PHOSPHATE ION AS A PROMOTER CATALYST OF RESPIRATION.

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I.

In other papers (Lyon, 1923–24, 1927) results have been reported which appeared to show that neutral solutions of sodium or potassium phosphate serve to catalyze the enzymatic production of CO₂ by plant tissues. The oxidising enzymes involved were those of Elodea canadensis, wheat seedlings, and potato tubers, the latter studied only in aqueous extracts. We shall now present additional proof of this promoter action through more careful analyses to determine the active component of mixtures of mono- and disodium phosphates.

The molar concentrations of the solutions which gave optimum results were somewhat high for the usual types of catalysis. The concentration most used was approximately 0.1 m, by which is meant a solution obtained by mixing 0.1 m monosodium phosphate with 0.1 m disodium phosphate. The complex nature of the components of such a solution suggested that some single element among them was the active, or at least the controlling factor of the catalysis. Since the ionization of even this concentration of the sodium phosphates is presumably complete, we are concerned primarily with the nature of the ionization products of phosphoric acid. An excellent statement of the conditions of equilibrium between \( H_2PO' \), \( HPO' \), and \( PO'' \) is given by Holt, La Mer, and Chown (1925), from which it is apparent that for \( H_2PO' \), \( K_1 \) is very large, \( K_2 \) is smaller, and \( K_3 \), which determines the relative amounts of \( PO'' \), is very small. These authors have also calculated the concentrations of \( PO'' \) in relation to pH over a wide range and have introduced the expression \( p[PO''] \) which may

1 Holt, La Mer, and Chown (1925) pp. 518 to 522.
be used to express the concentration of this ion just as $p[H^+]$ is used for the hydrogen ion.

The relative concentration of $PO_4^{3-}$ is so low that it must be admitted that it is no higher than that of well known catalysts such as the $H^+$ ion. At pH 7.0 only 1/500,000th of the total P present is present in this ion. On the alkaline side of neutrality the relative concentration of $PO_4^{3-}$ increases rapidly and on the acid side it falls off. A plot of tables of Holt, La Mer, and Chown (1925) shows that the graph of $p[PO_4^{3-}]$ (we prefer to use the form $pPO_4$) against pH is not a straight line but a curve between pH 6 and 9, which correspond to $pPO_4$ 7.44 and 3.4, respectively. Thus $pPO_4$ will vary with both pH and the molar concentration of the acid or its sodium salts.

Similar statements could be worked out for the other ions of the solutions but it is this ion which proves to be related to catalysis through mathematically simple and exact rules.

As we shall show later, the concentration curve for the rate of $CO_2$ production by *Elodea canadensis* (Lyon) in different concentrations of neutral phosphate solutions after an exposure of 1 hour does not afford the best data by which to test the conception of catalysis by the $PO_4^{3-}$ ion. At the two extreme concentrations other factors intervene to mask the real effect. When the concentration is low the element of rate of penetration of the phosphate into the living cells limits the observed effect at the end of 1 hour. At the higher concentration there is opportunity for a deleterious effect through either the osmotic effects or some other result of the presence of such a high concentration of salts. The intermediate data are too few to use.

Accordingly, we have performed the experiments necessary to provide data for the effect of change of $pPO_4$ through change of pH on the alkaline side of neutrality. Similar experiments were attempted for the acid side, but the presence of carbonates or bicarbonates gave rise to such an increase in the liberation of $CO_2$ that such readings are not comparable with those at a higher pH. In Fig. 1 are shown individual time curves of experiments on the alkaline side of neutrality. The significant values for our purpose are the levels at which each curve flattens out. These experiments were performed according to

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the technique described in previous papers, the main apparatus being a suitable form of the Osterhout respirometer (Osterhout, 1918–19, 1919–20). Hence, the preliminary rise in most of these curves is probably not due to a serious error such as the introduction of atmospheric CO₂ at the time of application of the alkaline solution. It is more likely that we have to do with a temporary change in equilibrium.

**Fig. 1.** The effect of alkaline phosphate solutions on the production of CO₂ by *Elodea canadensis*. Each curve represents a typical experiment in which the normal (taken as 100 per cent) is obtained with an 0.106 M neutral phosphate solution and followed by the application of phosphate mixtures of the same molar concentration but with the pH as indicated for each curve.
PHOSPHATE ION AS A PROMOTER

within the system though there may possibly be a real temporary increase in rate of CO₂ production above that at which a level is attained.

In Fig. 2 are shown the mean values (of all experiments) of the rate of CO₂ production at the end of 1 hour, plotted against pPO₄, the latter calculated from the tables of Holt, La Mer, and Chown, and against pOH. The solid line shows the regularity of the relationship between the PO₄³⁻ ion and the rate of production of CO₂. From the

![Graph showing the mean rates of production of CO₂ by Elodea canadensis in 0.106 M alkaline phosphate solutions of different pH in relation to pPO₄ (solid line) and to pOH (broken line), after 1 hour.](Image)

nature of the relationship between pPO₄ and pH it is to be expected that the relationship to pOH (broken line) should also be regular. We are attempting to show that the principal effect is that of the PO₄³⁻ ion rather than the OH⁻ or H⁺ ion.

The curve for the relationship to the PO₄³⁻ ion resembles that of a hyperbola with the general equation (for these coordinates)

\[(CO₂ - a) (pPO₄ - b) = K,\]

where a and b represent the fact that the asymptotes of this hyperbola may not be CO₂ = 0 and pPO₄ = 0 but CO₂ = a and pPO₄ = b. The mathematical solution of this equation for the five measured points on
the curve was accomplished by a reliable method that depends upon the general method of least squares. The equation which results is

$$(\text{CO}_2 - 68.475) (\text{pPO}_4 - 2.13) = 114.43.$$  

**Fig. 3.** The rates of production of CO$_2$ by Elodea canadensis in 0.106 M alkaline phosphate solutions of different pH in relation to pPO$_4$. The ordinates are logarithms of per cent CO$_2$ while the abscissae are logarithms of pPO$_4$ - 2.13. The straight line is the plot of values calculated from the equation in the text.

The closeness of fit to the data may be seen in Fig. 3 in which the logarithm of the per cent CO$_2$ is plotted against the logarithm of pPO$_4$. If the hyperbolic relationship holds the points should lie in a straight line in this type of plot. The straight line we have drawn represents the calculated equation while the points indicate the locations of the measurements.

The meanings of the constants $a$ and $b$ are interpreted as follows: Under the conditions by which the relationship was derived (viz. with
PHOSPHATE ION AS A PROMOTER

the pH also increased) the one asymptote locates the point beyond which further increase in concentration of PO$_4^{3-}$ ions fails to give an increase in rate of CO$_2$ production; the other asymptote denotes the fact that the enzyme may function apart from PO$_4^{3-}$ ions to the extent of 68.475 per cent, where 100 per cent is the rate measured in neutral phosphate solution (0.1 M). Not much emphasis can be attached to these constants, however, since they were not obtained by changing only one variable—an impossible step in this work.

We may now observe the relation of this equation to the points on the concentration curve for *Elodea* (Lyon, 1923-24, Fig. 2). The horizontal form of the curve at molecular concentrations approaching the threshold of plasmolysis could not be expected to check with an equation derived from measurements at optimum salt concentrations. Some limiting factor may also prevent a close adherence to a hyperbolic relationship.

Likewise, the lowest concentrations might not exhibit the same relation. Here it is found that if the increase in molar concentration be thought of as a slow increase in concentration of PO$_4^{3-}$ ions (as is the case), the slope of the curve for CO$_2$ production is greater than that of Fig. 2 and is sensibly uniform over the first third of the graph. This linear, or very nearly linear relationship, which denotes direct proportionality, is typical of catalytic processes over the range of low concentrations and does not conflict with the relationship expressed by our derived equation. At the midrange of concentration, however, we should expect some conformity and such was found to be the case. The total difference between the $K$'s computed for the two known midpoints is only 1.5 per cent of the mean $K$.

This is as far as we can go in the analysis of our own data which seem to point to the PO$_4^{3-}$ ion as the effective catalyst of oxidising enzymes. It would be very desirable to obtain similar concentration curves for other plant material, by experiments involving change of concentration of the phosphate ion by changing first the pH and then the molar concentration. We plan to conduct such studies at a later date.

The results of Bode (1926), who sought to measure the dependence of respiration upon hydrogen ion concentration, show a qualitative agreement with our results in so far as his data may be converted into data for pPO$_4$. His use of calcium and magnesium phosphates to regulate the pH introduces new variables affecting the exact concent-
tration of the phosphate ion and involving the influence of the metal. In general, however, the presence of higher concentrations of phosphate ion correlates with a higher rate of respiration as measured by the absorption of oxygen.

II.

A further confirmation of the promoter action of the phosphate ion is afforded by the results of analyses of some existing records in the literature of other enzyme actions. Appropriate treatment of such records demonstrates the same hyperbolic relationship.

The widespread practice of using phosphate buffers for controlling the pH of enzyme reactions has led to the statement of some records in a form which allows the transposition of either molecular concentration or change of pH into data for pPO₄. Unfortunately it is common practice to report but a few "typical" series of measurements. Mean values of two measurements were found for some cases and of course these as well as data covering both change of pH and change of molar concentration at constant pH in the same type of experiments carry more weight. ⁴

The method of procedure in the conversion of data into terms of pPO₄ will be described for only the first set of readings to be dealt with—those given by Smirnoff (1925) in a study of the effect of neutral salts on peroxidase. The enzyme was obtained from ground wheat seeds. The substrate was pyrogallol. The criterion of enzyme action was the amount of purpurogallin formed, estimated by titration with KMnO₄. The data for the effect of the phosphate solution of concentration N/80 were given for only one set of experiments, in the following tabular form.

<table>
<thead>
<tr>
<th>pH</th>
<th>Activity of enzyme, per cent.</th>
<th>Activity, per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>1.9</td>
<td>8.7</td>
</tr>
<tr>
<td>4.0</td>
<td>2.84</td>
<td>9.3</td>
</tr>
<tr>
<td>4.98</td>
<td>55.79</td>
<td>9.5</td>
</tr>
<tr>
<td>6.5</td>
<td>100</td>
<td>183.64</td>
</tr>
<tr>
<td>7.1</td>
<td>135.0</td>
<td>173.86</td>
</tr>
<tr>
<td>7.5</td>
<td>148.16</td>
<td>139.68</td>
</tr>
<tr>
<td>7.86</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>8.3</td>
<td>157.07</td>
<td></td>
</tr>
</tbody>
</table>

⁴ The measurements, however, are all recorded in terms of amounts of product after equal periods of time. That this is not always the proper measure of enzyme action was shown in the recalculation by Northrop (1924-25) of the results of Morgulis (1921) in a study of the kinetics of catalase action on peroxide.
From these data it is obvious that below pH 4.98 there is a powerful inhibition of enzyme action and above pH 8.7 there is a depressive action. These effects are undoubtedly due to a specific effect of the pH value and we can only use the intermediate data. The necessary data for a logarithmic plot in terms of pPO₄ is given in the following table. The pPO₄ was obtained by interpolation from a plot of the table of pPO₄ for various pH values.

**FIG. 4.** The logarithmic plot of the relation of the concentration of PO₄³⁻ ions to the percentage rate of oxidation of pyrogallol to purpurogallin by peroxidase. The straight line is the plot of the equation

\[(\text{Activity of enzyme}) \ (\text{pPO₄})^{1.24} = K.\]

The indicated points represent a single set of readings as reported by Smirnoff (1925).

<table>
<thead>
<tr>
<th>pH</th>
<th>pPO₄</th>
<th>Log pPO₄</th>
<th>Enzyme activity in per cent</th>
<th>Log enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.98</td>
<td>9.43</td>
<td>0.9745</td>
<td>55.79</td>
<td>1.7465</td>
</tr>
<tr>
<td>6.5</td>
<td>6.51</td>
<td>0.8136</td>
<td>100.0</td>
<td>2.0000</td>
</tr>
<tr>
<td>7.1</td>
<td>5.53</td>
<td>0.7427</td>
<td>135.0</td>
<td>2.1303</td>
</tr>
<tr>
<td>7.5</td>
<td>5.00</td>
<td>0.6990</td>
<td>148.16</td>
<td>2.1707</td>
</tr>
<tr>
<td>7.86</td>
<td>4.58</td>
<td>0.6609</td>
<td>150.53</td>
<td>2.1775</td>
</tr>
<tr>
<td>8.3</td>
<td>4.10</td>
<td>0.6128</td>
<td>175.07</td>
<td>2.2430</td>
</tr>
<tr>
<td>8.7</td>
<td>3.72</td>
<td>0.5705</td>
<td>183.64</td>
<td>2.2640</td>
</tr>
</tbody>
</table>
Fig. 4 shows the plot of these values. The points lie as near to a straight line as could be expected for a single set of readings. The slope of this line is not -1, however, but -1.34. Therefore the equation is

$$\text{(Activity of enzyme)} \times (\text{pPO}_4)^{1.34} = K.$$ 

Fig. 5. Logarithmic plot of the relation of concentration of PO$_4$ ions to the rate of hydrolysis of ethyl butyrate by pancreatic lipase in glycerin extracts of the pig pancreas. Data are taken from the work of Platt and Dawson (1925) who varied pPO$_4$ by change of pH. The straight line is fitted to the points (●) obtained from the readings for an experiment involving the use of low concentration of phosphate salts. The abscissa are those from the scale at the bottom of the figure and the equation for the line is

$$(\text{Hydrolysis}) \times (\text{pPO}_4)^{1.31} = K.$$ 

The other points (○) are referred to the scale of abscissa at the top of the figure, this scale differing from the lower one only by a displacement to the right by four units. These points were taken from a second experiment which differed only in the use of five times as much phosphate salts in the buffer solutions.
Smirnoff did not study in sufficient detail the effect of the concentration of phosphate solutions to warrant a similar test of the phosphate ion relations at constant pH. The method of calculation of pPO₄ under those conditions is fundamentally the same and will appear in connection with lipase analyses.

For another of the oxidising enzymes—laccase from alfalfa—we have found a single set of four readings for change of pH as recorded by Bunzel (1915). Its action was tested only on hydroquinone. The "unnaturalness" of the conditions is attested by the fact that there is no absorption of oxygen in neutral solutions. Therefore no conclusions can be reached as to the relation of laccase to the phosphate ion in living cells in which the substrate is quite different and the enzyme is effective at the pH of protoplasm. Actually, the graph on the logarithmic plot was a straight line with slope -7.46 for three of the four readings, the fourth one being nearest the point of no oxidation.

The most complete data to be found for enzyme action in relation to phosphate are those for pancreatic lipase. In all, the results of five independent sets of experiments have been found to be available for analysis. The most recent workers (Platt and Dawson, 1925) recognized a specific function of the phosphate buffers which were also used by the previous workers. Their technique was likewise more refined and their data cover both change of pH and change of concentration. Hence, their results carry more weight and are considered first.

Platt and Dawson estimated the action of pancreatic lipase of the pig by titrating the butyric acid released through the hydrolysis of ethyl butyrate. They were concerned partly with the optimum pH and found it to be about 7.0 for phosphate buffers and purified lipase. By using glycerin extracts of the pancreas they were able to carry the pH as high as 8.0 with steadily increasing hydrolysis. They consider that a protection of the enzyme is afforded by some constituent of the glycerin extract. These experiments (Nos. 5 and 6) are therefore the ones from which we have obtained data for a wide change of pH with constant concentration of phosphate salt. They are shown in Fig. 5 from which it is evident that their Experiment 5, in which only 5 cc. of phosphate solution was used, gives an unbroken straight line through a wide range of pPO₄. Experiment 6 involved the use of 25 cc. of phosphate solution and the points (shown by o) are somewhat irregularly distributed. In both experiments the points represent single readings and additional experiments would probably remove the irregularities. The general trend of the points is not far from that for the lower concentration of salt, the equation for which is

\[(\text{Hydrolysis}) \cdot (p\text{PO}_4)^{1.91} = K.\]

After a specific effect of phosphate was observed the point was carefully studied by using different molar concentrations of the buffer salts \(\text{(no hydrolysis could be observed in the absence of phosphate).}\) In the first of these studies (their Experiment 9) low concentrations were employed at pPO₄ 4.9 (pH 7.6). The results show that for the range 0.005 to 0.05 M there is a hyperbolic relationship between the
Fig. 6. Logarithmic plot of the relation of pPO₄ to the rate of hydrolysis of ethyl butyrate by pancreatic lipase. Data are taken from the work of Platt and Dawson (1925) in which the pPO₄ was varied by the use of varying concentrations of phosphate salts at constant pH. Each point represents the mean value of two readings. The equations for the lines, together with the pH used for each, are as follows:

Curve A (Hydrolysis) (pPO₄)⁵.⁶⁴ = K \[ \text{pH} = 7.6 \]

" B (Hydrolysis) (pPO₄)⁶.₃₂ = K \[ \text{pH} = 7.2 \]

" C (Hydrolysis) (pPO₄)².₉ = K \[ \text{pH} = 7.6 \]

" D (Hydrolysis) (pPO₄)³.₅⁹ = K \[ \text{pH} = 7.6 \]

The misprint indicated in connection with Curve C was obviously a mistake in one figure of the number given in the original paper.
amount of hydrolysis and the pPO₄. The logarithmic plot is given in Fig. 6, Curve A, which indicates the relation

\[(\text{Hydrolysis}) (\text{pPO}_4)^{1.24} = K.\]

The method of obtaining the pPO₄ for the various concentrations is the same as that used in similar conversions for pH and was done by the use of the table of factors given by Clark (1922) for this purpose. Thus the pPO₄ at 0.01 M (all calculations were corrected to this concentration) is 4.9 while that at 0.03 M is 3 × 4.9 or 0.3 × 3.9 which is pPO₄ 4.42.

In Fig. 6 there are also shown the results of the remainder of the tests with change of concentration at constant pH. For the experiment shown by Curve B the pH was 7.2. This is nearer the optimum than was the case for Curves C and D in which the pH was 7.6. Platt and Dawson comment on the difference in

\[^1\text{Clark (1922), p. 456.}\]
form between these two types of curves as plotted using molar concentration, and say that the farther the pH is above the optimum, the more pronounced the curvature. This is somewhat noticeable in Curves C and D when the data are converted to a logarithmic plot. However the conditions in the experiments were

![Logarithmic plot of the relation of pPO₄ to the hydrolysis of olive oil by pancreatic lipase of the pig. Data are taken from a single experiment by Umeda (1915). The pPO₄ was varied by the use of buffer solutions of pH 4.494 to 7.731. The equation for the line as drawn is (Activity of enzyme) (pPO₄)².54 = K.](image)

essentially the same as for those shown by Curve A. (This was also our justification for suggesting the indicated misprint in the data used for Curve C; see legend).

The equations for the four experiments in which the pPO₄ was altered by change of molar concentration are given in the legend of Fig. 6. They differ from the
equation for the same relationship derived by change of pH only in the slope of the line, which is also indicated by the exponent of pPO₄ in the equation. This difference is directly attributable to the attendant differences in molar concentration and hydrogen ion concentration. The straight lines on the logarithmic plots attest the hyperbolic relationship between pPO₄ and activity of the lipase, whether the concentration of the PO₄³⁻ ion be increased by decrease of pH or by increase of total salt concentration. The slope merely measures the sensitivity of the given sample of enzyme to the change in concentration of the PO₄³⁻ ion under the experimental conditions imposed by other factors.

FIG. 9. A composite logarithmic plot of the relation of pPO₄ to the percentage hydrolysis of tributyrin by the pancreatic lipase from beef pancreas. Data are taken from five single experiments by Rona and Bien (1914), each of which is represented by a broken line. The equation for the mean line as drawn (solid line) is

\[(\text{Hydrolysis})(\text{pPO}_4)^{1.08} = K.\]

The pPO₄ was varied by the use of buffer solutions of pH 4.87 to 8.58.

Platt and Dawson also noticed that both α- and β- sodium glycerophosphate promoted the action of lipase to the same degree. They state their opinion that this indicates an effect by the phosphate ion. The present analysis confirms this observation.

Another recent measurement of the action of pancreatic lipases (purified) on tributyrin is reported by Rona and Pavlović (1922). The available data are very meager but the uniformity of the results is evident from the form of the plots in Fig. 7. Only three measurements are available for the lipase from dog pancreas and there are but five for that from the human pancreas. Each set of points, however, lies along a straight line, the slope of which differs for the two types of enzymes. The "k" used as a measure of the activity of the enzyme was calculated by the authors from a monomolecular equation for the hydrolysis. In each case the pPO₄ was altered by change of pH.
The work of Umeda (1915) furnished a single set of readings of the hydrolysis of olive oil by purified pancreatic lipase of the pig, in relation to pPO₄ as calculated from the pH. The lipolysis was estimated by titration of the acid produced after 20 hours. Fig. 8 shows the same hyperbolic relationship to pPO₄. Here the equation is

\[(\text{Activity of enzyme}) \cdot (\text{pPO}_4)^{3.44} = K.\]

Fig. 10. Logarithmic plot of the relation of pPO₄ to the percentage hydrolysis of fat by pancreatic lipase of the pig (shown by (●) and solid lines) and by lipase of the duodenal juice (shown by (○) and broken line). Data are taken from single readings by Davidsohn in 1913.

Rona and Bien (1914) used a glycerin extract of lipase from beef pancreas and estimated the hydrolysis of tributyrin by a stalagmometric method. This method is not so accurate as titration. The results are converted into percentage of hydrolysis and plotted against pPO₄ as usual in Fig. 9. This is a composite of several experiments, each of which is indicated by a broken line. The variation from the average line is due largely to the variation in the activity of the sample of enzyme used. Each experiment gave a straight line plot and the mean slope is -1.08. Therefore the equation is the same as those derived from later measurements.

In Fig. 10 are shown the results from a still earlier report (Davidsohn, 1913) of hydrolysis of fat by pancreatic lipase of the pig. This is the only case we have
found in which all the points do not lie along a straight line on this type of plot. The two solid straight lines in the figure are drawn in respect to the readings from three experiments with lipase direct from the pancreas, one sample of which was obviously more active than the other two. These data clearly exhibit the same type of relationship as has been demonstrated in the previous analyses.

The other points (○) on this composite plot represent data from a number of single measurements with lipase contained in the duodenal juice. The curved, broken line roughly indicates the trend of these points as plotted over a wide range of pH and pPO₄. The points at the extremes could be accounted for on the basis of the unfavorable pH of the media. However, the distribution of all these points is not only very irregular but uncertain. This is due to the composite nature of the data, the use of the stalagmometric method of analysis, the variation in the total concentration of phosphate buffer salts, and the inability to correct for all such factors in converting to pPO₄ because of the incompleteness of the statement of experimental procedure. The relationships exhibited by our other analyses of lipase studies and the recent demonstration by Platt and Dawson of an absolute dependence of pancreatic lipase, also of the pig, upon the presence of at least some phosphate, appears to outweigh the doubtful evidence from these older results as regards the lipase obtained in a different medium.

From these analyses of the relation of lipase, peroxidase, and possibly laccase to phosphate solutions, it seems quite certain that the PO₄³⁻ ion acts as a promoter of their activities. The mathematical statement of the relationship is like that for the effect of phosphate on the production of CO₂ by living cells. There is then every reason to believe that the active component of such phosphate solutions is the PO₄³⁻ ion, acting as a promoter catalyst. The very fact that the mathematical expression of the relationship is of the form

\[(\text{Activity of enzyme}) \cdot (\text{pPO}_4)^n = K\]

is of itself an additional proof; for the term "pPO₄" is a direct measure of the potential of the PO₄³⁻ ion in a given solution. The inverse proportionality expressed by the equation is really a direct proportion because of the peculiar method of statement of the potential.

In the case of plant respiration the exponent of the pPO₄ term was found to be 1. The corresponding exponent in the case of peroxidase was essentially the same (1.34). Although an exponent of this order was also found for a few cases in the lipase analyses (cf. Figs. 5, 7, and 9), the value 3 or 6 was more characteristic of lipase (cf. Figs. 6 to 8). The agreement of the numbers of the "oxidase" group of enzymes is significant while the question of sensitivity of lipase (measured by
the value of the exponent, \( n \) to the phosphate ion is beyond the immediate scope of our problem.

III.

SUMMARY.

The active component of phosphate solutions, in relation to promoter action on oxidising enzymes, is the \( \text{PO}_4^{3-} \) ion. This is shown by the demonstration of a hyperbolic relationship between per cent production of \( \text{CO}_2 \) (of \( Elodea \)) and \( \text{pPO}_4 \), the measure of the phosphate ion potential. This is consistent with the rate of respiration as affected by changing \( \text{pPO}_4 \) through change of total phosphate concentration while pH is kept constant. The equation for this relationship is

\[
(\text{CO}_2 - a) \ (\text{pPO}_4 - b)^n = K
\]

where \( a, b, n, \) and \( K \) are constants and \( n = 1 \).

The same relationship to phosphate ion concentration, expressed by the equation

\[
(\text{Activity of enzyme}) \ (\text{pPO}_4)^n = K,
\]

where \( n \) and \( K \) are constants and \( n \) varies from 1 to 6 under different conditions, appears to hold for some other enzyme actions, including those of peroxidase and pancreatic lipase.

CITATIONS.