action currents increases on stronger stimulation up to a maximum of about 200 a second.

Though it has been conclusively shown that the twitch and the slow contraction are evoked by different nerve fibres, we have reasons to believe that both kinds of contractions occur in the same muscle fibres.

¹ K. Lucas, *Jour. Physiol.*, 51, 1 (1917).

² H. Blaschko, McKeen Cattell and J. L. Kahn, *Ibid.*, 73, 25 (1931).

³ C. A. G. Wiersma, *Zeits. vergl. Physiol.*, 19, 349 (1933).

⁴ C. F. A. Pantin, *Jour. exp. Biol.*, 13, 111 (1936).

THE KINETICS OF PENETRATION. XIII. EFFECT OF pH ON THE ENTRANCE OF POTASSIUM INTO NITELLA AT LOW CONCENTRATIONS

BY A. G. JACQUES

LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Communicated January 17, 1936

In a recent paper¹ it was shown that the rate of entrance of potassium into *Nitella* is not appreciably influenced (1) by the external pH when the external concentration of potassium is constant and not below 0.0001 *N*, or (2) by the external concentration of potassium, at constant pH, down to a concentration of 0.0003 *N*. Below this concentration the rate fell off with the decrease in the external concentration of potassium. These findings suggested that a pH effect might be found if the external concentration of potassium were made very small.

Enough experiments have now been performed to determine that such a pH effect occurs, and to illustrate its general nature.

The cells in strings, as described in a previous paper,¹ were exposed to solutions containing 0.00001 *N* potassium,* at pH's from 6 to 8. The pH was maintained in most cases by the sodium bicarbonate-CO₂ buffer. The

concentration of the latter was fixed by being brought into equilibrium with the CO_2 of the atmosphere and the proportion of the former varied from 0.00001 to 0.001 N in order to produce the required pH.

In these cases there was a very great difference in concentration of sodium added as NaHCO_3 . In order to eliminate this, in some cases sodium chloride was added to the solutions of lower pH to bring the sodium concentration to the same value throughout. As a consequence there was about a hundredfold difference in chloride content between pH 6 and pH 8.

In other cases the chloride was kept constant and the sodium was permitted to vary about one hundredfold between pH 6 and pH 8. No marked difference in results was observed between the two types of experiments. The same may also be said of calcium chloride which was added in very low concentrations in some cases.

The results may be briefly generalized in the following statement. The lower the pH in the range studied (6 to 8) the greater the tendency for po-

TABLE 1
POTASSIUM CONCENTRATION OF EXTERNAL SOLUTION = 0.00001 N

DAYS	pH OF EXTERNAL SOLUTION	CONC. K	CONC. CL	ADJUSTED CONC. K
0	6, 7, 8	0.0438	0.1255	0.0434
2	6	0.0472	0.1250	0.0470
	7	0.0436	0.1278	0.0425
	8	0.0422	0.1241	0.0423
4	6	0.0477	0.1229	0.0483
	7	0.0467	0.1238	0.0470
6	8	0.0384	0.1211	0.0395
	6	0.0504	0.1237	0.0507
	7	0.0468	0.1265	0.0460
	8	0.0384	0.1242	0.0385

Av. 0.1245

tassium to enter the sap, and, indeed, when the external concentration of potassium was 0.00001 N the cell invariably lost potassium at pH 8. At pH 6 and 7 the sap usually gained potassium or the concentration remained constant. But the loss (if it occurred) was less rapid at pH 6 than at pH 7, and when the cell gained potassium this happened more rapidly at pH 6 than at pH 7. When the external concentration of potassium was 0.000015 N , the cells gained potassium at pH 6, 7 and 8, but the rate decreased as the pH increased.

In all cases the chloride concentration remained substantially constant, as indeed might be expected since Hoagland found that *Nitella clavata* is capable of removing the chloride ion quantitatively from extremely dilute solutions.² Such variations as occurred showed no trend.

The results of a typical experiment are given in table 1. (Column 5,

"Adjusted conc. K," has been calculated on the assumption that the chloride content remained constant at 0.1245 *N* and where the measurements do not show this an adjustment has been made to eliminate the effect of accidental variations.)

At present it does not seem possible to give a reasonable interpretation of these unexpected results. Among other unknown factors is the internal pH which has not yet been investigated. However, in the previous work on *Nitella*, where the pH effect was studied at concentrations down to 0.0001 *N* potassium, scattered determinations of the internal pH failed to show marked changes, 6.0 being the highest encountered. Unfortunately such a negative finding as this is inconclusive because the pH of the gross sample of sap may be very different from that of the layer in contact with protoplasm, especially when, as in this case, the sap is somewhat viscous. However, if the pH gradient really is the factor which controls entrance or exit it would be necessary to suppose that at pH 8 the internal pH in the layer adjacent to the protoplasm is considerably higher than pH 8. But since at pH 8, as far as we know, no cation** is entering, the pH change, if it occurs, must result from operations in the cell itself, e.g., photosynthesis, which would use up the CO₂ of the sap and so raise the pH. This reaction might conceivably be favored as the pH rose. But the experiments were carried out practically in darkness so that photosynthesis was probably not a factor.

It seems possible that secondary factors other than the pH gradient may be important. Thus the distribution of the carriers upon which the entrance and exit of bases seems to depend might be altered by changing pH in such a way as to concentrate them at the inner surface of the protoplasm as the external pH is increased.

Finally there is the possibility that the permeability of the sap to organic salts of potassium is increased as the external pH is increased, so that KA can come out as such. Such a change need not be considered as injury since injury would also further the exit of KCl, which does not occur.

Summary.—Cells of the fresh water plant *Nitella* were exposed to very dilute solutions of potassium at pH 6, 7 and 8. The lower the pH the greater the tendency of potassium to enter the sap. The chloride concentration of the sap remained practically constant.

The interpretation of these results must await further investigation.

* In one case 0.000015 *N*.

** It is, of course, possible that sodium goes in as potassium comes out, but it is not likely that an excess of sodium enters so that from this cause there should not be any increase in the sap pH.

¹ Jacques, A. G., and Osterhout, W. J. V., *Jour. Gen. Physiol.*, 18, 967 (1934–35).

² Hoagland, D. R., and Davis, A. R., *Ibid.*, 5, 643 (1922–23).