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The effect of phosphates on respiration

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THE EFFECT OF PHOSPHATES ON RESPIRATION.

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This is a preliminary report of a study of the action of phosphate upon the production of CO_2 by plants. The phosphate caused a marked and sustained acceleration of both the aerobic and anaerobic respiration.

The experiments were carried on by the use of the apparatus described by Osterhout[1] modified by replacing the rubber valves by two glass bead valves that handle a large current of air more easily and with less danger of a reverse current that often ruins an experiment. The third tube suggested by Osterhout[2] to hold the reagent with a buffer effect while the normal is being determined, was placed so that all CO_2 passed through it on the way to the indicator tube. An extra section of rubber tubing extending between the glass tubes (with its ends flush with the lower side of the rubber stoppers) is clamped off until the normal has been determined. Without opening the system or stopping the air current, the clamp is removed and the tube of phosphates is tilted until the solution passes over into the reaction chamber.

The well known function of phosphate in alcoholic fermentation as shown by Harden and Young (1910) and the generally accepted theories as to the relations between fermentation and respiration make it advisable to use modern methods in studying respiration as it may be affected by phosphates, with the pH and other factors carefully controlled. Palladin[3] believed that respiration is accelerated by phosphate but most of his authority for this seems to lie in the

[1] Osterhout, W. J. V., *J. Gen. Physiol.*, 1918–19, i, 17.
[2] Osterhout, W. J. V., *J. Gen. Physiol.*, 1919–20, ii, 1.
[3] Palladin, V. I., Plant physiology, Philadelphia, 1918, 194.

writings of a number of contemporary workers, L. Iwanoff,[4] N. Iwanoff,[5] Zaleski,[6] and others. Study and comparison of these papers only bring out the conflicting conclusions, the lack of data as to salts and concentrations employed and the pH of the reaction mixtures, and the inaccuracy and incompleteness of such experiments due to the failure to follow the time curves as can be done with the apparatus used in my experiments.

More recently there has appeared a paper by Witzemann[7] in which phosphate is shown to be a catalyst for the oxidation of glucose by H_2O_2. This would lead one to suspect that phosphate might accelerate aerobic respiration. My experiments (which were well started before this paper was published) show this to be true. In fact, certain of my experiments to be reported later seem to indicate that phosphate may catalyze an oxidation in the absence of a peroxide.

Recent papers by Embden and his students[8] and by Meyerhof[9] have appeared as the result of studies of the part played by H_3PO_4 in the production of the energy used in the muscles of animals. A compound containing carbohydrate and phosphoric acid is considered to be the controlling substance of muscular contraction. To account for these results, Meyerhof in particular has outlined a scheme of oxidation of glucose that involves the formation and decomposition of a hexosephosphate in both the aerobic and the anaerobic phases. In so far as it is possible to compare the effects of phosphate action in my experiments on plant respiration with those of H_3PO_4 on muscular action, the action appears to be the same in both cases, an acceleration of the metabolic processes involved. But it is not clearly indicated that the series of chemical reactions in plant respiration are the same as those suggested to explain the rôle of H_3PO_4 in oxidation in animal

[4] Iwanoff, L., *Biochem, Z.*, 1910, xxv, 171.

[5] Iwanoff, N., *Biochem. Z.*, 1911, xxxii, 74.

[6] Zaleski, W., *Ber. bot. Ges.*, 1910, xxviii, 319. Zaleski, W., and Reinhard, A., *Biochem. Z.*, 1910, xxvii, 450.

[7] Witzemann, E. J., *J. Biol. Chem.*, 1920–21, xlv, 1.

[8] Embden, G., and others, *Z. physiol. Chem.*, 1921, cxiii. Embden, G., and Lawaczeck, H., *Biochem. Z.*, 1922, cxxvii, 181; and other papers.

[9] Meyerhof, O., *Arch. ges. Physiol.*, 1920, clxxxv, 11; 1921, clxxxviii, 114; 1921, cxci, 128; and other papers.

muscles, though they have this in common; both the aerobic and the anaerobic phases are affected.

The first experiments were carried out with *Elodea canadensis*. Fig. 1 shows the results of three typical experiments in which different concentrations of neutral phosphate solutions[10] were used. For convenience these solutions will be referred to as having a definite concentration in the sense that when a 0.1 M solution is mentioned a solution is meant which was obtained by mixing 0.1 M monosodium phosphate with 0.1 M disodium phosphate.

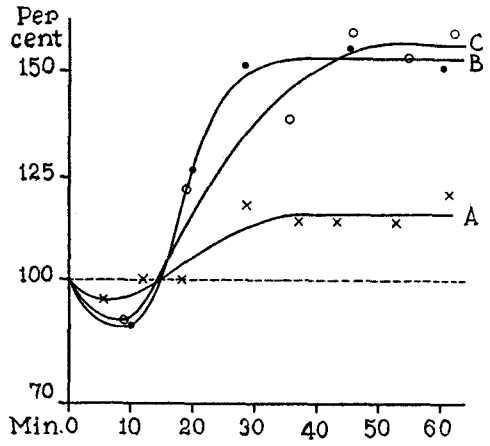

FIG. 1. The effect of neutral phosphate solutions on the production of CO_2 by *Elodea*. Each curve represents a typical experiment. Curve *A* shows the effect of a concentration of 0.021 M; Curve *B*, 0.085 M; Curve *C*, 0.106 M. The normal rate before the addition of phosphate is taken as 100 per cent.

An experiment with potassium salts gave identical results with those obtained with the sodium salts which were used throughout the work. The first readings usually fall below normal: this is either the result of a preliminary depressing action on respiration or is due to

[10] From a paper by Beysel, W., and Löb, W., *Biochem. Z.*, 1915, lxviii, 368. I obtained Sörensen's table for the mixtures of equimolecular solutions of monosodium and disodium phosphates to give solutions of definite pH. Interpolation and checks with indicators (phenolsulphonephthalein and rosolic acid) showed that a mixture of 6.2 parts of Na_2HPO_4 plus 3.8 parts of NaH_2PO_4 gives a neutral solution.

the buffer effect of the phosphate mixture being slightly changed by the dilution of the mixture as the result of its transfer to the reaction chamber. However, the equilibrium is soon reestablished and the curve rises, flattening out after an hour. Fig. 2 shows the concentration curve of acceleration after an hour's exposure, each point being the average of several experiments. Higher concentrations gave plasmolysis and were not tested. At the point where the curve becomes horizontal the acceleration is 56 per cent.

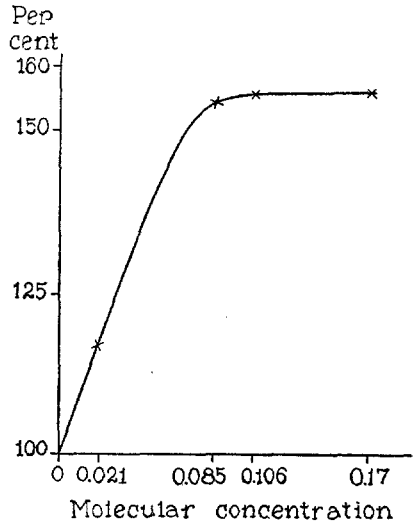

FIG. 2. The rate of production of CO_2 by *Elodea* in different concentrations of neutral phosphate solutions after an exposure of 1 hour. Each point is obtained by averaging from four to nine experiments (such as are shown in Fig. 1). Probable error of the mean is less than 3.5 per cent of the mean. The normal rate before the addition of phosphate is taken as 100 per cent.

After it was shown that all concentrations of neutral phosphate solutions as high as 0.17 M markedly accelerate the rate of production of CO_2, it became of interest to see how long this effect lasted. Many experiments were continued for 2 and 3 hours and in general the rate remained the same. In order to test it for longer periods the plants were removed from the reaction chamber after a measurement had been taken, and kept in an open dish of neutral phosphate mixture until about 2 hours before another measurement was desired. Then

the CO_2-free current was run through them in the closed system for $1\frac{1}{2}$ to 2 hours before the next observation was made. This removed all accumulation of CO_2, even when the plants had been left for 10 hours, as was shown by the constancy of the readings obtained.

Fig. 3 shows the individual curves and the average of three experiments, all of which agreed in general but varied in the time required

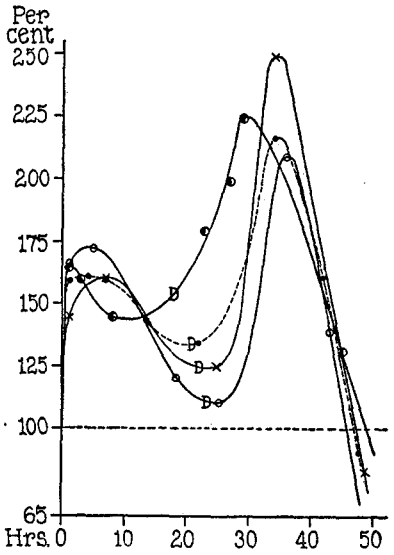

FIG. 3. The individual time curves and the average curve (dotted line) of three typical experiments on the effect of a 0.106 M neutral phosphate solution on the production of CO_2 by *Elodea*. The *D* on each curve indicates the approximate time of death of the cells as tested by plasmolysis. Probable error of the mean is less than 10 per cent of the mean. The normal rate before the addition of phosphate is taken as 100 per cent.

to arrive at corresponding parts of the curve. The preliminary acceleration gradually lessens, then rises sharply to a new maximum, and later is rapidly lost. Plasmolytic tests for the death point showed that death occurred 18 to 24 hours after exposure to the phosphate, at the time when the curve had nearly or just reached its first minimum. Thus the second rise followed the death of the plant cells[11] and oxida-

[11] Regarding respiration after death see Haas. A. R. C., *Bot. Gaz.*, 1919, lxvii, 347.

tion appeared to go on under the influence of the phosphate. The rate of production of CO_2 fell below the normal after several hours; presumably all the easily oxidisable substances were used up.

To meet the possible objection that rapid multiplication of bacteria after the death of the *Elodea* was responsible for the second rise, efforts were made to kill the *Elodea* in such a way as to kill the bacteria and the *Elodea* and leave the enzymes of the latter unharmed. Toluene is, of course, the obvious reagent to try and was used with the result that subsequent addition of phosphate caused no increase in production of CO_2. Yet microscopic examination of the *Elodea* leaves before and after death in the duration experiments always failed to show an increase in number of the very few bacteria that are not removed by washing, at the beginning of the experiment.

In order to see whether the toluene in killing the plants had not used up the materials available for oxidation, time curves were obtained of the production of CO_2 before and after exposure to toluene.[12] It was found that toluene induces an immediate, large acceleration, as do many other killing agents. From previous work[13] it was known that a solution of $CaCl_2$ always lowered the rate of production of CO_2 so it was here used to kill the *Elodea* and bacteria by a 2 hour exposure to a 1 M solution. Subsequent washing and exposure to phosphate resulted in a large increase in the production of CO_2. This confirmed the results of the duration experiments.

The experiments described above dealt with normal respiration which may be regarded as the sum of the anaerobic and aerobic processes going on in the plant. When the apparatus was filled with hydrogen gas, no production of CO_2 was observed in several experiments on *Elodea*. This shows that phosphate accelerates the aerobic phase. In order to test the effect on the anaerobic phase it was necessary to use another plant and sterile wheat seedlings (caulicle $\frac{1}{4}$ to $\frac{3}{4}$ inch long) were selected. Fig. 4 shows the average curves for the normal (or combined) respiration and for the anaerobic phase alone, as affected by phosphate. It is evident that much of the production of CO_2 is to be assigned to the anaerobic phase, as is shown

[12] Toluene destroys rubber valves and only the bead valves made these experiments possible. They will be reported later.

[13] Lyon, C. J., *Am. J. Bot.*, 1921, viii, 458.

by the fact that the anaerobic is only 28 per cent less than the aerobic (when no phosphate is added). The exposure to the phosphate solution results in an increase in production of CO_2 whether the oxygen is present or absent.

Curve *A* is for the normal respiration while Curves *B* and *C* are for the anaerobic phase alone. Curve *C* is Curve *B* moved up by 28 per cent so that it starts at 100 per cent and is more easily compared

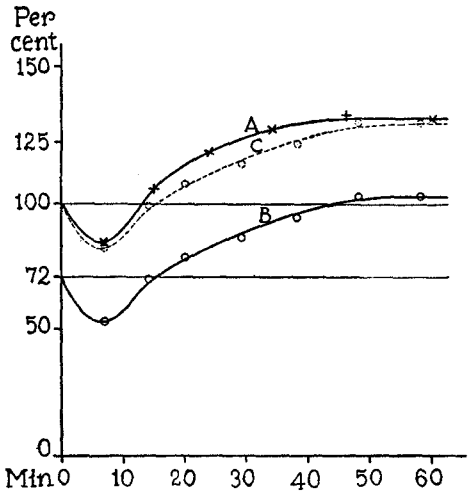

FIG. 4. The effect of a 0.04 M neutral phosphate solution on the production of CO_2 by wheat seedlings in air (Curve *A*) and in hydrogen (Curves *B* and *C*). The normal rate is taken as 100 per cent for Curve *A*. For Curve *B*, the average rate before exposure to the phosphate was 72 per cent of the rate before the oxygen was removed; hence the curve for anaerobic action starts at 72 per cent. Curve *C* is Curve *B* with its first point moved up to 100 per cent for comparison with Curve *A*. Average of three experiments for Curve *A* and of four for Curve *B;* probable error of the mean is less than 3.5 per cent (except for one point which is less than 9 per cent) of the mean.

with Curve *A*. It will be seen that the levels of acceleration are about the same at the end of the hour, the exact figures being 136 per cent for the normal (combined) and 132.25 per cent for the anaerobic alone. This proves the acceleration of the anaerobic phase of wheat seedlings and shows again (but less conclusively) that the aerobic phase is accelerated for if only the anaerobic were affected the

per cent of acceleration of the combined respiration would have been less than that for the anaerobic alone and not slightly more as the curves show.

The nature of the phosphate action will be further studied by experiments now being carried out. A full report and discussion of the above results is reserved until all the information is obtained. It seems probable, however, that phosphates will be shown to be necessary for plant respiration.

SUMMARY.

Phosphate accelerates both aerobic and anaerobic respiration. The acceleration almost disappears when the plant dies (in phosphate solution) but subsequently becomes greater than in life.