ACCUMULATION OF BRILLIANT CRESYL BLUE IN THE SAP OF LIVING CELLS OF NITELLA IN THE PRESENCE OF NH_3.

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I.

INTRODUCTION.

It has been shown by several investigators[1] that when the pH value of the external dye solutions is increased, the rate of accumulation of a basic dye in the cell sap is accelerated. Two different explanations for this have been given. Some have accounted for it on the basis that the basic dye enters the cell in the form of a dye hydrate and combines with the substances in the cell; and that, therefore, the increase in the rate of accumulation of the dye is due to the increase in the concentration of the dye hydrate. Others[2] have assumed that the increase in the rate of accumulation of the dye is due primarily to the increase in the concentration of the combining substances in the cell caused by the increase in the pH value of the cell contents. From these standpoints it is of decided interest to see what will happen if we increase the pH value of the cell sap, while keeping that of the external solution constant. An investigation of this sort was made by McCutcheon and Lucke,[3] but from their

* This work was done in part while the writer held a fellowship in the biological sciences, National Research Council, Washington, D. C.

[1] Overton, E., *Jahrb. wissensch. Bot.*, 1900, xliii, 669. Harvey, E. N., *J. Exp. Zool.*, 1911, x, 507. Robertson, T. B., *J. Biol. Chem.*, 1908, iv, 1. McCutcheon, M., and Lucke, B., *J. Gen. Physiol.*, 1923–24, vi, 501.

[2] Bethe, A., *Biochem. Z.*, 1922, cxxvii, 18. For other recent papers on the influence of pH on vital staining see Rohde, K., *Arch. ges. Physiol.*, 1920, clxxxii, 114. Pohle, E., *Deutsch. med. Woch.*, 1921, xlvii, 1464. Collander, R., *Jahrb. wissensch. Bot.*, 1921, lx, 354. Irwin, M., *J. Gen. Physiol.*, 1922–23, v, 223, 727.

[3] McCutcheon, M., and Lucke, B., *J. Gen. Physiol.*, 1923–24, vi, 501.

experiments it is not possible to draw a definite conclusion.[4] They placed living cells of *Nitella*[5] in dye solutions at pH 8, containing in one case NH_4OH and in another case NaOH, and found that the dye accumulated in the sap less rapidly in the former than in the latter. Since the pH value of the cell sap increased in NH_4OH solution (without dye) while it did not change from the normal in NaOH solution (without dye) they concluded that the decrease in the rate of accumulation of the dye in the sap was due to the increase in the pH value of the sap, and that therefore the dye entered in the form of a dye hydrate, DOH, and combined with weak acids in the sap. This conclusion, however, does not seem to be entirely justified for the following reason. The change in the pH value of the sap is merely an indication that NH_3 (for convenience the term NH_3 will be used in this paper to represent aqueous NH_3 which includes undissociated ammonium compounds and NH_4 ions), has combined with substances in the sap, thus decreasing their power to combine with the dye when it enters. In other words, there is a competition between the dye and NH_3 for the substances in the sap and it is quite possible that the competition may exist without noticeable increase in the pH value of the sap. If there is sufficient buffer action, a considerable amount of NH_3 might accumulate without raising the pH value, but this would diminish the rate of accumulation of the dye since the NH_3 would compete with the dye for the substances in the cell. The presence of such a competition does not necessarily mean that the dye enters the cell in the form of DOH, and that the combining substances are weak acids. In case the dye salt, DCl, unites with the salts of proteins or of weak acids, the rate of accumulation of the dye will be decreased when NH_3 is present in the cell, provided the affinity of NH_3 for the substances in question is greater than the affinity of the dye for the same substances. The reaction might be of the ordinary type where NH_3 combines with proteins or weak acids to form salts which are ionized and which can combine with the dye or NH_3 might combine with a salt to form a compound which

[4] *Cf.* Irwin, M., *J. Gen. Physiol.*, 1925–26, viii, 147.

[5] The same results were obtained by them with *Gonionemus* and starfish eggs. See Foot-note 3.

cannot combine with the dye, similar to the compound[6] formed by the union of copper hydrate with sodium tartrate, with which sodium hydroxide cannot react.

The possibility must also be borne in mind that NH_3 in the external dye solution may hinder the dye from entering the cell. McCutcheon and Lucke concluded, on the ground of experiments with the effect of NH_3 on the partition of dye between oil of sweet almonds and water, that the presence of NH_3 had no effect upon the taking up of the dye by the oil, but this may not necessarily prove to be the case with living cells.

In order to determine the cause of the decrease in the rate[7] of accumulation of dye in the presence of NH_3 it is desirable to carry out experiments which will show the rate of accumulation of the dye in the sap; (1) when the pH values of the sap are the same, while the concentrations of NH_3 in the sap are varied, which will show if there is a competition between NH_3 and the dye in the sap without a change in the pH value of the sap; (2) when the pH values of the sap and the concentrations of NH_3 in the sap are the same while the concentrations of NH_3 in the external solutions are varied, which will show if the presence of NH_3 in the dye solution can hinder the penetration of the dye into the cell; and (3) when the pH values of the sap are varied, while the concentrations of NH_3 in the sap remain practically constant, which will show if an increase in the pH value of the sap alone can bring about a decrease in the rate of accumulation of the dye.

[6] Norris, J. F., The principles of organic chemistry, New York, 1912, 272.

[7] It is evident that the competition of NH_3 would affect both the rate of accumulation of the dye in the sap and the concentration of the dye in the sap at equilibrium. In this paper the rate alone is studied since the cells die before the final equilibrium is attained. The rates are taken as near the beginning as possible and represent the concentrations of the dye in the sap at a given time in all cases. It is assumed that such rates would be approximately proportional to the concentrations of dye in the sap at equilibrium, if the reaction were not complicated by secondary processes as described in the writer's previous paper (see Foot-note 4).

II.

Accumulation of Brilliant Cresyl Blue in the Sap When Living Cells Are Placed in a Solution of Dye Containing NH_4Cl.

In order to see how much decrease occurs in the rate of accumulation of the dye in the sap when the cells are placed in a solution of dye containing NH_4Cl, cells were divided into three lots. The

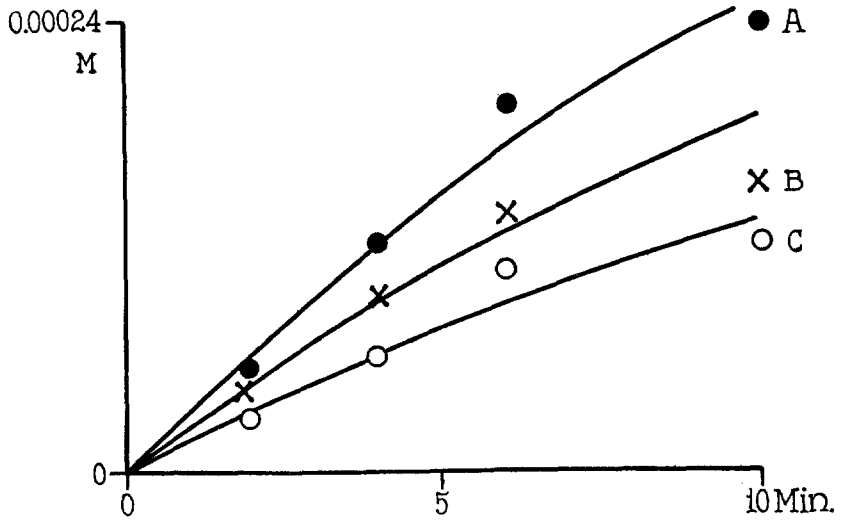

FIG. 1. Curves showing the rate of accumulation of dye in the sap of living cells of *Nitella*. The ordinates represent the concentrations of dye in the sap, and the abscissæ represent time. Curve A shows the rate of accumulation in 0.00014 M dye solution at pH 6.9; Curve B shows the rate in 0.00014 M dye solution at pH 6.9 containing 0.005 M NH_4Cl; Curve C shows the rate in 0.00014 M dye solution at pH 6.7. Each point on every curve is an average of forty experiments and the probable error of the mean is less than 7 per cent of the mean.

first lot was placed in 0.00014 M dye solution at pH 6.9, the second lot in the same concentration of dye solution at the same pH containing 0.005 M NH_4Cl, and the third lot in the same concentration of dye solution at pH 6.7. The concentration of the solutions was kept constant. All solutions were made up with $\frac{M}{150}$ phosphate buffer mixtures.[8] The experiments were made at 25 ± 0.5°C.

[8] The writer wishes to thank Mr. E. S. Harris for determining the pH values of the solutions by means of the hydrogen electrode.

At definite intervals, a few cells were removed, wiped, and the end of each cell was cut and the sap gently squeezed out on a glass slide. The sap was then drawn up into a capillary tube, and the color of the

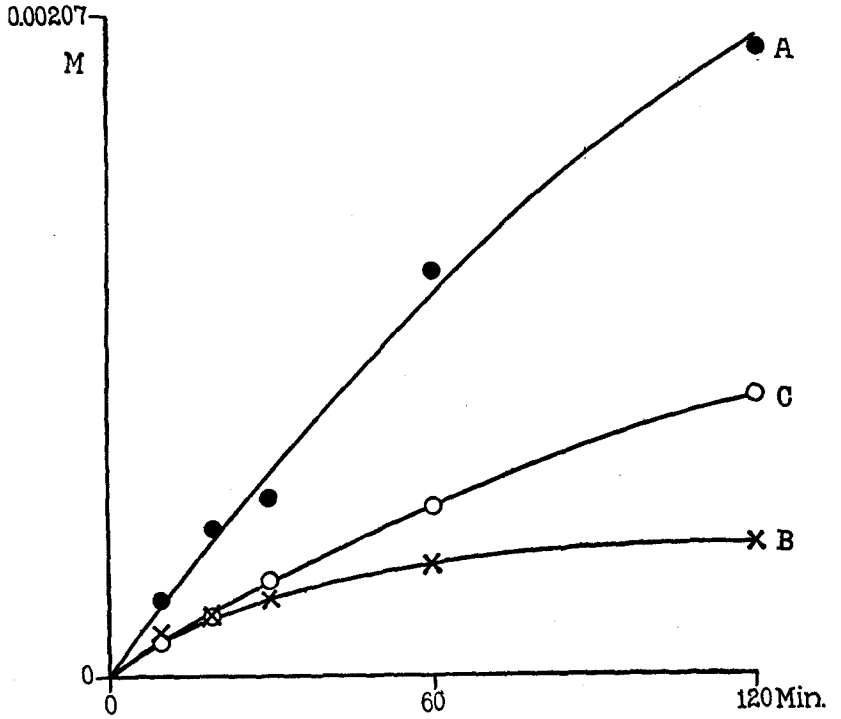

FIG. 2. Curves showing the rate of accumulation of dye in the sap of living cells of *Nitella*. The ordinates represent the concentrations of dye in the sap, and the abscissæ represent time. Curve A shows the rate of accumulation in 0.00014 M dye solution at pH 6.9; Curve B shows the rate in 0.00014 M dye solution at pH 6.9 containing 0.005 M NH_4Cl; Curve C shows the rate in 0.00014 M dye solution at pH 6.7. Each point on every curve is an average of forty experiments and the probable error of the mean is less than 7 per cent of the mean.

tube was matched[9] with that of a tube of the same diameter containing a known concentration of the dye. The results are shown in Figs. 1 and 2.

On comparing the rate of accumulation of the dye in these three

[9] For details of technique see the writer's previous paper, *J. Gen. Physiol.*, 1925–26, viii, 147. In all the figures the curves are drawn free-hand through the points to give an approximate fit.

different dye solutions, it is found that in the first lot the rate is the highest from the start, while in the second lot the rate is higher at the start than in the third, but it becomes lower after about 25 minutes, as shown in Figs. 1 and 2, Curves A, B, and C. These curves indicate clearly that when the cells are placed in a solution of dye containing NH_4Cl, the rate of accumulation of the dye in the sap falls off from the start and this decrease becomes greater as the time goes on.

III.

Change in the pH Values of the Cell Sap.

In order to carry out such experiments as are discussed in the introduction it is first of all necessary to obtain time curves showing the changes in the pH values of the cell sap when living cells are placed in solutions with[10] and without NH_3.

The pH of the sap was determined by the colorimetric method as follows: A capillary tube was filled for 1 inch with the sap; another capillary tube of the same diameter was filled for $^1/_{10}$ of an inch with about 0.005 per cent brom-cresol purple. The contents of the two tubes were then mixed on a glass slide and the entire amount of mixed sap and indicator was drawn up into a third capillary tube the color of which was carefully matched with that of a fourth tube having the same diameter as the third and filled with a standard phosphate buffer solution of known pH value containing the same concentration of the indicator as the third tube. The sap contains about 0.1 M halides so that the salt error should be corrected to obtain absolute values, but since the importance of these experiments lies in the relative values, this correction was omitted.

The solutions of NH_4Cl were made up in $\frac{M}{150}$ phosphate buffer mixtures at pH 6.9, and the concentration was kept constant throughout the experiment. The experiments were made at 25 $\pm$ 0.5°C.

[10] Hoagland, Davis, McCutcheon, Lucke, and the writer have found that the increase in the pH value of the cell sap takes place when cells are placed in a solution containing NH_3. See Foot-notes 3 and 4, and also, Hoagland, D. R., and Davis, A. R., *J. Gen. Physiol.*, 1922–23, v, 629.

When the cells were placed in 0.005 M NH_4Cl solution at pH 6.9 and the change in the pH value of the sap was followed at definite intervals until death took place, it was found that after 5 minutes the pH value of the sap began to increase and in about half an hour it changed from pH 5.6 (normal) to pH 5.94 beyond which there was very little change in the pH value as shown in Fig. 3, Curve A, until almost all the cells were dead (within 6 hours after they were placed in the solution).

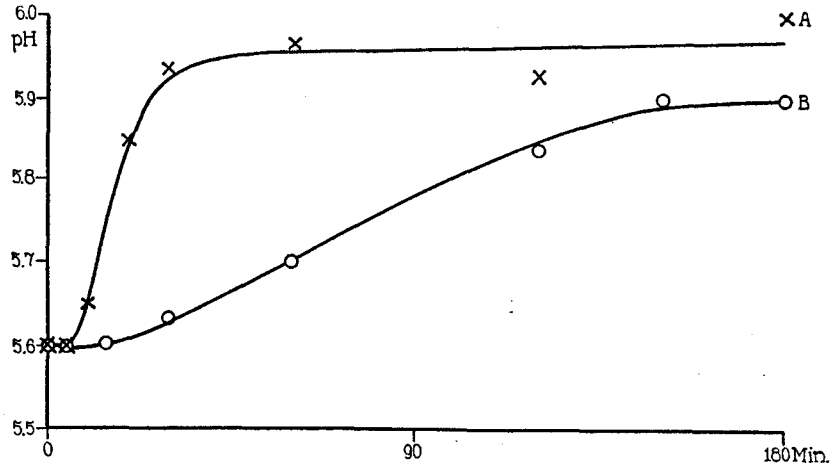

FIG. 3. Time curves showing the change in the pH value of the sap of living cells of *Nitella*. The ordinates represent the pH values of the sap, and the abscissæ represent time. Curve A shows the changes in the pH value when the cells are placed in 0.005 M NH_4Cl at pH 6.9. Curve B shows the changes in the pH values when the cells are placed in boric acid and sodium hydrate mixtures at pH 10.1. Each point on every curve is an average of forty experiments and the probable error of the mean is less than 7 per cent of the mean.

When living cells were placed in a buffer solution at pH 10.1 $\left(\frac{M}{40}\right.$ boric acid + NaOH), and the pH value of the sap was determined at intervals, it was found that the pH value began to change in about 20 minutes and in 2½ hours increased from 5.6 to 5.9, after which there was a very little change as shown in Fig. 3, Curve B, until almost all the cells were dead (in about 5 hours).

On comparing Curves A and B in Fig. 3, it is found that the pH value of the sap changes more rapidly, and the flattening of the curve is reached more quickly in the case of the cells placed in 0.005 M NH_4Cl solution at pH 6.9 $\left(\frac{M}{150}\text{ phosphates}\right)$ than in the case of the cells placed in pH 10.1 $\left(\frac{M}{40}\text{ boric acid} + NaOH\right)$ without NH_4Cl.

If we base our notion of injury to the cells on the rapidity of death in the solution, then there is a very little difference between the cells placed in these two solutions, in that the cells in 0.005 M NH_4Cl solution at pH 6.9 die in about 6 hours while the cells placed in pH 10.1 solution without NH_4Cl die in about 5 hours. But if we base the criterion of injury[11] on the power of recovery there is a considerable difference as shown by the following experiment. When the cells that had been placed in 0.005 M NH_4Cl solution for 1 hour and other cells that had been in pH 10.1 buffer solution for 2½ hours were removed from the solutions, wiped, and placed in distilled water (in which the cells normally live for days), almost all the former cells were found living after 24 hours, while the latter were almost all dead in 2 hours. In all probability cells become more or less injured when the pH value of the sap is appreciably changed but the injury to the cells under these conditions in NH_4Cl solution is much less and the recovery is more apt to occur than when the cells are placed in NaOH plus boric acid buffer solution. This injury increases and the cells die if left in these solutions for several hours. It is not possible to change the pH values of the cell sap in solutions containing NH_3 or NaOH in such a way that one can definitely say that the cells are not injured at the time the pH value of the cell sap is altered. The conditions described above are the most favorable which the writer has been able to find.

[11] If we base the criterion of injury in the appearance of masses of chlorophyll in the sap, the observation is not accurate, though in general we may state that there are greater masses of chlorophyll in the sap of an injured cell than in the sap of a normal cell.

IV.

Accumulation of NH_3 in the Sap When Living Cells Are Placed in NH_4Cl Solution.

Since the experiments in Section III show that when the cells are placed in NH_4Cl solution the pH value of the sap increases progressively until an apparent equilibrium is established, it is desirable to see what type of curve is followed in the accumulation of NH_3 in the sap.

Cells were placed in 0.005 M NH_4Cl solution at pH 6.9 $\left(\frac{M}{150}\text{ phosphates}\right)$ at 25 $\pm$ 0.5°C. The concentration of the solution was kept constant. The concentration of NH_3 in the sap was determined by means of Nessler's reagent in the following manner. A few cells were removed from the solution and wiped with a wet cloth (free from NH_3). The ends of the cells were cut and the sap was gently squeezed out on a glass slide. Then the sap was drawn up into a capillary tube (about 10 inches in length), until it filled the tube for the distance of 1 inch. The sap was then blown into the Nessler tube containing 50 cc. of distilled water and 1 cc. of the Nessler reagent. The solution was then carefully shaken. A standard solution was made by taking the same amount (as in the case of the sap) of a known concentration of NH_4Cl solution and putting this into a Nessler tube containing 50 cc. of distilled water and 1 cc. of the Nessler reagent in the same manner as in the case of the sap. The colors of the two tubes were then compared by looking into the solutions from the top of the tubes. Since the color of the solution deepened on standing in both cases, it was necessary to make determinations at a definite time after the solutions were made up. For the present purpose the color was matched immediately.

The sap is not a clear liquid like the standard solution but it contains a viscous substance, which rises to the top of the tube and makes the readings difficult so that an accurate determination of an absolute value of NH_3 is not possible but this did not interfere with the present experiments since we needed only such relative values as could be obtained by this method.

The distilled water did not contain a measurable amount of NH_3 so that this was considered to be at a zero concentration for the sake of comparison since this distilled water was used for the Nessler test.

Tests showed that NH_3 adhering to the surface was not sufficient

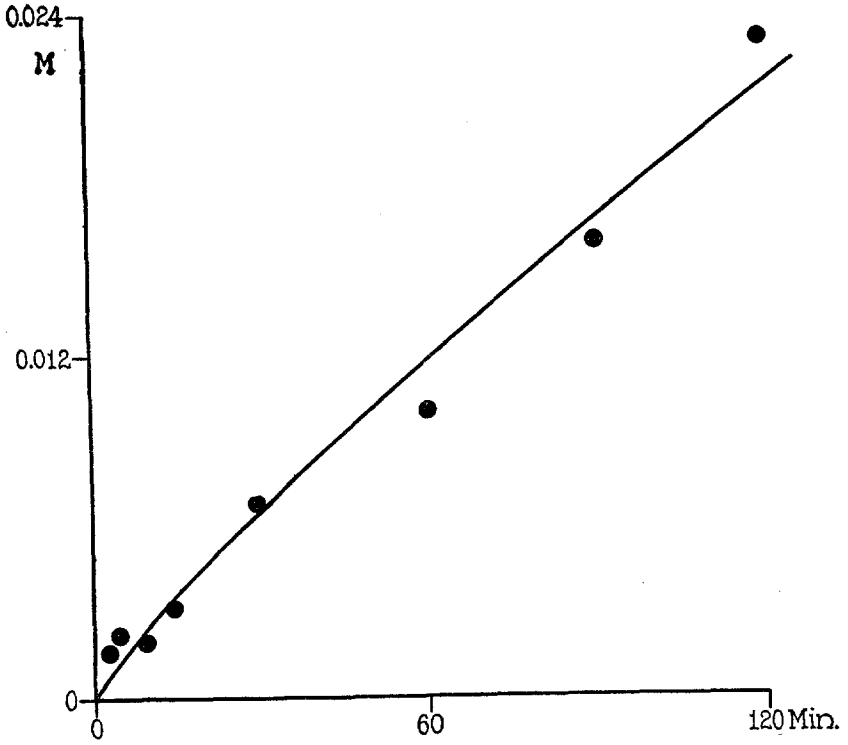

FIG. 4. Curve showing the rate of accumulation of NH_3 in the sap when living cells of *Nitella* are placed in 0.005 M NH_4Cl at pH 6.9. The ordinates represent the concentrations of NH_3 in the sap and the abscissæ represent time. Each point on the curve is an average of forty experiments and the probable error of the mean is less than 7 per cent of the mean.

to bring about noticeable errors. When the cells were dipped for a second in 0.005 M NH_4Cl and then wiped, and the sap was examined for NH_3 as described above, it was found that the sap gave no test for NH_3.

When living cells were placed in 0.005 M NH_4Cl at pH 6.9 and a few were removed at definite intervals for the determination of NH_3

in the sap, it was found that the accumulation of NH_3 took place gradually in the sap without reaching an equilibrium before the cells died (in about 6 hours). See Fig. 4.

When the relation between the concentration of NH_3 in the sap and the extent of the change in the pH value of the sap is considered,

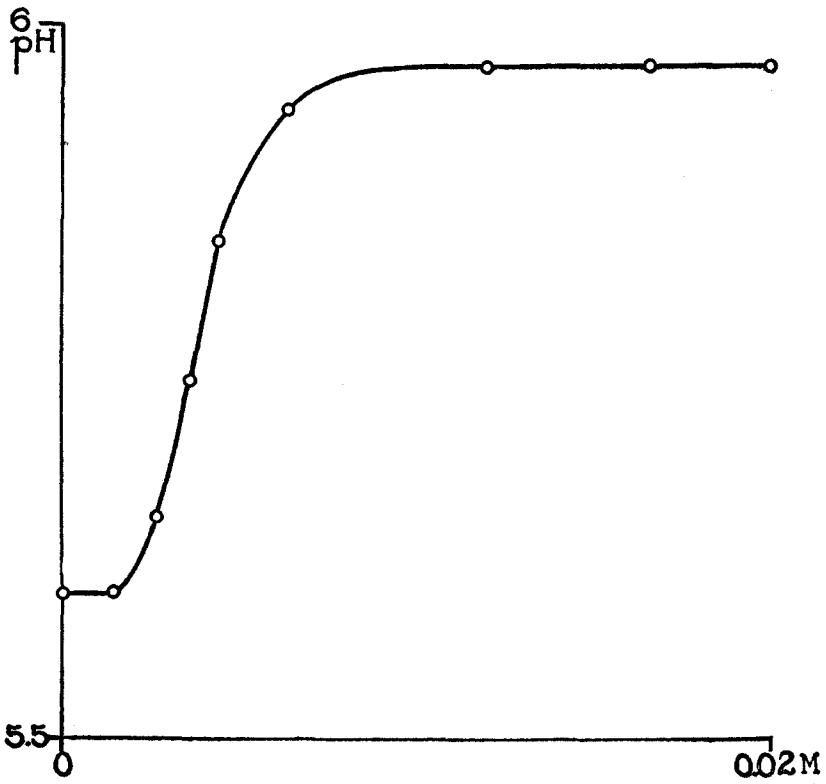

FIG. 5. Curve showing the relation between the concentration of NH_3 in the sap and the pH value of the sap. The ordinates represent the pH values and the abscissæ represent the concentrations of NH_3. The points on the curve are obtained from the curves as drawn in Fig. 3 (Curve A) and in Fig. 4.

the following is found to be true, as shown in Fig. 5. At the start the pH value of the sap remains unchanged until the concentration of NH_3 in the sap has reached 0.0014 M; this may be regarded as due to the buffer action of the sap (provided it is not NH_4Cl which enters the cell). Above this concentration the pH of the sap

increases but when the concentration of NH_3 in the sap has reached about 0.0064 M further accumulation of NH_3 brings about no appreciable change in the pH value of the sap until the cells die.

V.

Accumulation of the Dye in the Sap When the pH Values of the Sap Remain Constant While the Concentrations of NH_3 Are Varied.

When Figs. 3 and 4 are compared it is seen that after the cells have been placed in 0.005 M NH_4Cl solution at pH 6.9 $\left(\frac{M}{150}\text{ phosphates}\right)$ the accumulation of NH_3 in the sap takes place for about 5 minutes without a measurable change in the pH value of the sap. This enables us to carry out experiments in which we can compare the rate of accumulation of the dye in two lots of cells, one having no NH_3, and the other having 0.0014 M NH_3 in the sap (NH_3 is found to remain[12] in the sap during the experiment) while the pH value of the sap is the same in both cases.

Cells were placed in 0.005 M NH_4Cl solution at pH 6.9 $\left(\frac{M}{150}\text{ phosphates}\right)$ for 5 minutes after which they were removed, wiped, and placed in 0.00014 M dye solution at pH 6.7 $\left(\frac{M}{150}\text{ phosphates}\right)$, at 25 $\pm$ 0.5°C. and the rate of accumulation of the dye was measured at different intervals for 6 minutes. It was found that the rate (Fig. 6, Curve B) was slightly lower than that obtained in the case of the cells dipped for a few seconds in NH_4Cl and placed in the same dye solution. (Fig. 6, Curve A.) The decrease is about 24 per cent which was about the extent of decrease found at the end of 5 minutes when the cells were placed in 0.00014 M dye solution at pH 6.9 containing 0.005 M NH_4Cl solution (Fig. 1, Curves A and B, in Section II). This indicates that the decrease is not due to the fact that NH_3 in the external dye solution hinders the entrance of the dye, but it was due to the presence of NH_3 in the sap. Since these

[12] On removing the cells (containing 0.0014 M NH_3 in the sap) from 0.00014 M dye solution at pH 6.9, after 6 minutes, and testing the sap for NH_3, it is found that the NH_3 has not come out at all.

experiments were carried out when the cells had been placed in the NH_4Cl solution for only 5 minutes, we may assume that the cells were not injured at this stage.

It is of interest to see what happens when we employ cells whose

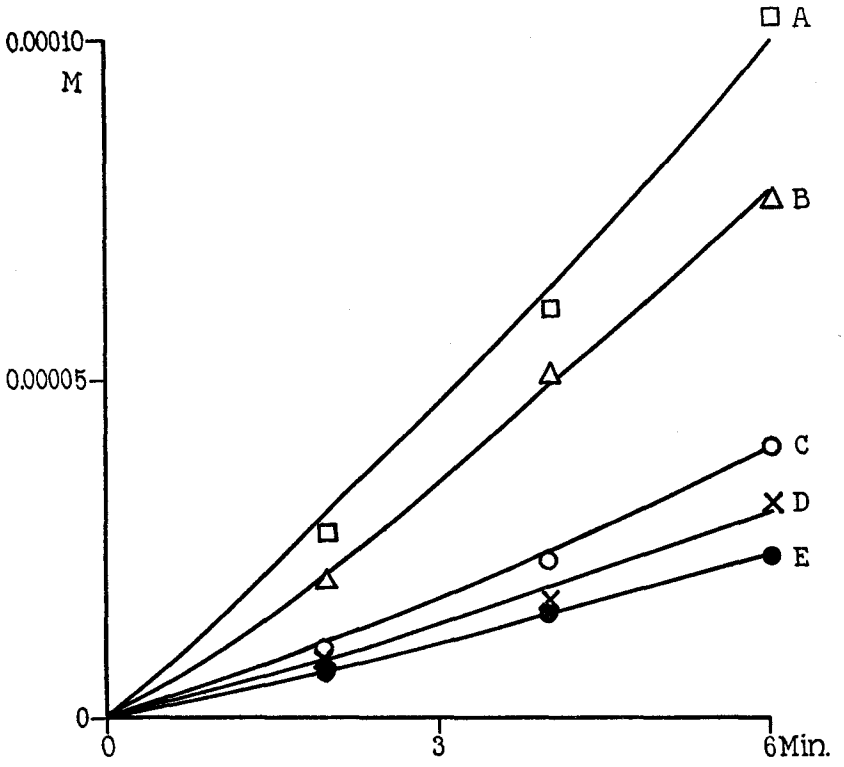

FIG. 6. Curves showing the rate of accumulation of dye in the sap of living cells of *Nitella* when the cells are placed in 0.00014 M dye solution at pH 6.7 after the cells have been treated for different lengths of time in 0.005 M NH_4Cl at pH 6.9. Curve A shows the rate when cells have been dipped for a few seconds in NH_4Cl; Curve B for 5 minutes; Curve C for 30 minutes; Curve D for 60 minutes, and Curve E for 120 minutes. Each point on every curve is an average of forty experiments and the probable error of the mean is less than 7 per cent of the mean.

sap has increased in alkalinity after a longer exposure, such as may possibly produce injury. The experiments in Sections III and IV show that the pH value of the sap is about 5.95, when the cells have been placed in 0.005 M NH_4Cl solution at pH 6.9 either for 30 min-

utes, 60 minutes, or 120 minutes though the concentration of NH_3 in the sap in the first case is 0.0064 M, in the second it is 0.0117 M, and in the third it is 0.0214 M. Such cells were removed from the NH_4Cl solution after 30, 60, and 120 minutes, wiped, and placed in 0.00014 M dye solutions at pH 6.7 $\left(\frac{M}{150}\text{ phosphates}\right)$ at 25 ± 0.5°C. When the rate of accumulation was measured at different intervals during 6 minutes (in this interval the concentration of NH_3 in the sap remained unchanged),[13] it was found that the rate decreased as the concentration of NH_3 increased in the sap as shown in Fig. 6, Curves C, D, and E. The rate of accumulation of the dye in the sap decreased about 24 per cent when there was 0.0014 M NH_3 in the sap, and when the pH of the sap was found to be 5.6 (normal value). There was about 62 per cent decrease in the case of the cells which contained 0.0064 M NH_3 in the sap (pH value of sap was about 5.93). There was a decrease of about 71 per cent when the cells contained 0.0117 M NH_3 in the sap (pH value of the sap about 5.97). At this concentration of NH_3 in the sap the extent of decrease seemed to have almost reached its maximum since in the case of the cells containing 0.0214 M NH_3 in the sap at pH 5.97 there was only about 76 per cent decrease. This seems to indicate that the effect of NH_3 on the rate of accumulation of the dye in the sap reaches a maximum at a definite concentration of NH_3.

VI.

Further Experiments to Ascertain If NH_3 in the External Solution Hinders the Entrance of the Dye.

It has been indicated by the experiments described in Section V that NH_3 does not hinder the entrance of the dye unless NH_3 penetrates into the sap, but in order to confirm this the following experiments were made. When the cells have been placed in 0.005 M NH_4Cl at pH 6.9 $\left(\frac{M}{150}\text{ phosphates}\right)$ for 1 hour it is found that

[13] When cells containing 0.0064 M, 0.0117 M, or 0.0214 M NH_3 in the sap are placed in 0.00014 M dye solution at pH 6.7 for 6 minutes, then removed, and the NH_3 of the sap determined, it is found that no NH_3 has come out of the sap.

0.0117 M NH_3 has accumulated in the sap; at this stage there is no measurable increase in NH_3 in the sap if the cells are left in the solution 6 minutes longer.

Cells were therefore placed in the NH_4Cl solution for 1 hour, removed, and wiped. One lot was now placed in 0.00014 M dye at pH

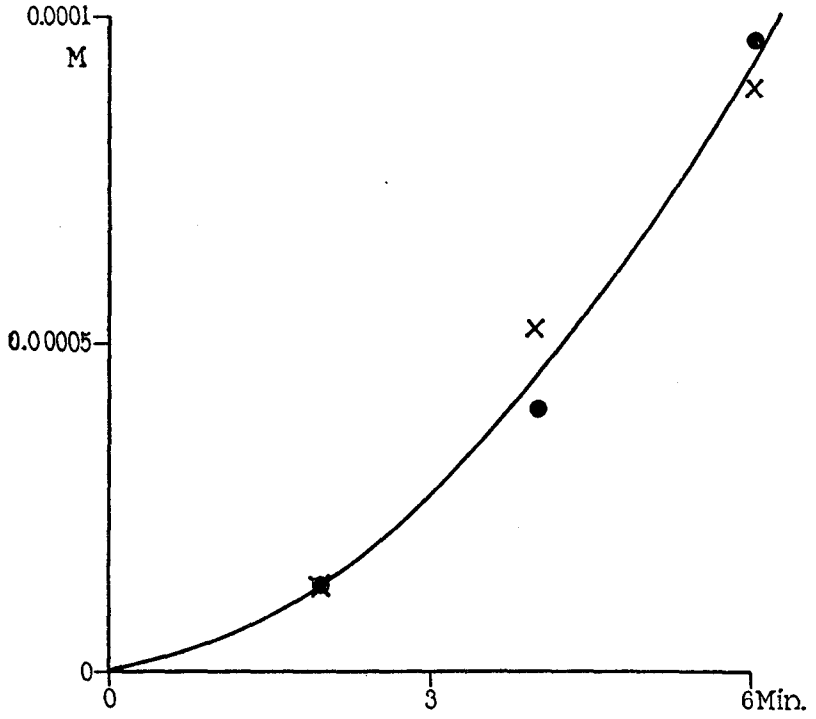

FIG. 7. Curves showing the rate of accumulation of dye in the sap of living cells of *Nitella* when cells are placed in a solution of dye after they have been placed for 1 hour in 0.005 M NH_4Cl at pH 6.9. The symbol (×) represents t he rate of accumulation in 0.00014 M dye solution at pH 6.9, while the symbol (●) represents the rate in 0.00014 M dye solution at pH 6.9 containing 0.005 M NH_4Cl. Each point on every curve is an average of forty experiments and the probable error of the mean is less than 7 per cent of the mean.

6.7 $\left(\frac{M}{150}\text{ phosphates}\right)$ and another lot in the dye solution of the same concentration and the same pH value containing 0.005 M NH_4Cl at 25 ± 0.5°C. When the rate of accumulation of the dye in the sap was measured at intervals during 6 minutes, it was found that

the rate was the same in both cases as shown in Fig. 7, symbol ● and symbol ×. Thus it is evident that it is only the NH_3 in the sap which affects the accumulation of dye and that so long as this is constant variations to the above extent in the concentration of NH_3 in the external solution are of no import.

VII.

Accumulation of the Dye in the Sap When the pH Values of the Sap Are Varied and the Sap Contains No NH_3.

Since it is evident from the experiments described above that the presence of NH_3 in the cell sap brings about a decrease in the rate of accumulation of the dye, the next step is to ascertain whether the increase in the pH value of the cell sap (in absence of NH_3) will have the same effect. Although we cannot obtain cells having the same concentrations of NH_3 in the sap, while the pH values of the sap are different, it is possible to change the pH values of the sap by placing the cells in solutions at pH 10.1$\left(\frac{M}{40}\right.$ boric acid + NaOH mixtures.$\left.\right)$ Fig. 3, Curve B shows that in this mixture the pH value of the sap increases but little in 30 minutes. This increase continues until the pH of the sap is changed from pH 5.6 to 5.9 in $2\frac{1}{2}$ hours after which there is very little change until the cells are dead.

Cells were placed in this mixture at 25 ± 0.5°C. for 5 seconds, 15, 30, 60, and 150 minutes, removed from the sol$_u$tions, wiped, and placed in the 0.00014 M dye solution[14] at pH 6.7$\left(\frac{M}{150}\right.$ phosphates$\left.\right)$ at 25 ± 0.5°C. An exposure of 15 minutes to the buffer solution at pH 10.1 caused a noticeable decrease in the subsequent accumulation

[14] It is not possible to determine the pH value of the cell sap after the dye has entered the sap, hence another experiment was made. When the cells which had been in pH 10.1, as described in the text, for different lengths of time, were removed, and placed in pH 6.7 $\left(\frac{M}{150}\right.$ phosphates$\left.\right)$ at 25°C. for 6 minutes, it was found that the pH values remained the same. From this result it is assumed that the pH values of the sap will not change when cells are placed in the dye solution at pH 6.7 for 6 minutes.

of the dye and this decrease became greater the longer the cells were left in the solution at pH 10.1 (Fig. 8).

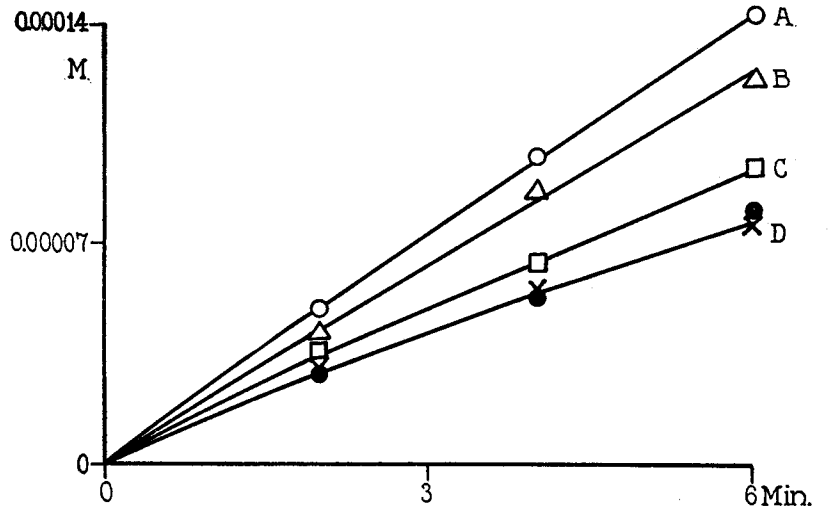

FIG. 8. Curves showing the rate of accumulation of dye in the sap of living cells of *Nitella* which have been placed in $\frac{M}{40}$ boric acid and sodium hydrate mixtures at pH 10.1 and then removed and placed in 0.00014 M dye solution at pH 6.7: Curve A, in the buffer solution for a few seconds; Curve B for 15 minutes; Curve C for 30 minutes; Curve D for 60 minutes (×) and 150 minutes (●). Each point on every curve is an average of forty experiments and the probable error of the mean is less than 7 per cent of the mean.

Fig. 8 shows that there may be a decrease of 13 per cent in the rate[15] of accumulation of the dye in the sap though the pH value of

[15] After the cells had been dipped in the $\frac{M}{40}$ buffer mixture of boric acid and sodium hydroxide at pH 10.1, wiped, and placed in 0.00014 M dye solution at pH 6.7 as already described, the rate of accumulation of the dye was greater than that found in the case of the cells which had been exposed in the same way to a buffer solution at pH 6.7 $\left(\frac{M}{150}\text{ phosphates}\right)$. When such cells were left for 2 minutes in the phosphate solution the rate was the same as in unwashed cells. Whether this increase is due to a preliminary stage of an injury, or to an adhering of the buffer mixtures to the surface of the cell, which cannot be readily washed out, and which produces an effect of the buffer mixtures on the surface of the cell apart from injury, cannot be stated definitely at present.

the cell sap remains normal (pH 5.6). There is a decrease of about 35 per cent when the pH value of the sap increases from 5.6 to 5.63. It is a striking fact that the decrease is only 44 per cent when the pH value of the sap reaches 5.7 or 5.9. From this it is evident that the decrease[16] in the rate may take place even when there is no change in the pH value of the sap, and that the extent of decrease reaches a maximum value when the pH of the sap is at 5.7. This may be due either (1) to the presence of substances, as in the case of NH_3, which compete with the dye for the combining substances in the sap, or (2) to injury to some part of the surface of the cell, which partly prevents the accumulation of the dye in the sap by allowing some to diffuse out of the cell, or (3) possibly to a combination of both.

VIII.

DISCUSSION.

The experiments described in the present paper show that there is a decrease in the rate of accumulation of the dye in the sap when NH_3 is present in the sap but that the presence of NH_3 in the external solution alone has no such effect.

The fact that NH_3 when present only in the external solution does not affect the entrance of the dye would seem to indicate that at the concentrations of the solution used there is no antagonism between NH_4^+ ions and D^+ ions in the sense that they might hinder each other from entering the living cell, and that there can be no tautomeric change in the dye brought about by NH_4Cl which could

[16] When cells which had been placed in the $\frac{M}{40}$ boric acid and sodium hydroxide mixture at pH 10.1 for 2½ hours were wiped, and placed in 0.005 M NH_4Cl at pH 6.9, 0.0007 M NH_3 was found to have accumulated in the sap in 5 minutes. This is much less than the concentration of NH_3 (0.0014 M) in the sap of a control cell placed directly in NH_4Cl solution at pH 6.9. When such cells were removed from the solution of NH_4Cl, wiped, and placed in 0.00014 M dye solution at pH 6.7 it was found that the rate of accumulation of the dye had decreased considerably as compared with cells which had been exposed to the buffer mixture for the same period but which had not been placed in NH_4Cl solution. The pH of the sap in both cases was about 5.9, so that the decrease was due to the presence of NH_3 in the sap.

produce such an effect. It is also evident that the dissociation of the dye is not affected by NH_4Cl, at the concentrations employed.

The decrease in the rate of accumulation of the dye in the sap may be interpreted as due to the fact that NH_3 and the dye compete for certain substances in the cell. The degree of competition as expressed by the decrease in the rate of accumulation of the dye may be dependent on the dissociation constants of the dye and of the NH_3 in the sap, and on the concentrations of these two substances. This is to be expected if we assume that the dye enters as DOH and, like NH_3, is capable of combining with weak acids and proteins in the sap. If the dye enters as a dye salt, *e.g.* DCl, and combines with a salt of a weak acid or of protein it may also be affected by the competition of NH_3 as already explained in the introduction.

Though it is not possible to determine experimentally at present, the same type of competition may exist in the protoplasm or in the surface membrane of the protoplasm, so that the assumption made in a previous paper[4] regarding the rôle of the surface membrane of the protoplasm may not be wrong.

It is evident from what has been said that it is not possible to determine experimentally the exact relation between the pH value of the sap and the decrease in the rate of accumulation which is found in the presence of NH_3.

Thus it is not yet possible to state definitely whether or not the dye enters the cell as the dye hydrate, but experiments are being carried on by the writer which may lead to a definite conclusion in the near future.

SUMMARY.

When the living cells of *Nitella* are placed in a solution of brilliant cresyl blue containing NH_4Cl, the rate of accumulation of the dye in the sap is found to be lower than when the cells are placed in a solution of dye containing no NH_4Cl and this may occur without any increase in the pH value of the cell sap. This decrease is found to be primarily due to the presence of NH_3 in the sap and seems not to exist where NH_3 is present only in the external solution at the concentration used.