

COMPARATIVE STUDIES ON RESPIRATION.

IX. THE EFFECTS OF ANTAGONISTIC SALTS ON THE RESPIRATION OF ASPERGILLUS NIGER.

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(Received for publication, July 21, 1919.)

The relation of antagonistic salts to the respiration of higher fungi has received no attention. As the problems involved are of considerable interest the writer has made a beginning in this direction by conducting a series of experiments on *Aspergillus niger*.

It may be of interest to compare the results with those obtained on other organisms.

In experiments on sea urchin eggs Warburg¹ and Meyerhof¹ found that NaCl causes a rise in respiration which is inhibited by the addition of CaCl₂. Loeb and Wasteneys¹ found no such rise. The results with *Aspergillus* show a rise with lower concentrations of NaCl and a fall with higher concentrations. Since the highest rise in NaCl (24 per cent) is very small as compared with the rise of several hundred per cent obtained by Warburg and by Meyerhof, the results as a whole agree much more nearly with those of Loeb and Wasteneys. Still closer agreement is found in the results of Brooks,² who has used both sodium and calcium in studies on the respiration of bacteria. She finds an increase in the rate of respiration with certain concentrations of NaCl and CaCl₂ and a decrease in higher concentrations. A mixture of NaCl and CaCl₂ shows antagonism.

In some experiments on *Aspergillus niger* Kosinski³ tested the effect of NaCl; although he seems to think that a 1.523 per cent (0.26M)

¹ For a summary of the experiments on sea urchin eggs see Osterhout, W. J. V., *J. Gen. Physiol.*, 1919-20, ii, 1.

² Brooks, M. M., *J. Gen. Physiol.*, 1919-20, ii, 5.

³ Kosinski, I., *Jahrb. wiss. Bot.*, 1902, xxxvii, 154.

solution does not affect respiration, his data show an increase in respiration of 41 per cent.

The writer, in his experiments on *Aspergillus niger*, used NaCl and CaCl₂, as well as mixtures of the two salts. For measuring the production of CO₂ the apparatus described by Osterhout⁴ was employed, in which the material is placed in one tube and the indicator in another.⁵ This method is very accurate and simple. Care must be exercised to prevent any alkali from passing over from the tube containing it into the indicator tube, as this would vitiate the results.

By adding an indicator to the tube containing the fungus it can be shown that an acid is produced which is non-volatile (or practically so) since it does not disappear when a current of air (free from CO₂) is passed through the liquid for 15 or 20 minutes (under these conditions CO₂ would disappear in 5 minutes or less).

The apparatus is so constructed that a non-volatile acid cannot affect the color of the indicator which is being matched by the observer. The question may arise, however, whether the production of such an acid has any bearing on the interpretation of the results. If it is borne in mind that the problem is to ascertain the changes produced in the output of CO₂ under the influence of reagents it is evident that we need not consider the production of other acids except as intermediate stages or as by-products whose study is a problem by itself. This problem always exists, for wherever respiration goes on organic acids and other substances are produced. It seems best for the present to confine the investigation to the production of CO₂, leaving the study of other substances to the future.

The rate of production of CO₂ is obtained by taking the reciprocal of the time required to produce a definite change of color in the indicator tube.⁶

The fungus was grown and handled as previously described,⁶ except that water distilled from a hard glass flask was used in place of tap water. The fungus, which forms a mat on the surface of the cul-

⁴ Osterhout, W. J. V., *J. Gen. Physiol.*, 1918-19, i, 17, 171.

⁵ In this investigation five drops of a 0.01 per cent solution of phenolsulfon-ephtalein were added to 10 cc. of water. This solution was then used for comparison with the buffer tubes, which contained the same amount of indicator.

⁶ Gustafson, F. G., *J. Gen. Physiol.*, 1918-19, i, 181.

tured solution, was rinsed in distilled water⁷ to free it from any adhering nutrient solution, before putting it in the apparatus. The proper amount of material was then wound around the glass tube dipping into the solution in the respiration tube and secured in this position by tying it with a thread. This exposed the surface of the fungus to the liquid and also kept it from moving about and insured the smallest amount of mechanical injury. When the apparatus was in motion the air bubbles passing through the solution containing the organism carried the CO₂ given off by the fungus into the indicator tube, where the change in color was noted.

In all experiments pH 7.75 was the starting point and pH 7.42 the end-point. This gave the same range for every reading and a uniform change of 0.33 pH. The time required to produce this change under normal conditions varied from $2\frac{1}{4}$ to $3\frac{1}{2}$ minutes, depending upon the amount and condition of the material.

A number of preliminary experiments were performed without giving the fungus any nutrient while they lasted. When no nutrient was present the rate of respiration gradually fell below normal. For this reason it was thought best to add enough food to keep the control up to normal, so that any deviation from normal would be due to the action of the salt under investigation.

In starting an experiment the rate of respiration was first obtained in a 0.1 per cent solution of dextrose in distilled water. Unless the rate remained practically constant for at least 20 minutes in this solution, the material was rejected. The rate obtained in the dextrose solution is called the normal rate of respiration and in all calculations is taken as 100 per cent.

Several concentrations of dextrose were tried, but as all seemed to give the same results 0.1 per cent was chosen, as low viscosity is advantageous in the prevention of foaming. When the reagent used in the experiment was introduced the concentration of dextrose was thereby diluted one-half, so that during the experiment with the reagent it was only 0.05 per cent. In the numerous controls which were made the same thing was done, but no effect on the respiration was

⁷ All water used in these experiments was distilled from a hard glass flask, and came in contact with hard glass only throughout the experiment.

noticed, and control experiments made in this way kept up to normal for several hours, or as long as the experiment lasted.

Various concentrations of NaCl were used. Lower concentrations such as 0.125M, 0.25M, and 0.5M caused a rise in respiration. At 1M the results were rather variable, some experiments showing a rise and others a fall. Solutions of 2M always gave a decided decrease which was followed by a small increase. The respiration then remained constant for more than an hour or to the end of the experiment. Figs. 1 and 2 give a graphic representation of the results obtained with various concentrations of NaCl.

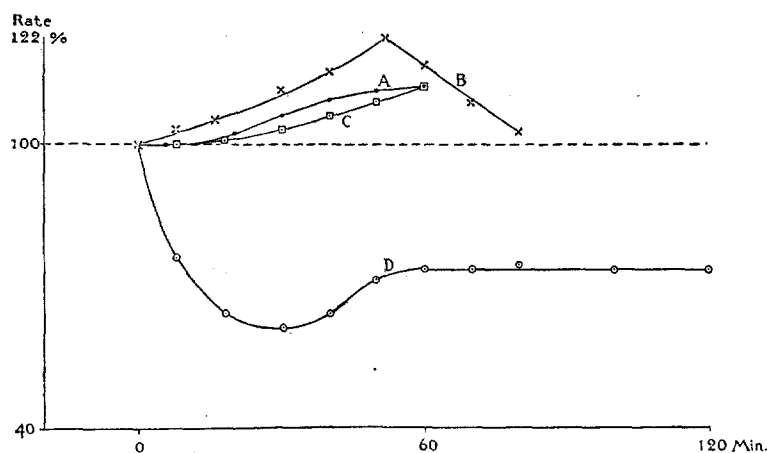


FIG. 1. Respiration of *Aspergillus niger*. The broken straight line to the left of the point marked 0 on the abscissa represents the normal rate of respiration before the addition of the salt. Curve A represents the respiration in 0.125M NaCl, Curve B respiration in 0.25M NaCl, Curve C respiration in 0.5M NaCl, Curve D respiration in 2M NaCl, and the broken line the control in 0.05 per cent dextrose. A, B, and C were in 0.05 per cent dextrose while D was in 1.5 per cent dextrose. Curves A, C, and D are each an average of 3, B of 4 experiments. Probable error less than 3 per cent of the mean.

The results with the lower concentrations are somewhat variable as to the amount of increase, as well as in respect to the time of maximum respiration. Thus in some experiments with 0.25M NaCl the maximum was reached at about 30 minutes after the introduction of the salt, while in most the maximum was not reached until at the end of 50 minutes.

This variability was of course to be expected with substances that are not more toxic than NaCl, where the least individual variation in the physiological activity of the cultures has a chance to exert its influence fully. This fact has already been noted in respect to some of the weaker anesthetics.⁶ Though there are differences in the amount of increase and in the time when the maxima occur, yet there

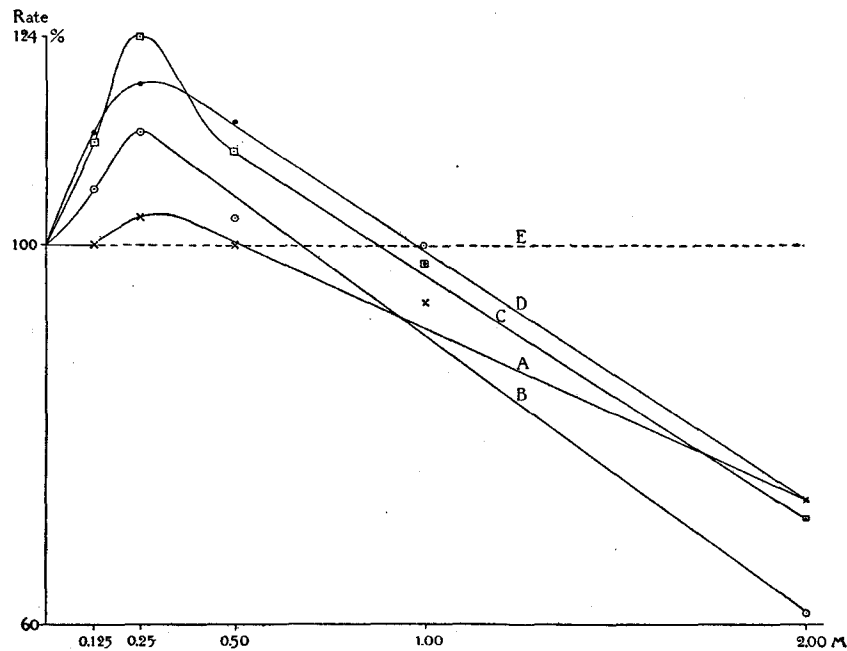


FIG. 2. Respiration of *Aspergillus niger*. The curves show rate plotted against concentration and represent the same experiments as shown in Fig. 1. Curve A represents the respiration at the end of 10 minutes exposure to NaCl, Curve B at end of 30, Curve C at end of 52, and Curve D at end of 60 minutes. Curve E represents the control in 0.05 per cent dextrose.

is no doubt about the general result. There is a distinct, though small, amount of increase in the rate of respiration, when *Aspergillus niger* is treated with NaCl at concentrations between 0.125 and 0.5M.

With 1M solutions of NaCl apparently contradictory results were obtained; *i.e.*, some experiments gave a large initial decrease followed by a rise to normal, others gave little or no initial decrease followed

by a rise above normal, while still other experiments gave only normal respiration.

The writer believes that these results are due to two factors acting on the fungus. One is the specific chemical action of NaCl which tends to stimulate, while the other, the osmotic pressure of the NaCl, tends to decrease the respiration. As is well known the abstraction of water from tissues lowers the rate of respiration. The fact that sometimes one factor prevails, and sometimes the other is probably due to physiological differences in the fungus.

Experiments with 2M NaCl show a large initial decrease followed by an increase of about 10 per cent. This is also found with 1M but is not so pronounced. The explanation of this fact may be that at first rapid withdrawal of water occurs, but as the salt penetrates and osmotic pressure within the cell increases, water is taken up, causing a rise in respiration. This supposition is further strengthened by experiments with 1.25M CaCl₂,⁸ which showed a large initial decrease, not followed by an increase in respiration, but by a slow steady decrease which might be expected if CaCl₂ does not penetrate readily. Osterhout⁹ in his experiments on *Laminaria* showed that NaCl increases the permeability, while CaCl₂ at first decreases it.

Only one concentration of CaCl₂ was used with dextrose, but several more concentrations without dextrose were employed. The concentration used with dextrose was 0.5M. This caused an increase in the respiration, giving a curve with rounded apex as shown in Fig. 3, Curve B. Concentrations used without dextrose were 0.3125M, 0.625M, and 1.25M. The first two caused a rise, while the last one only a decrease in respiration.

At this point it may be of interest to note that the effect of the salt seemed to be more pronounced when dextrose was absent than when it was present. This was especially the case with CaCl₂. Kosinski found that *Aspergillus niger* does not store up raw food material, and that as soon as it is taken out of a solution containing nutrient material it is in a starving condition.¹⁰ He states that when this happens

⁸ This concentration of CaCl₂ has approximately the same osmotic pressure as 2M NaCl.

⁹ Osterhout, W. J. V., *Science*, 1911, xxxiv, 187; 1912, xxxv, 112.

¹⁰ In the writer's experiments the rate of respiration began to decrease about 30 minutes after transfer from nutrient solution to distilled water.

plastic material is used in oxidation. If this is true it is easily conceivable that the oxidation of such material might not be affected in the same way by CaCl_2 as the oxidation of dextrose.

Experiments on antagonism between NaCl and CaCl_2 were also made: A solution containing 19 cc. of NaCl and 1 cc. of CaCl_2 (both 0.5M) gave the best results. In this mixture the rate of respiration was practically normal (Fig. 3, Curve C). Other proportions gave more or less increase.

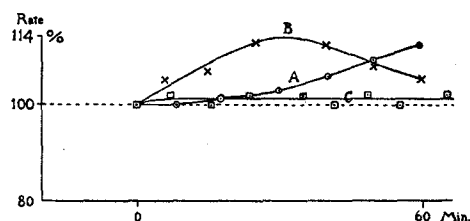


FIG. 3. Respiration of *Aspergillus niger*. The broken straight line to the left of the point marked 0 on the abscissa represents the normal rate of respiration in 0.1 per cent dextrose before the addition of the salt. Curve A represents the respiration in 0.5M NaCl , Curve B in 0.5M CaCl_2 , and Curve C in a mixture of 19 cc. of NaCl and 1 cc. of CaCl_2 (both 0.5M). The control (dotted line) and C are practically identical. All these experiments were made in 0.05 per cent dextrose. Curve A is an average of 3, Curves B and C of 4 experiments. The probable error was less than 3 per cent of the mean.

In order to compare these results with the effects on growth spores were sown on solutions of NaCl and CaCl_2 (with 0.05 per cent dextrose), on various mixtures of these,¹¹ and on 0.05 per cent dextrose alone. For these experiments Petri dishes were used. The extent of germination was determined by microscopic examination.

The following results were noted: In NaCl there was no germination; in 49 cc. of NaCl +1 cc. of CaCl_2 some germination; 24 cc. of NaCl +1 cc. of CaCl_2 , and 9 cc. of NaCl +1 cc. of CaCl_2 showed a growth which increased in proportion to the amount of CaCl_2 ; 4 cc. of NaCl +1 cc. of CaCl_2 seemed to produce the best growth; 1 cc. of NaCl +1 cc. of CaCl_2 and CaCl_2 alone showed fairly good growth; 0.05 per cent dextrose produced rather poor growth.

¹¹ Both NaCl and CaCl_2 were 0.5M and were dissolved in 0.05 per cent dextrose.

From these data it would seem that effects of CaCl_2 and NaCl on respiration are different from their effects on growth. This difference cannot be an osmotic effect, as the CaCl_2 solution, having a stronger osmotic pressure, would in that case be the one that would prevent growth to a greater extent, but this is not the fact.

SUMMARY.

1. In the presence of 0.05 per cent dextrose the respiration of *Aspergillus niger* is increased by NaCl in concentrations of 0.25 to 0.5M, and by 0.5M CaCl_2 .

2. Stronger concentrations, as 2M NaCl and 1.25M CaCl_2 , decrease the respiration. The decrease in the higher concentrations is probably an osmotic effect of these salts.

3. A mixture of 19 cc. of NaCl and 1 cc. of CaCl_2 (both 0.5M) showed antagonism, in that the respiration was normal, although each salt alone caused an increase.

4. Spores of *Aspergillus niger* did not germinate on 0.5M NaCl (plus 0.05 per cent dextrose) while they did on 0.5M CaCl_2 (plus 0.05 per cent dextrose) and on various mixtures of the two. This shows that a substance may have different effects on respiration from those which it has upon growth.