RESPIRATION OF PHYCOMYCES

by

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INTRODUCTION.

METHODS AND APPARATUS.

In the following pages a description is given of experiments on the respiration of Phycomyces Blakesleeanus Burgeff. This fungus is wellknown under the name of Phycomyces nitens Kunze, but two years ago, Burgeff (16) showed that the latter name really belonged to another Phycomyces, so he called the former Phycomyces Blakesleeanus.

In all the experiments transfers were used from a one spore culture of a + strain, obtained from the „Centraalbureau voor Schimmelcultures" at Baarn, Holland.

Two kinds of respiration apparatus were used, one of the air current type and the other of the closed space or Regnault type.

Respiration, consisting in evolving carbon dioxide and absorbing oxygen, is generally measured.

1° by determining the CO_2 given off
2° „ „ „ O_2 taken in
3° „ using both methods simultaneously.

The first method has already been worked out by Pettenkofer (69); later on Pfeffer (70, 95) introduced it into plant-physiology. This socalled Pettenkofer-Pfeffer method consists in forcing or sucking a current of air deprived of carbondioxide through the respiration vessel. In leaving it the air has to pass a solution of bariumhydroxide in long horizontal glass tubes, called Pettenkofer-tubes. The quantity of CO_2 given off by the plant can be deter-

mined from its absorption by the barytawater and subsequent titration. The air current can be led alternately into different tubes. Another absorber with barytawater behind the Pettenkofer-tubes will tell that all the CO_2 has been absorbed in the tube, if the solution in the absorber remains clear.

This method is a very simple and handy one and can always be used when the only thing to be settled is the amount of the CO_2, given off by the plant. It was in such cases that I made use of it, the air being sucked through the system by an aspirator of the kind described by Fernandes (31). Attached to it was an open manometer with mercury which enabled me to check the sucking force of the aspirator.

The respiration chamber was placed in a glass basin filled with water, kept at a constant temperature by electrical heating. Inside the glass basin there was also a metal spiral tube through which the air had to pass before entering the respiration vessel in order to take on the same temperature.

Before entering the vessel the air was purified in different washing-bottles filled with solutions of sodium hydroxide, strong sulphuric acid, silver nitrate and potassium permanganate. In leaving the respiration vessel the air passed a small absorbing flask with sulphuric acid. Without this precaution, the air, rich in watervapour given off by the respiring plant, would render the bariumhydroxidesolution less concentrated. The sulphuric acid should not be taken too strong, for in this case an absolutely dry air enters the Pettenkofer-tube and a humid one leaves it, also changing the strength of the barytawater.

Half a century ago A d. M a y e r (61, 96) determined the O_2- absorption by measuring the decrease in a volume of air, which was shut off from the atmosphere by means

of mercury, a strong alkaline solution absorbing all the CO_2 evolved by the plant.

By measuring the CO_2 absorbed by the alkaline solution, Godlewsky (35) determined the O_2 taken in and the CO_2 given off by the plant at the same time. Bonnier and Mangin (11) examined the O_2 absorbed and the CO_2 given off by the plants by analysing samples of air. It is obvious that in both methods a lack of oxygen may effect the respiration. Moreover, in the last method, a lot of CO_2 may be formed in the experimental vessel, which may injure the plants. Besides this, the more there is of CO_2, the more will be absorbed by the water and the plants, so the amount of CO_2 found will be too small.

In zoo-physiology an apparatus is used without these drawbacks, the prototype of which was published by Regnault and Reiset (76). It has subsequently been modified and improved by Benedict (7) and by Fredericia (33). In these apparatus a supply of oxygen has to compensate the decrease in the oxygen pressure inside, so as to keep it as nearly as possible to the original pressure. Various devices are in use. Connected with the chamber a volume recorder is often arranged, which will at a certain point close an electric circuit and admit oxygen from a cylinder and reduction valve.

In 1923 Fernandes (30 and 31) introduced into plant-physiology an ingenious respiration apparatus on the Regnault-Reiset principle. The oxygen is obtained by a new method, i.e. by hydrolyzing a sodium hydroxidesolution. As much O_2 as is taken up by the plant is formed and led to the respiration vessel. So the amount of oxygen in the closed space always remains the same. The hydrogen formed at the other electrode is collected in an eudiometer. It is equal to twice the amount of oxygen absorbed by the plant. A pump circulates the constant volume of air through the system. By means of tubes filled with barytawater the

CO_2 can be absorbed. As both O_2 and CO_2 are determined the respiratory quotient is known.

Fernandes did not pay much attention to the respiratory quotients. I made the determination of the respiratory quotients an important part of my investigations. I soon discovered that in order to be able to determine the quotients accurately, certain alterations had to be made.

For a detailed description of the original apparatus I beg to refer the reader to Fernandes' publication. Here I am only going to give a schematic description of the apparatus and its improvements as shown in fig. 1. The closed space with the constant volume of air is formed by the system A, B, etc... H. By means of H the air is pumped in the direction indicated by the arrows. A is the respiration chamber, the air entering at the top and leaving the vessel at the bottom. Then the air has to pass the washing-bottle B filled with a solution of sulphuric acid of which the same may be said as on p.118. D and E are Pettenkofer tubes, long 35 c.m., diameter 2.1 c.m., each filled with 80 ccm. barium-hydroxide solution, absorbing the CO_2 given off by the plant. G is the control barytatube. The level in the right leg of the manometer L would rise if oxygen was not formed in the electrolyzing vessel M. The electrolyzing current can be regulated by means of a resistance. In the circuit I placed a milliamperemeter which was a great convenience as will be proved afterwards.

It appears from the diagram that the most important improvement made is the compensating vessel K. In starting an experiment the vessels A and K are shut off from the atmosphere by closing the taps I and J. Changes in barometric pressure can have no influence then. It stands to reason that the electrolyzing vessel M must be shut off too. The glass tube at the left in which the hydrogen is formed and conducted to the eudiometer N is in connection with the atmosphere. O is a levelling device consisting of a glass

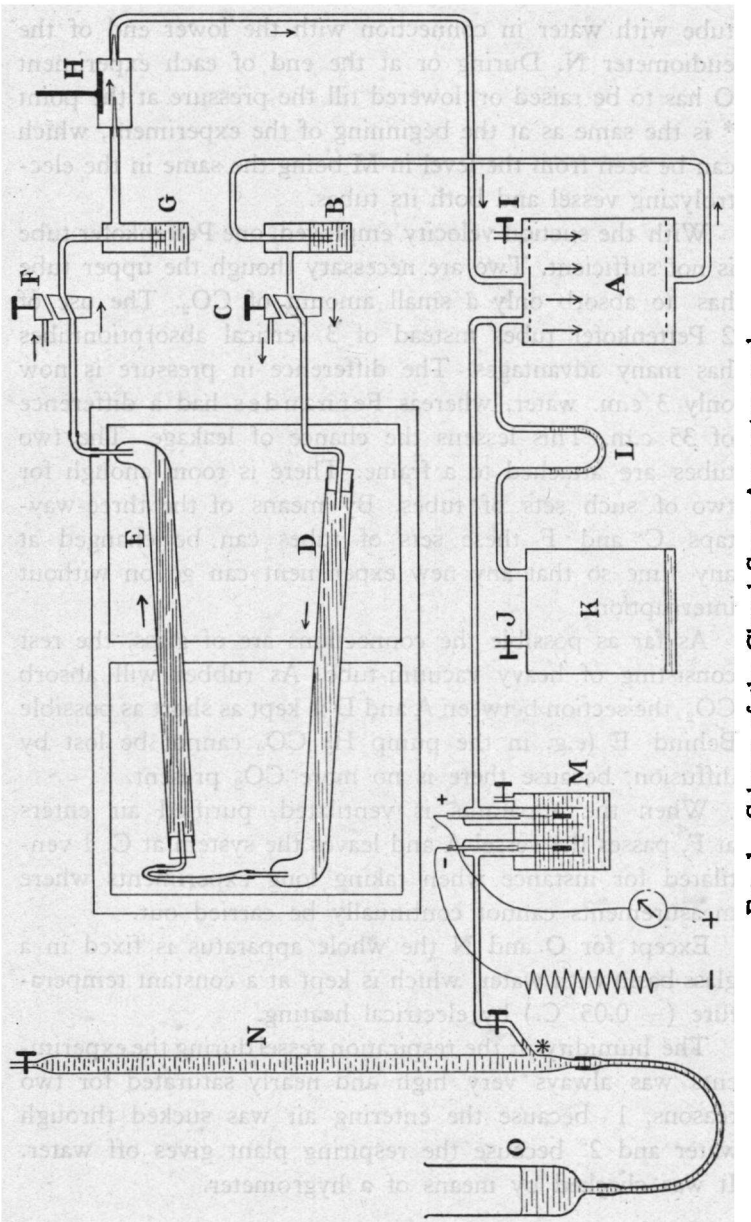


FIG. 1. Scheme of the Closed Space Apparatus used.

tube with water in connection with the lower end of the eudiometer N. During or at the end of each experiment O has to be raised or lowered till the pressure at the point * is the same as at the beginning of the experiment, which can be seen from the level in M being the same in the electrolyzing vessel and both its tubes.

With the suction velocity employed, one Pettenkofer tube is not sufficient. Two are necessary though the upper tube has to absorb only a small amount of CO_2. The use of 2 Pettenkofer tubes instead of 3 vertical absorptiontubes has many advantages. The difference in pressure is now only 3 c.m. water, whereas Fernandes had a difference of 35 c.m. This lessens the chance of leakage. The two tubes are attached to a frame. There is room enough for two of such sets of tubes. By means of the three-way-taps C and F these sets of tubes can be changed at any time so that any new experiment can go on without interruption.

As far as possible the connections are of glass, the rest consisting of heavy vacuum-tube. As rubber will absorb CO_2 the section between A and D is kept as short as possible Behind E (e.g. in the pump H) CO_2 cannot be lost by diffusion, because there is no more CO_2 present.

When the apparatus is ventilated, purified air enters at F, passes the vessel A and leaves the system at C. I ventilated for instance when taking long experiments where measurements cannot continually be carried out.

Except for O and N the whole apparatus is fixed in a glass basin with water, which is kept at a constant temperature ($\pm$ 0.03° C.) by electrical heating.

The humidity in the respiration vessel during the experiments was always very high and nearly saturated for two reasons, 1° because the entering air was sucked through water and 2° because the respiring plant gives off water. It was checked by means of a hygrometer.

During my experiments I also investigated the influence of pure oxygen on respiration. The oxygen entered at tap F, passed absorber G, the respiration vessel and absorber B, and left the system at tap C. In this way the liquid in the system, for instance that in B and G, was saturated with oxygen. It stands to reason that, when starting an experiment, the barytawater in the Pettenkofer tubes also had to be totally saturated with oxygen before connecting them to the taps C and F. Otherwise oxygen would be absorbed, because oxygen is more soluble in water than air. In 160 ccm. of solution at 25° C., 2.8 ccm. air and 4.6 ccm. oxygen are absorbed at a pressure of 1 atm. It was necessary to lead an oxygen current of the same temperature as that of the basin through each new set of tubes for 10 minutes before using it in a new experiment. Otherwise oxygen would dissolve and one might get the impression that there was a higher respiration than was actually the case.

An analogical difficulty was encountered when using different temperatures. In 160 ccm. of solution, air is dissolved to an amount of 3.7 ccm. at 10°; 3.05 ccm. at 20°; 2.65 ccm. at 30° and 2.25 ccm. at 40°. Suppose the temperature in the room is 15°. Then the temperature of the solution in the Pettenkofer tubes before putting them in the waterbasin will be the same. When experiments are carried out at 37°5 about 1 ccm. of air will be given off. A mistake of 1 ccm is a very large one, as may be seen afterwards. So whenever there was a difference between the temperature in the room and the temperature of the water an air current was pumped through each set of tubes for 10 minutes before starting the new experiment, because in this way the equilibrium was much more quickly attained than when the liquid remained undisturbed. This air first passed through a metal spiral in order to assume the temperature in question.

The rectangular respiration vessel in the circulating apparatus had a width of 3.5 c.m., a length of 8.5 c.m. and

a depth of 10.5 c.m. These were the inside dimensions. With the washing-bottle B its contents were 325 to 350 ccm. The lid and the bottom were double. The inner walls were perforated at different places, except near the openings of the tubes. This aids the distribution of the air current. The sides of 10.5 × 8.5 c.m. were of glass. The glass was provided with horizontal lines at a distance of ¼ c.m. so that the growth of the sporangiophores could be observed. Two slides, impenetrable to light, were usually covering the glass walls, preventing the phototropic curving of the sporangiophores.

Many blank experiments were carried out in order to test the apparatus. The decrease in the strength of the barytawater in the Pettenkofer tubes, probably owing to a condensation of water vapour, on an average did not exceed an amount corresponding to 0.1 ccm per hour. I neglected it, the more readily as the decrease in the constant volume of air in the apparatus was on an average 0.15 ccm per hour. These two facts compensated each other. It seems that the metal of the vessel was oxydized to a slight degree. In pure oxygen the decrease in volume was much larger, namely 0.6 ccm per hour, whereas the change in the barytawater had remained the same as in air. With the experiments in pure oxygen the error of 0.6 — 0.15 = 0.45 ccm per hour was taken into account.

In the air current apparatus I sometimes used another vessel measuring 13 × 12 × 5 c.m. inside, the contents being about 800 ccm. By means of different blocks of metal exactly fitting in the vessel I could reduce the volume to any required degree.

It takes some time for the CO_2 given off by the plant to reach the Pettenkofer tubes. This will take longer, as the experimental chamber is bigger and the suction-velocity smaller. Therefore the relation between the suction velocity and the volume of the respiration vessel is of the utmost

importance in studying the influence of some external factor on respiration. I studied this relation by liberating in the vessels of 325 and 800 ccm one milligrammolecule of CO_2 (22.4 ccm). At a certain moment I emptied a tube with 3 ccm of n. hydrochloric acid in a small glass basin, containing one milligrammolecule of sodiumcarbonate (106 mgm). Air was sucked through the vessel at different velocities. The tubes were very often changed. The results are to be found in table 1 and 2.

In my experiments I mostly used the respiration vessel of 325 ccm at a suction-velocity of 3 or 3½ l. per hour.

TABLE 1.

Vessel of 325 ccm. cubic contents.

1*a*. Suction velocity 1.8 l. per hour.	1*b*. Suction velocity 2¼ l. per hour.
After 5 min. 7.4 ccm. CO_2.	After 5 min. 9.15 ccm. CO_2.
„ 10 „ 13.1 „ „	„ 10 „ 14.9 „ „
„ 20 „ 18.7 „ „	„ 20 „ 19.4 „ „
„ 30 „ 21.1 „ „	„ 30 „ 21.1 „ „
„ 60 „ 22.8 „ „	„ 60 „ 22.1 „ „
Half the amount has disappeared after 8 min.	Half the amount has disappeared after nearly 7 min.
1*c*. Suction velocity 3 l. per hour.	1*d*. Suction velocity 3½ l. per hour.
After 5 min. 11.55 ccm. CO_2.	After 5 min. 12.75 ccm. CO_2.
„ 10 „ 17.3 „ „	„ 10 „ 17.95 „ „
„ 20 „ 20.8 „ „	„ 20 „ 20.75 „ „
„ 30 „ 21.7 „ „	„ 30 „ 21.65 „ „
„ 70 „ 22.15 „ „	„ 70 „ 22.1 „ „
Half the amount has disappeared after about 4¾ min.	Half the amount has disappeared after about 4 min.

TABLE 2.

Vessel of 800 ccm. cubic contents.

2*a*. Suction velocity 1.8 l. per hour.	2*b*. Suction velocity 2.7 l. per hour.
After 10 min. 5.45 ccm. CO_2.	After 10 min. 10.4 ccm. CO_2.
„ 20 „ 10.0 „ „	„ 20 „ 16.0 „ „
„ 30 „ 13.25 „ „	„ 30 „ 18.8 „ „
„ 50 „ 17.5 „ „	„ 50 „ 21.35 „ „
„ 70 „ 19.85 „ „	„ 90 „ 22.95 „ „
„ 130 „ 22.5 „ „	
Half the amount has disappeared after 24 min.	Half the amount has disappeared after 11 min.
2*c*. Suction velocity 3 l. per hour.	2*d*. Suction velocity 3½ l. per hour.
After 10 min. 12.3 ccm. CO_2.	After 10 min. 13.4 ccm. CO_2.
„ 20 „ 17.4 „ „	„ 20 „ 18.6 „ „
„ 30 „ 19.7 „ „	„ 30 „ 20.7 „ „
„ 50 „ 21.7 „ „	„ 50 „ 22.2 „ „
„ 95 „ 22.35 „ „	„ 90 „ 22.9 „ „
Half the amount has disappeared after 8¾ min.	Half the amount has disappeared after 7¾ min.

It follows from table 1 that in this case the CO_2 on an average reaches the barytawater after fully 4 minutes.

As regards former investigators K u y p e r (52) used a cylindrical vessel, height 16 c.m., diameter 10 c.m., so measuring about 1250 ccm. The air was sucked through at the rate of 3 l. per hour. The relation was about the same as in table 2a. So in Kuyper's experiments CO_2 newly formed needed on an average 25 minutes to reach the Pettenkofer tubes.

Usually I employed a solution of about 0.05 n. of barium-hydroxide, equal to about 7.9 grm. per l., the molecular

weight of $Ba(OH)_2 + 8$ aq. being 315.6. As the commercial bariumhydroxide is impure I had to dissolve about 9 grm. per l. I dissolved the hydroxide in hot water in flasks of about 8 l. and allowed the insoluble carbonates to settle. The clear solution was then siphoned. A little more than 1 grm. $BaCl_2$ per l. was added in order to repel the solubility-product of the $BaCO_3$.

The titer of the barytawater before and after an experiment was determined by means of 0.05 n. HCl. The latter was made to correspond to a 0.05 n. NaOH solution, in its turn corresponding to a 0.05 n. solution of oxalic acid.

CHAPTER I.

THE RESPIRATION OF PHYCOMYCES ON DIFFERENT MEDIA.

§ 1. Introduction.

At first my purpose was to carry out respiration experiments with cultures of Phycomyces on a culture medium of a definite well-known composition. It is possible to cultivate Phycomyces on sugar media. Lindner (56) for instance found that the + strain grew very well on maltose, raffinose and dextrinesolutions, the — strains moreover also on glucose, fructose and saccharose. Grete Orban (65) on the contrary thought that the + strains were less particular and consumed more different kinds of sugar than the — ones. More contradictions are to be found in literature as to which kind of sugar is the best medium. The reason may be the existence of a considerable number of races with different physiological properties, as Satina and Blakeslee (e.g. 84) have shown, so that the investigators probably have worked with different races. The race I investigated for instance grew better on saccharose than on maltose, but probably this fact does not hold good for all + races.

The more sugar has been dissolved in the liquid, the better the fungus will grow. Of course there are limits, because at higher concentrations the osmotic pressure prevents the growth. The same is to be seen on agar media. Neither can the growth be continually increased by taking thicker layers, as the aerophilous mycelium remains in the uppermost part of the agar. Schmidt (86) has investigated the same subject in detail. The growth was therefore never strong enough to work with when the fungus was cultivated on sugars.

On starchagar the growth of the fungus can be increased by enlarging the amount of starch, as the harmful influence of higher osmotic pressure does not exist in this case. On starchagar media however, oxygen cannot enter sufficiently. With fatty media we see the same thing. F l i e g (32) found that Phycomyces did not grow very well on fat; probably there was also a lack of oxygen in this case. With the above media sufficient respiration can of course be obtained by taking a great quantity of them in thin layers. But in doing this a large respiration vessel is necessary, the drawback of which has been shown on p. 126. Secondly the larger the amount of culture medium the more CO_2 will remain dissolved in it.

In my experiments I therefore used such solid culture media as always contain a sufficient amount of food and allow the air to enter freely. For a starch-medium I used bread, for an oil-medium ground linseed, both soaked in water. Some of the culture media were analysed in the pharmaceutical laboratory of Prof. S c h o o r l, to whom I am very much indebted for his kind assistance.

In the carbohydrate medium there are proteins and some fats, in the oil-medium we also find carbohydrates and proteins. With the apparatus described in the introduction I was able to measure the respiratory quotients very accurately. My purpose next was to determine by means of these quotients what kind of food Phycomyces would take from such a heterogeneous culture medium. When carbohydrates are consumed the respiratory quotient will ordinarily be about 1.00, when fats or proteins are combusted it will be smaller as more oxygen has to be absorbed in comparison with the carbohydrates.

During the growth of the fungus the p_H of the culture medium is changing. As the principal subject of my investigations was respiration I only examined the change in the acidity of the medium qualitatively by means of p_H measure-

ments. If examining the whole metabolism of Phycomyces, I should also have had to determine the quality and quantity of the acids formed, as p_H is not a measure for the quantity of acids in a culture medium. For instance it depends on its buffer-capacity.

I determined p_H by means of the quinhydroneelectrode. I am very thankful to Dr. K o l t h o f f for helping me with this part of my investigations. Media with a small buffer capacity cannot be measured, except when very pure quinhydrone is used (46). My culture media, however, had a large buffer capacity so that these precautions could be omitted.

The fungus was cultivated in two ways. Firstly in small earthenware troughs, containing about 13 ccm, area 6 sqcm, depth about 2 c.m. The dry weight of the bread in it was about 6 grm. and of the ground linseed, etc. about 4½ grm. Secondly the food was given to the fungus in a thin layer on rough linen, which was stretched over a small glass frame of 7 × 2½ c.m., in such fashion that the medium was in contact with the air both above and below. I always used two of such frames fitted above each other in the respiration vessel. For the sake of brevity they will be spoken of in the tables as "two layers".

All experiments in this chapter were carried out at 25° C.

§ 2. Respiration on Carbohydrate Medium.

The analysis of the bread[1]) used per dry weight was as follows: carbohydrates 77 %, proteins about 10 % and fat nearly 1 %. Of the carbohydrates about 10 % are soluble, such as maltose.

On bread first a generation of thin sporangiophores appears, followed by a generation of thick ones. In course of time

[1]) In Holland the bakers sometimes mix the flour with water. I always used this "waterbread". If milk is used the percentage of fat is higher (see p. 139.)

new generations of sporangiophores follow. Fig. 2 (table 3) shows the whole grand period of the respiration of Phycomyces on a small trough with bread. Both the amount of CO_2 given off and of O_2 taken in increase very rapidly,

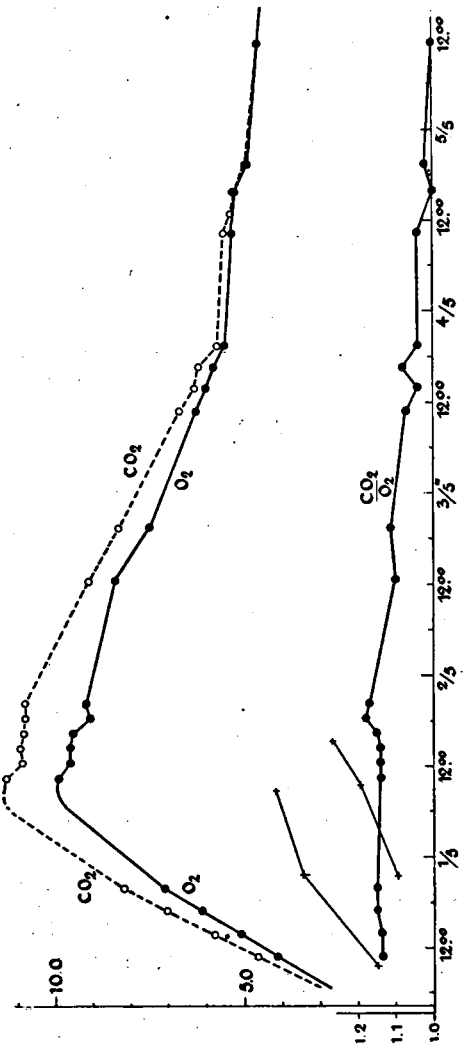


FIG. 2. Grand Period of Respiration on a Small Earthenware Trough with Bread.

As in the other figures in this chapter the length of the sporangiophores is indicated in c.m. by means of the lines plotted through the small crosses.

The abscissa-axis has been taken as the time axis, graduated into periods of 6 hours. The ordinates represent the rate of respiration in ccm. per hour. The solid lines are the "O_2-lines", the dashes the "CO_2-lines".

In the same figures the respiratory quotients during the grand period are also indicated by a solid line. Here the ordinates represent the magnitude of the respiratory quotients.

For further explanation see text.

the more the mycelium penetrates through the culture medium. About 3½ days after transferring, i.e. 2 days after the surface has been covered by mycelium, the respiration reaches a maximum. The sporangiophores of the first generation are now very thin and have nearly stopped growing, the thick ones are about 2½ c.m. long. From this moment respiration decreases gradually: to half the amount after 3 or 4 days.

As for the respiratory quotient, at the moment of the most intensive respiration it is higher than 1.00. As respiration decreases the quotient drops from about 1.15 to about 1.00. I wondered whether in the long run the quotient would continue decreasing or remain at 1.00. I therefore carried out an experiment with an old culture, which had remained at room-temperature for about 2 or 3 weeks. From table 4 it appears that old cultures also show a quotient of about 1.00 on these starch media.

Why is the quotient higher than 1.00 when respiration is at its full strength? The thought occurred to me, that the cause might be that the air could not enter sufficiently into the culture troughs, which are relatively deep. Thus CO_2 might have been formed anaerobically. If so, the quotient would be smaller if the air could enter better. I now used for the first time the frames with the thin layers of culture medium, mentioned above. The result of the experiment with $2 \times 1\frac{1}{4}$ grm. bread is shown in fig. 3 (table 5). Contrary to expectation the quotient is raised and fully 1.20. I therefore carried out many experiments on this point and the quotient always turned out to lie between 1.20 and 1.25.

Also from fig. 3 it follows that in the end the quotient approaches the value 1.00. As here the amount of culture medium is smaller, the grand period of the respiration is less extended than in fig. 2, whereas the sporangiophores are yet smaller during the maximum respiration.

Phycomyces does not behave like the Mucoraceae of the Mucor group, which are able to ferment sugars, as Brefeld (15) already showed. Kostytschew (48) grew Mucoraceae on bread without finding any fermentation; on sugar being added to the bread fermentation appeared. In case the high respiratory quotient of Phycomyces were due to a fermentative process, the quotient should rise if the bread were soaked in a sugar solution instead of in water. This is not so, however, no such rise being found for instance with bread soaked in a 5 % solution

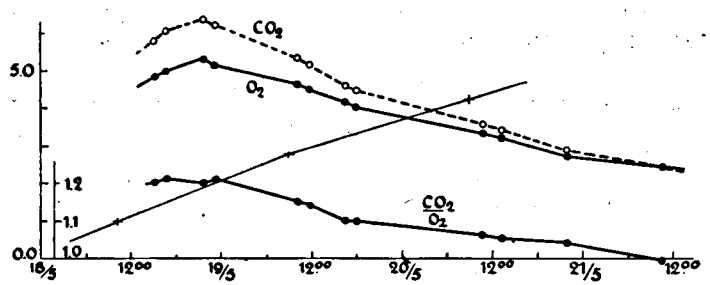


FIG. 3. Grand Period of Respiration on 2½ grm. of Bread in Two Layers. (See table 5).

of saccharose. Respiration as such however is more intense in this case.

As the high respiratory quotient on bread is a normal characteristic of the fungus, substances are apparently manufactured containing less oxygen than carbohydrates. The presence of alcohol etc. cannot be demonstrated, so I suppose that the value of the respiratory quotient is probably at first higher than 1.00 because carbohydrates are changed into fats. In the mycelium fats can be demonstrated. Also the facts mentioned in chapter III render the supposition probable.

The more respiration decreases the lower sinks the respiratory quotient. In the troughs the respiration in the

upper part of the culture medium will be in the descending part of the grand period, whereas below the respiration is at its maximum. So the average respiratory quotient never reaches the value measured on the thin layers of culture media. In examining different external influences afterwards, I therefore always used the last culture method, as processes are simpler here.

As for the acidity of the medium, Schmidt (86) found that Phycomyces turned the medium acid. This holds good for the above carbohydrate medium. The p_H of bread is about 5.85 and is made smaller by the fungus, for instance after 2 weeks it is 5.3, after 3 weeks 4.5.

§ 3. Respiration on Media with a varying Amount of Fat.

As has already been stated I used ground linseed as an oily medium. Here the fat and also the other food proteins and carbohydrates are distributed through the medium, so that the air can enter better than on pure fat. The composition of linseed per dry weight is as follows: fat 35 % and proteins 25 %. According to the text-books (e.g. 99) carbohydrates occur as much as 9 % sometimes, the greater part however consists of pectin-mucilage, the smaller part of sugars. Phycomyces thrives exceedingly well on fatty media. Fat is well-known to have a high combustion energy (Hoeber 40). Flieg (32) cultivated Aspergillus niger on fatty media. On sugar media however respiration was more intensive. Phycomyces on the contrary is a true fat consumer as will be shown presently. Respiration is intensive on fatty media. The fungus prefers fat out of a mixed culture medium. The sporangiophores also grow better. As a rule a thin generation is not to be observed on ground linseed. One generation of very thick sporangiophores appears, growing faster than the ones on bread. The sporangiophores are very numerous and arise as it were all

at the same time, leaving no space for following generations, for in contradistinction to the starch-media new generations hardly follow.

In fig. 4 (table 6) the grand period of respiration is shown

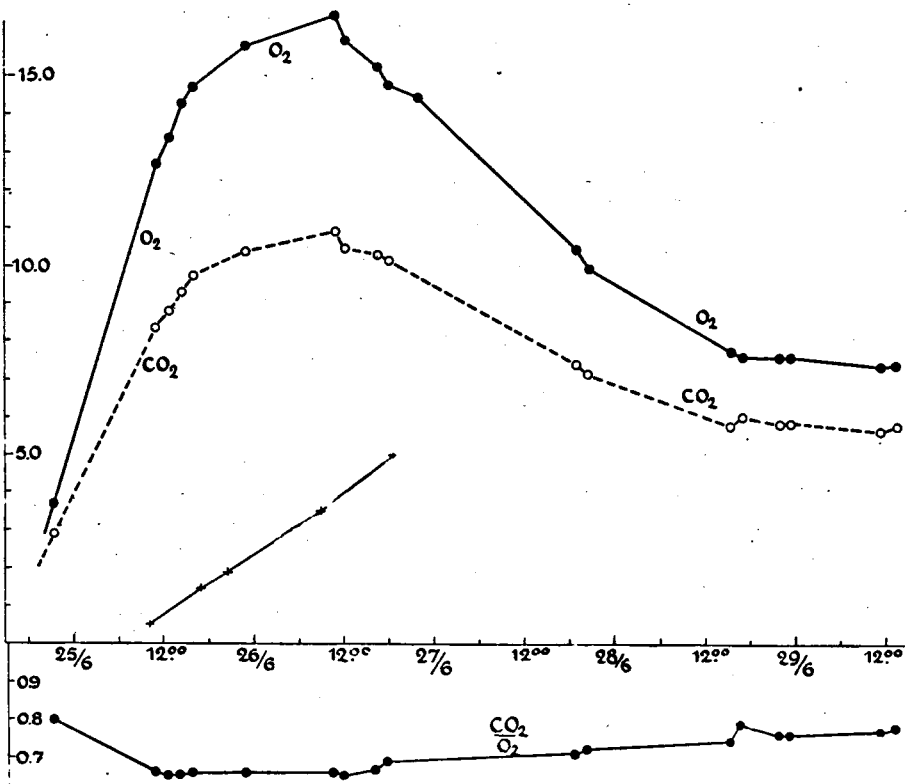


FIG. 4. Grand Period of Respiration on a Small Earthenware Trough with Ground Linseed (oil 35 per cent.). See table 6.

on a small trough with ground linseed. Respiration increases very rapidly and about 3½ days after transferring, i.e. nearly 2 days after the surface was covered with mycelium, it reaches its maximum. The sporangiophores then have a length of from 4 to 5 c.m. Then respiration decreases

gradually as on the starch-media. In consequence of fat-combustion the respiratory quotient is low. It drops to about 0.66 and remains there during the maximum respiration. When respiration decreases the respiratory quotient rises in a few days to about 0.75.

From the composition of linseed-oil (93) it appears that in consuming only the oil, the quotient should be about 0.72. As will be proved hereafter carbohydrates are also consumed in some measure. Therefore the quotient should be found still higher, whereas it is lower. Phycomyces on oil-media apparently takes up more oxygen than is necessary for the combustion of fats. Oxygen is apparently fixed by changing fats into carbohydrates.

This fact has been mentioned in earlier literature for seedlings. Sachs (82) found microchemically that, in germinating fatty seeds, fat was changed into carbohydrates. Godlewski (35) determined the respiration of fatty seeds and found the respiratory quotient falling from 1.00 to about 0.60. So carbohydrates were manufactured. Later on the quotient again approached 1.00, so the carbohydrates formed were consumed. Fungi, growing on fatty substances, show this phenomenon very often in a very strong degree as Flieg (32) records.

I also examined the respiration on linseed-meal, containing 14½ % oil. Fig. 5 (table 7) shows the respiration on a small earthenware trough. The course of the grand period resembles that on ground linseed. Respiration increases less rapidly at first and remains a little lower. The maximum of respiration again takes place when the sporangiophores are about 4 c.m. long. As there are less fatty substances, the influence of the carbohydrates is more obvious, as the respiratory quotient is much higher. It falls during the most intensive respiration only to 0.76 and rises during the following days to about 0.85.

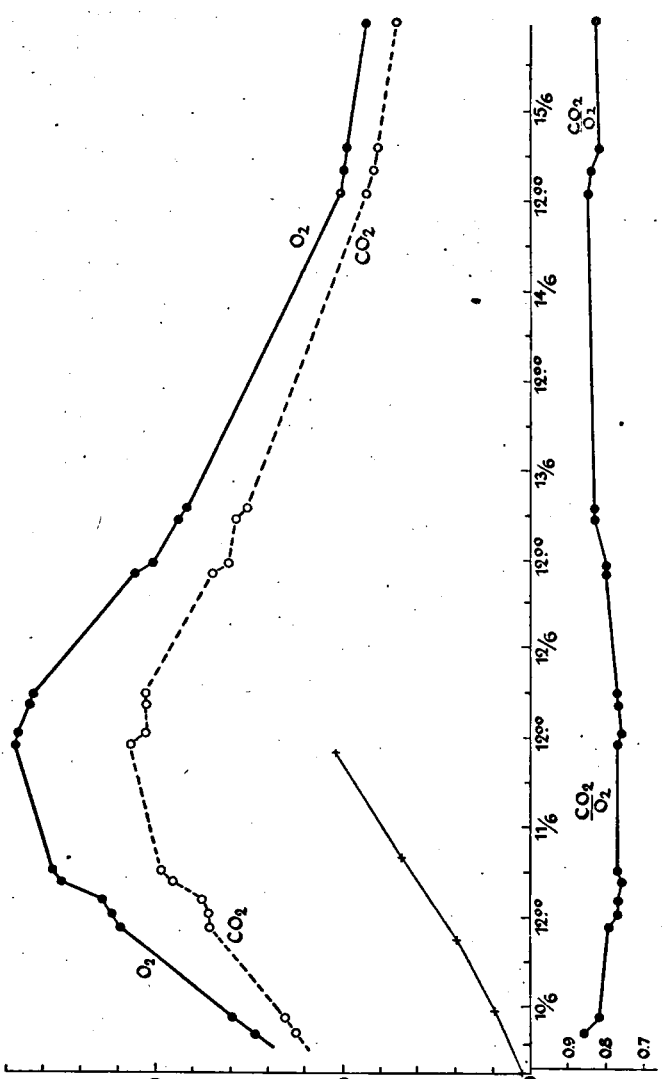


FIG. 5. Grand Period of Respiration on a Small Earthenware Trough with Linseed-meal, containing 14½ per cent. of Oil. (See table 7).

In order to get a medium as the above with still less oil, I kept linseed-meal for a considerable time in a bottle with petrol ether. Now and then I renewed the ether. After it had been removed quantitatively, the medium contained

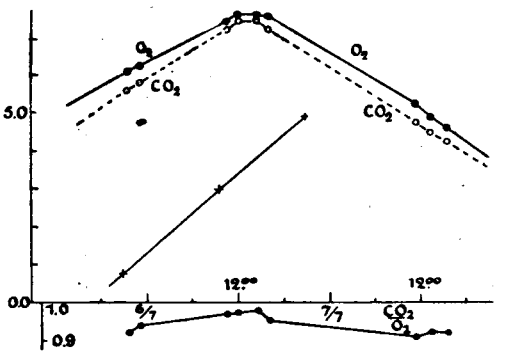


FIG. 6. Grand Period of Respiration on a Small Earthenware Trough with Linseed-meal, containing about 2½ per cent. of Oil. (See table 8)

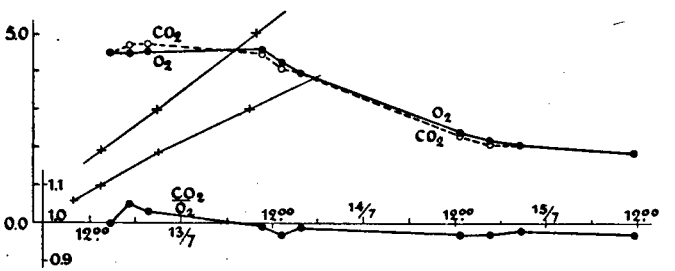


FIG. 7. Grand Period of Respiration on a Small Earthenware Trough with Linseed-meal, totally freed from Oil (See table 9).

about 2½ % fat. I grew Phycomyces on this medium. The respiration now was completely changed as may be shown in fig. 6 (table 8). Fat with the best nutritive value has

nearly disappeared so that the respiration is much lower, though the remaining 2½ %, still have great influence, as the respiratory quotient is about 0.95 and the respiration much higher than in the next case without fat.

On this medium appear always more generations with fewer and thinner sporangiophores, and not one numerous generation with thick sporangiophores as in the former two cases.

I totally deprived a little of the above named medium of fat by extracting it for 4 days in a Soxhlet with petrol ether. The respiratory quotient is now about 1.00. Fig. 7 (table 9). A kind of carbohydrate-respiration takes place. It seems that the abundance of proteins has no influence. The respiration is smaller than on the medium with 2½ % oil, which proves that Phycomyces uses oil by preference when it is there.

This also appears from the following experiment. Once by mistake instead of waterbread, the so-called "milkbread" was used, which contained about 2½ % fat. This fact lowers the respiratory quotient very much as may be seen from table 10.

Afterwards, whenever I studied the influence of external factors on the respiration of Phycomyces on media rich in oil, I did so on the method of thin layers on a set of frames. In fig. 8 (table 11) the grand period of respiration is given for such a culture on 2 × 1¼ grm. of ground linseed. The respiratory quotient is again about 0.65. In consequence of the smaller quantity of culture medium and the larger surface of it as compared with fig. 4, the mycelium relatively spreads more quickly and reaches the maximum respiration sooner, namely about 2½ days after transferring. At this moment the sporangiophores are not yet visible. When they appear the respiration is already decreasing. The curve descends more rapidly than the one in fig. 4. The whole curve is sharper and shorter.

§ 4. Consumption of Proteins.

Butkewitsch (17) showed that fungi only consume proteins when no carbohydrates or fats are present. In this case Aspergillus niger liberates a lot of ammonia. Penicillium and fungi like Mucor especially amino-acids. Kostytschew (48) carried out respiration experiments

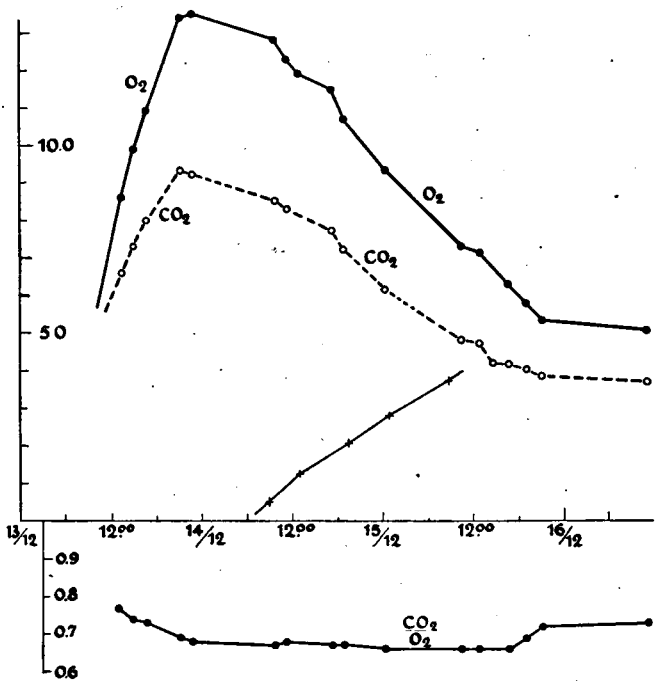


FIG. 8. Grand Period of Respiration on 2½ grm. of Ground Linseed in Two Layers (See table 11).

with fungi on proteins as a culture medium and found the respiratory quotient to be about 0.50. Klotz (44) did not find an ammonia production with fungi until the carbohydrates had entirely been consumed.

As has already been remarked it follows from fig. 7 that Phycomyces does not consume proteins either if carbohy-

drates are to be had, as the respiratory quotient would otherwise be much smaller. I investigated this question in another way by baking bread containing respectively 10 % and 20 % peptone. Moreover, it was just possible that in

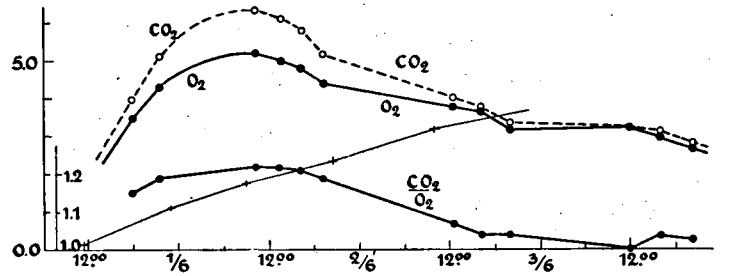


FIG. 9. Grand Period of Respiration on 2½ grm. of Bread plus 10 per cent. of Peptone in Two Layers. (See table 12).

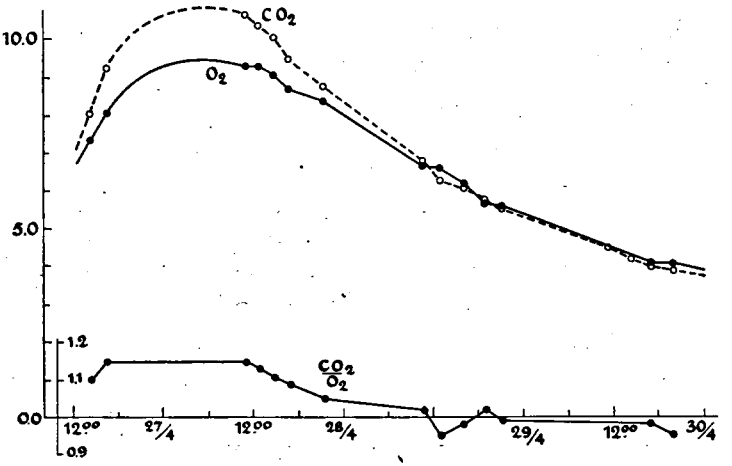


FIG. 10. Grand Period of Respiration on a Small Earthenware Trough with Bread plus 20 per cent. of Peptone.

the presence of simple proteins the whole respiration would be raised. Fig. 9 (table 12) shows the respiration on two layers of bread plus 10 % peptone. The curve looks like fig. 3,

where bread without peptone was used. Neither the respirationvelocity, nor the respiratory quotient has changed. The same is to be seen in fig. 10, giving the respiration on a small trough with bread plus 20 % peptone. The curve corresponds on the whole to fig. 2. At last the respiratory quotient seems to be lower than on bread.

So proteins take no essential part in the respiration of Phycomyces. They are probably taken in a small measure, as follows also from the change in the acidity of the medium. Whereas on bread the p_H decreases, as stated above, on bread plus peptone the p_H rather increases, especially on bread with 20 % peptone. The p_H of linseed meal is about 5.5, after one week of growth the p_H has risen to about 6.0, after 2½ weeks to 6.8 and later on to 8.0, so that the p_H cannot be measured well by means of the quinhydrone-electrode.

When a culture of Phycomyces on linseed medium is kept sterile for several weeks, ammonia is at last produced, as indicated by its strong odor.

§ 5. Discussion.

In the above experiments, by measuring very accurately the respiratory quotients, I determined the substances consumed by Phycomyces in its respiration when cultivated on heterogeneous culture media. Fat has a high nutritive value, carbohydrates also are good culture media. Secondly the grand period of the respiration was determined on food substances of different quantities and qualities. The curves ascend rapidly to a maximum of respiration, then descend more or less slowly.

What may be the cause of this dropping of the curve? Is it the decrease in food or the formation of harmful substances, products of metabolism? The same phenomenon was already observed half a century ago with the respiration of seedlings. Rischawi (78) found that with Vicia Faba

seedlings, which have big cotyledons and therefore a lot of food, respiration remained constant for a long time. So want of food might be the cause of the decrease in the respiration of other objects of investigation. Lately it was Fernandes (31) who said that, when respiration decreases with seedlings of Pisum sativum, there is no question about want of food. The fall is probably the result of the abnormal conditions of the seedlings in the respiration vessel. Krzemieniewski (51) managed to raise the respiration by adding minerals to the plants.

In our case abnormal conditions cannot be responsible. One gets the impression that the decrease in the respiration results from the food being more difficult to obtain at the moment. When the mycelium starts to grow over the surface of the culture medium, respiration increases very strongly till the maximum has been attained. By that time the mycelium has penetrated the whole culture medium, if the method of the two thin layers is used (fig. 8). A small part of the food has indeed been consumed, but the hyphae cannot spread in an untouched medium, so that the hyphae approach each other and have to grow in every hole and corner. When a small trough with medium is used this process is more gradual, because the mycelium can grow downwards for a long time. The curve in fig. 4 is therefore less sharp than in fig. 8 and the maximum of respiration is later.

It seems therefore probable that the shape of the curves is governed by the available food supply, though special investigations would be necessary to prove that no harmful metabolic substances play a part.

In examining in the following chapters the influence of external factors such as temperature, air of different composition, etc. on respiration, it should be borne in mind that I always used cultures at the maximum of the grand

period. The experiments as a rule take about 12 hours, during which time the respiration is nearly constant. Sometimes I employed the small earthenware troughs, where the maximum appears about 3½ days after inoculation at 25°. For the greater part I proceeded on the method of the thin layers of culture medium on a set of two frames, and mostly with 2 × 1¼ grm. medium. In this case the maximum of respiration begins about 2½ days after the transfer on the linseed medium. The medium is penetrated by the mycelium, the thick sporangiophores already mentioned being however not yet visible. Fully 12 hours later, when the experiment is generally finished, they are about 1 c.m. long. On bread, where the respiration is less intensive and does not rise so rapidly as on oil-media, the cultures have to be half a day older before an experiment can be begun. The sporangiophores are then about 1 c.m. in length.

As stated above the length of the sporangiophores is an index of their stage of development corresponding to a specific point in the grand period. After some training this can be seen at a glance.

When fungi are grown on liquid solutions the dry weight of the fungous mat can be determined and so the respiration can be compared per dry weight, although this method is not at all an exact one, as dead cells etc. do not respire and are included in the dry weight. As Phycomyces was cultivated on solid media, dry weights could not be determined. Notwithstanding this the different experiments can very well be compared as, by cultivating on the described lines, all cultures respired almost with equal strength.

In my cultures two factors affect the rate of respiration, first the method of transferring and secondly the amount of water in the culture medium.

The influence of the former is especially visible on the starch-media. Ordinarily the thick generation of sporangio-

phores is preceded by one with very thin sporangiophores as has been described above. When the medium is inoculated with very few spores, for instance by means of a strongly diluted spore suspension, on bread only thick sporangiophores arise. On the other hand when the whole surface has been inoculated with a great number of spores, a great number of sporangiophores appears, which are all very thin. No thick ones appear. In this case the respiration is also lower. All my cultures were therefore inoculated in the same way: each frame was just touched in two spots with the platinum needle, covered with spores.

The second influence is still of more importance. As stated above the bread and the oil-media are mixed with water, as no growth takes place on dry media. But when too much water is used, the respiration is also too low, as may be seen in table 13, where the respiration does not reach 75 % of the amount of table 5 (fig. 3). The difference sometimes is still larger. Therefore always the same amount of water has to be mixed through the medium, namely about 2 grm. of water per 1¼ grm. of ground linseed and fully 1½ grm. per 1¼ grm. of bread.

After some time it is easy to tell how much the respiration of a culture will be, from the humidity of the culture medium, as also the amount of water necessary for an intensive respiration. On an average a culture on 2 × 1¼ grm. of bread takes in during the maximum of respiration 5.75 ccm. O_2 and gives off 7.0 ccm CO_2, a culture on ground linseed absorbs 15 ccm O_2 and evolves 10 ccm CO_2. (50 seedlings of Pisum sativum, dry weight about 8 grm., evolve about 12.5 ccm CO_2 at 25°).

In former investigations on respiration with seedlings etc. as a rule no allowance was made for the fact that bacteria might accompany the objects of investigation; Fernandes (31) proved that this may very often be the case. As I

worked with a pure culture of a fungus this did not occur in my experiments. I always took as many precautions as possible. Before starting an experiment the respiration vessel was sterilized by means of a 1 $^0/_{00}$ corrosive sublimatesolution. Whenever the apparatus was ventilated the air was sucked through a Pettenkofer tube with strong sulphuric acid keeping back to some extent spores of the atmosphere. After finishing an experiment I always examined the cultures for the possibility of an infection by fungi or bacteria. I inoculated on malt media and broth, the latter being an excellent medium for all kinds of bacteria. Only once in all my experiments did an infection take place, but never again when I transferred from below the surface. When I transferred from the surface only in a few cases bacteria appeared in the broth after some days, Phycomycesmycelium having already been formed. Apparently bacteria had fallen on the culture but had not developed.

Considerable mistakes may be made by neglecting the amount of CO_2 dissolved in the culture medium (especially in certain respiration apparatus, see p. 119). It seems to me that this was the case with Puriewitsch (74) who carried out determinations of the respiratory quotients with Aspergillus, by analysing air-samples.

I tried to make sure whether a great amount of CO_2 was absorbed by the solid medium I used.

To begin with, CO_2 may be bound chemically to substances in the medium. By adding acid the CO_2 will be liberated as the compounds are decomposed. This can be done in a Barcroft-apparatus (5). The increase in volume indicates the CO_2 evolution. I found that CO_2 was only chemically bound in old cultures on linseed medium and on bread plus 20 % peptone, where the medium finally becomes alkaline as stated on p. 142. I never used these old cultures in my experiments however.

Secondly an amount of CO_2 will be dissolved in the water of the culture medium. I determined it as follows. The culture was placed in a glass flask with a wide neck. First I measured respiration for some hours. Then CO_2-free water was poured into the flask. The water was boiled, which took about 5 minutes. The CO_2 dissolved in the medium was driven out and absorbed in the Pettenkofer tube. In a trough with bread at the utmost 4½ ccm CO_2 appeared to be dissolved, with linseed somewhat more. With the 2½ grm. media on the set of frames the amount of CO_2 dissolved was probably about half the amount.

At all events the amount of CO_2 absorbed by the culture media cannot have had any influence on the figures found.

CHAPTER II.

THE INFLUENCE OF LIGHT ON RESPIRATION.

I started my investigations by studying the influence of light on respiration. Opinions are still divided on this point, although the question was already studied half a century ago. I chose Phycomyces because it possesses strongly developed sporangiophores, and in consequence of their rapid growth an energetic metabolism should take place in them. Also a part of the aerophilous mycelium remains above the culture medium, so that in contrast with other fungi a large part of the whole will receive light, namely sporangiophores and mycelium above the surface of the culture medium.

I was only acquainted with the publications of Bonnier and Mangin (11) on the influence of light on the respiration of Phycomyces. They found a decrease in respiration in light. If I had known about Shorawski's publication (87) I should certainly have chosen a different fungus.

As regards the other publications I will not discuss those by Drude (26), Pauchon (67) and Pringsheim (72) because they are not accurate enough for the present time. Wolkoff and Mayer (96) studied the influence of light on the consumption of oxygen by seedlings. Whereas a small increase in the respiration was discovered, a decrease was never determined. Wilson (95) found no influence of light on the respiration of seedlings or mushrooms.

The experiments of Bonnier and Mangin (11, 12) were detailed and apparently very accurate. With all their objects of observation (mushrooms, fungi, rhizomes, roots, flowers, etc.) they found a strong decrease in respiration

in light. It amounted to 10 %, 20 % or more, and became stronger as the intensity of the light increased. Puriewitz (73) confirmed these results for mushrooms. With roots and rhizomes the decrease was not always noticeable. With flowers and etiolated plants there was rather an increase in respiration.

Elfving (29) found no influence on the respiration of older cultures of fungi such as Penicillium. The dry weight of cultures grown in light was however far less than the dry weight of cultures grown in the dark. Therefore the light seemed to have influenced the respiration of the fungi when they were still growing. Accordingly Elfving analysed the gas from the closed flasks in which the spores of the fungus had germinated and grown. It now appeared that not only the dry weight remained smaller but also that the respiration was lower in light. Elfving therefore agreed with Bonnier and Mangin, whose objects, according to him, all indicated growth.

It is questionable in Elfving's experiments whether, if the respiration had been calculated per dry weight, it would then also have been smaller in the light.

Aereboe (1, see also Detmer, 24) could not discover the slightest influence of light on respiration. As for mushrooms he did not state his opinion on the influence of light because his material was unfavourable. He often used the petals of flowers. There is room for doubt whether in his experiments the petals were not too closely packed together so that only part of them received light.

I had a translation made of a paper by Shorawski (87), the original of which appeared in Russian. For young heads of Agaricus campestris he found a decrease in respiration of about 20 % in light, with Mucor, cultivated on bread, there was an increase of about 20 %. With Phycomyces the respiration was independent of the light. Bonnier and Mangin may have found a decrease

through making no allowance for the grand period of the respiration.

Finally Kolkwitz (45) found an increase in the respiration in light of about 10 % for Penicillium, Mucor and others. For Aspergillus and Mucor, Maximow (60) failed to discover any influence on young cultures where there was sufficient food, while there was an increase in respiration of the older cultures in light, especially at first.

According to Löwschin (57) the increase of respiration in light, found by Kolkwitz and Maximow, is due to a rise in temperature.

Summarizing it therefore looks as if light has a retarding influence on the respiration of mushrooms. For the lower fungi there may be an accelerating influence on the respiration under special circumstances. It is possible that the investigators did not allow for the fact that there may be certain substances in the culture media which develop CO_2 under the influence of light, as for instance oxalic acid. This substance is indeed often formed by fungi such as Aspergillus, with which the experiments are often carried out.

Like Shorawski, I have not been able to find any influence of light on the respiration of Phycomyces. I tried the effect of daylight at different times of the day and the year and also of strong and weak electric light. As the lamps were placed at a distance of only 20 c.m. from the basin, a large glass cuvette, 10 c.m. thick, was filled with water and placed between the lamp and the basin to absorb the heat. The light intensities used, were measured photometrically and were about 800 and 6000 M. C. The different intensities of the daylight were not measured as the influence was negative. All the experiments in this chapter were carried out at 20° C.

The tables 14, 15 and 16 illustrate some of the many experiments with Phycomyces. It follows from the tables

that the results are negative, but we also see that Phycomyces is a poor object for studying the influence of light on respiration, because contrary to expectation the respiration of the sporangiophores and the mycelium that receive light forms only a small part of the total respiration.

In table 15 and 16 the sporangiophores were namely removed at 20.20 and 19.00 respectively. We see that afterwards respiration has diminished very little.

The supposition might be made that for some time after the removal of the sporangiophores, respiration is found too high in consequence of the woundstimulus. But it seems to me that this is not the case, because if so, respiration would be sure to decrease in the long run. Separate experiments showed however that it does not.

Table 15 shows that the removal of the culture for a few minutes (from 18.00 to 18.03) out of the respiration vessel as such has no influence.

These experiments are not of great consequence for answering the question as to the influence of illumination on respiration. In the experiments described in this chapter I therefore mainly used the higher fungi namely mushrooms, where a decrease in respiration has often been stated. Besides the mushrooms found in nature, I used pure cultures of mycelium. It has to be remembered however that in nature the fructifications are exposed to the light, whereas this is not the case with the ordinary mycelium.

I am giving some tables of the respiration of *Polyporus destructor* on flat pieces of carrot on which it grows well. These pieces are easily illuminated on both sides.

Of the experiments with mushrooms I only give those carried out with little ones. The respiration of the larger ones, such as Boletus species and others is more intensive, but with the little ones the surface illuminated is larger in proportion to the weight.

It follows from the tables 17, 18, 19, 20 and 21 that light does not influence the respiration of mushrooms, either of the naturally-occurring fructifications, or of the pure cultures, in contrast with the findings of Bonnier and Mangin, Puriewitsch and Shorawski. Our results are in agreement with those of Wilson.

Moreover some time ago a paper of Rischards (77) appeared, who also failed to discover any influence.

It therefore seems to me that there is no direct influence of light on respiration, except of course under accessory circumstances. Spoehr (89) for instance found a higher respiratory activity caused by ionizated air due to the ultra-violet rays of the sunlight. So in my experiments this factor was eliminated, the light always being deprived of ultra-violet rays.

The influence of light on the respiration of green plants is quite a different question. Borodin (14) already showed that the respiration is the more intensive the more carbohydrates are present. As carbohydrates are formed in light, the light will indirectly increase the respiration of green plants.

CHAPTER III.

THE INFLUENCE OF GASMIXTURES CONTAINING DIFFERENT PERCENTAGES OF OXYGEN ON THE RESPIRATION OF PHYCOMYCES.

It may be concluded from the behaviour of Phycomyces as described in chapter I that the fungus will grow better the more it is exposed to the air. It was therefore important to determine how Phycomyces will behave in pure oxygen, in gas with different percentages of oxygen and in the total absence of it.

§ 1. Literature.

Many researches about respiration under the above conditions were carried out with other objects, also with some fungi. The respiration in the total absence of oxygen, especially in connection with the intramolecular respiration, has repeatedly been made a matter of investigation. In the absence of oxygen the evolution of CO_2 does not as a rule cease directly, but goes on for some time owing to the splitting of carbohydrates in the culture medium. This was already noticed by Lechartier and Bellamy (54 cf. also 58) with fruits, by Dehérain and Moissan (23) with leaves and by Borodin (13) with seedlings.

The quotient, giving the proportion between the amount of CO_2 given off anaerobically and the amount given off in air, varies in different plants. It is 1.0 for seedlings of Vicia Faba (97) but mostly smaller, e.g. ¼ for Lupinus (70, 95). Moeller (64) found this quotient to be independent of the reserve food in the seeds. It may be the same for fatty seeds as for those containing starch. Chudiakow (18) also found that fatty seeds give off much CO_2 anaerobically. On the other hand Diakonow (25) showed

that the amount of CO_2 given off anaerobically is larger with seeds containing starch than with fatty ones. Godlewski and Polszenius (36) explained these contradictions: Chudiakow first soaked the seeds in water, allowing the air to enter freely, by which fats changed into carbohydrates.

As far as fungi are concerned, Mucor species give off a good deal of CO_2 in the absence of oxygen (Brefeld, 15), Penicillium and Aspergillus one fourth of the amount given off in air (Diakonow, 25). This holds for cultures on sugar. On chinic acid and tartaric acid they die. Saccharomyces is also unable to give off intramolecular CO_2 without sugar (Chudiakow 19).

Tissues, poor in carbohydrates, give off a lot of CO_2 anaerobically on sugar solutions (etiolated leaves: Palladin 66; seeds rich in proteins: Godlewski 37, 38). The smaller the number of carbohydrates, the sooner therefore, the fungi and the tissues of higher plants will die in the absence of oxygen.

If CO_2 is given off anaerobically on media without carbohydrates, it generally is supposed that these have been manufactured by the fungus itself. Kostytschew (49) demonstrated sugars in cultures of Aspergillus niger on chinic acid and tartaric acid (cultures on peptone appear to behave otherwise). Flieg (32) cultivated Aspergillus niger on pure oil-media and found that young cultures died very soon in the absence of oxygen, older ones could stand it better, carbohydrates having alreadyb een manufactured from the fat.

As for high oxygentensions, the older investigators like Bert (8) and Boehm (10) expected from them an increase in growth and respiration but neither of the two happened. On the contrary in the long run they found a decrease. Dehérain and Moissan (23) discovered no influence of

oxygen on respiration. Proceeding on Pettenkofer's method, at different temperatures, Rischawi (78) sent air and oxygen through his experimental vessel in turns, without finding the slightest influence on the respiration of seedlings. Godlewski (35) found, at least with seeds containing oil, that in pure oxygen the respiration increased a little in the beginning and afterwards went down. Johannsen (42) worked with different oxygentensions up to a few atmospheres. At first the amount of CO_2 given off became greater. If the pressure continued the respiration became weaker and weaker until death occurred. The higher the pressure the sooner death was brought about. During the experiments rapid changes appeared in the tension and this may also have affected the plants.

Kolkwitz (45) found that in pure oxygen the respiration of fungi such as Aspergillus is at the least double its usual amount.

Flieg (32) investigated the influence of pure oxygen on fungi cultivated on fat. In this case the respiration was doubled and decreased very gradually in course of time. On sugar where ordinarily the respiration is much higher, the increase in oxygen was small. Moreover oxygen is here more harmful than on fat, as within few days the respiration was reduced to a minimum.

Th. de Saussure (85) discovered no difference in respiration when the oxygentension was reduced to half its amount. Wolkoff and Mayer (96) afterwards also stated that in a gasmixture with 10 % oxygen the amount of oxygen taken in did not vary. Borodin (14) did not agree with them. Wilson (95) found no change in the CO_2 evolved in 4 % oxygen with Helianthus, where the quantity of CO_2 given off anaerobically equals only one fourth of that given off in air. The decrease was considerable in 1 % oxygen.

At low oxygentensions CO_2 will probably be given off

intramolecularly. Stich (91) found that with different seedlings the respiratory quotient sometimes begins to increase when the amount of oxygen is 3 to 4 %. Puriewitsch (75) affirmed that in a 4 to 5 % oxygen-mixture the intramolecular respiration has not yet begun.

The influence of different oxygentensions on the growth of the sporangiophores of Phycomyces has been the subject of two examinations. Jentys (41) found that they grow as well in pure oxygen as they did in air. Wieler (94) said that the growth ceases at 0.2 % oxygen. This fact does not say anything about the respiration, because in the absence of oxygen an intensive intramolecular respiration can take place, without any noticeable growth.

The influence of different oxygentensions on the respiration of Phycomyces has never been studied. Other Mucoraceae may give off a large amount of CO_2 in the absence of oxygen (48) but this does not say anything for Phycomyces, as the latter also behaves quite differently as regards the culture conditions (see chapter I). It is not possible to say beforehand how Phycomyces will behave in different gas-mixtures. I will therefore proceed to the description of my own experiments.

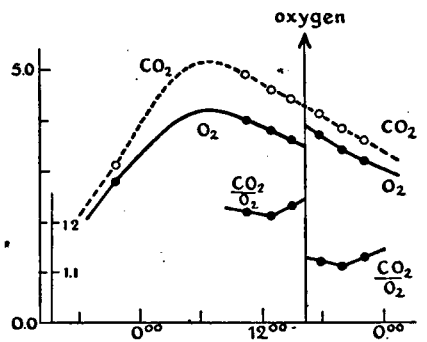
§ 2. The Influence of pure Oxygen.

Commercial "oxygen" taken from a bomb, containing more than 96 % oxygen, was purified by means of washing-bottles with solutions of permanganic soda, strong sulphuric acid and strong alkaline solutions.

A. Starch-medium. Table 22 shows the effect of a long exposure to the action of pure oxygen of a culture on bread. After the maximum in the grand period, the respiration continues to decrease in the same way as in the air. Apparently oxygen neither raises the evolution of CO_2 nor lowers it, no matter how long the exposure.

Fig. 11 (table 23) gives the influence on the respiratory quotient. Compared with the CO_2 given off, the O_2 absorbed is a little more than in the air. The quotient therefore has diminished. Table 24 shows the same for an older culture.

B. Oil-medium. The grand period of the respiration on ground linseed also takes about the same course in pure oxygen as in the air, as is shown in table 25.



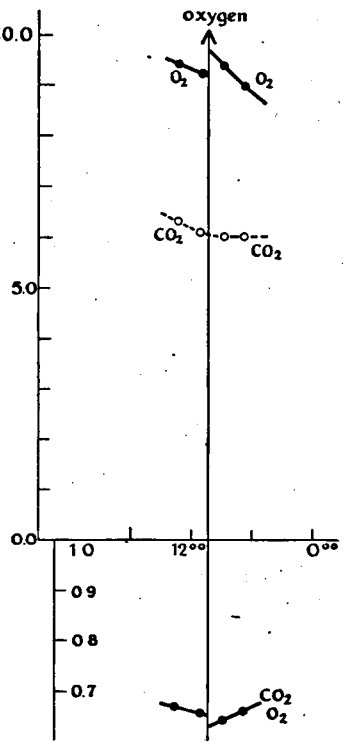


FIG. 11 and 12. Influence of Oxygen on the Respiratory Quotient on Starch- and Oil-media (see table 23 and 26).

Fig. 12 (table 26) gives the influence on the respiratory quotient. Table 27 shows another experiment. As on starch, the quotient is a little lower in pure oxygen, as some more oxygen is absorbed.

§ 3. The Influence of Gasmixtures containing no Oxygen or less Oxygen than Air does.

I used commercial bombs of "nitrogen" containing a gas consisting of nearly 98 % nitrogen and fully 2 % oxygen.

By mixing different amounts of this gas with air, I obtained mixtures of different compositions. The percentage of oxygen was determined by means of the Jordan pipet (43).

The nitrogen from the bomb was purified in washing-bottles containing strong sulphuric acid and a strong solution of sodium hydroxide.

Some of the experiments were carried out with the small earthenware troughs. Others with the medium on a set of frames. The quantity mostly used in this case was about 1½ grm.

A. Starch-medium. The result of the experiments on the effect of different percentages of oxygen on bread-cultures of Phycomyces is summarized in the following table.

TABLE 28.

The influence of different percentages of oxygen on cultures of Phycomyces on bread.

12 % oxygen	no influence.			
9½ % „	influence?			
8½ % „	94 % of the CO_2-evolution in air.	Table 36.	Fig. 17.
6½ % „	85 % „ „ „ „ „ „	„ 29.	
3 % „	64 % „ „ „ „ „ „	„ 37.	„ 18.
		„ 38.	
2 % „	50 % „ „ „ „ „ „	„ 30.	„ 13.
		„ 31.	„ 14.
1½ % „	40 % „ „ „ „ „ „	„ 32.	„ 15.

It follows from fig. 13 that in a 2 % oxygenmixture where the respiration has been reduced to half the normal amount, it will remain nearly constant. In young cultures where in the air there is a rapid increase, the respiration also becomes constant in 2 % oxygen (fig. 14).

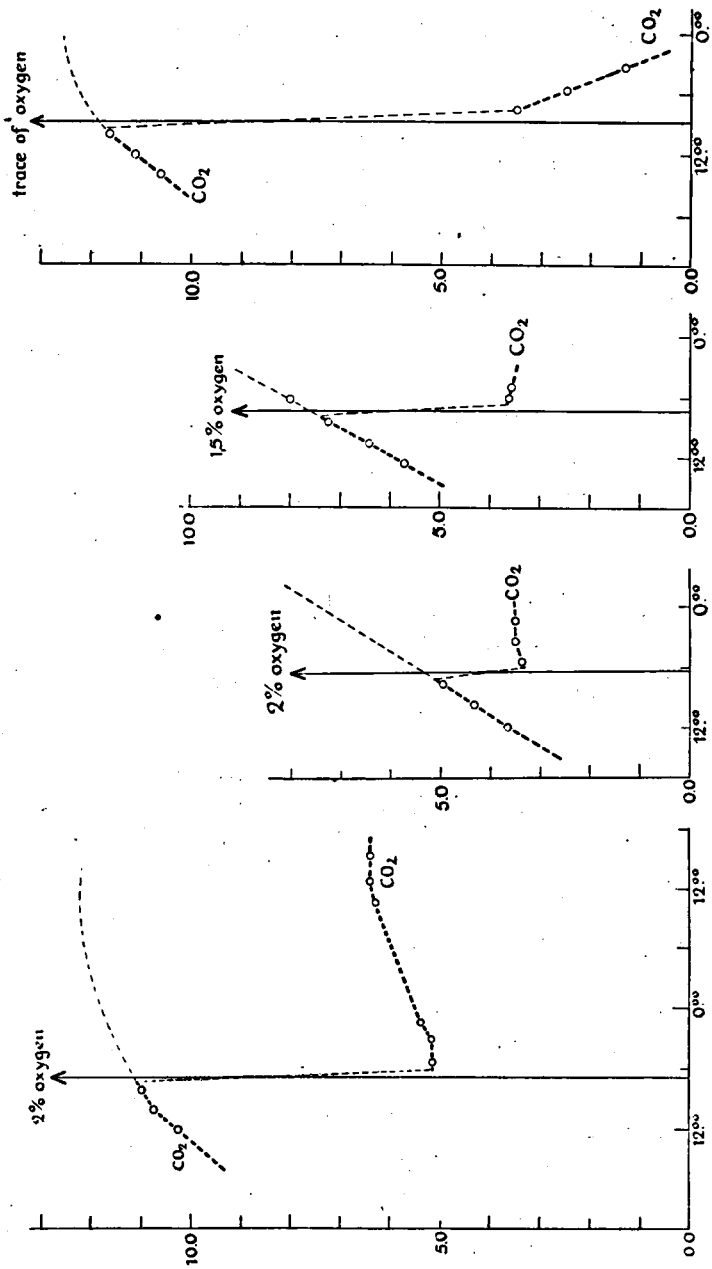


Fig. 13.

Fig. 14.

Fig. 15.

Fig. 16.

Influence of Low Oxygen Tensions on the Respiration on Starch-media, see Text and Table 30, 31, 32 and 33.

Fig. 15 shows the respiration of such a young culture in 1½ % oxygen.

From the values given in table 28, it appears that the CO_2-evolution gradually decreases to about 3 %, where the fall becomes faster so that probably at a very low oxygen tension respiration will stop.

By forcing the gas containing 2 % of oxygen through two Pettenkofer tubes, filled with a strongly alkaline pyrogallic solution, I obtained nitrogen with only a trace of oxygen.

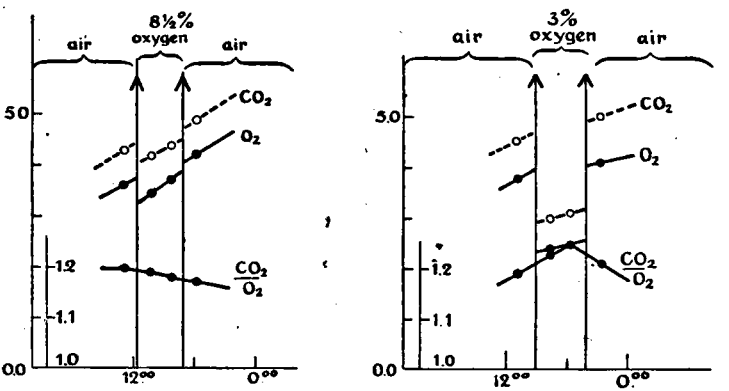


FIG. 17 and 18. Influence of an Atmosphere containing 8½ and 3 per cent. of Oxygen on the Respiratory Quotient on Starch-media. (See table 36 and 37).

It follows from fig. 16 (table 33) that in this case respiration indeed soon falls to a minimum.

Tables 34 and 35 show the transition from air to a mixture containing no oxygen at all. I used hydrogen, liberated in a Kipp-apparatus by means of pure zinc and 5-normal sulphuric acid. The hydrogen was passed through a washing-bottle with potassium permanganate and Pettenkofer tubes with alkaline pyrogallic solutions. In the total absence of oxygen Phycomyces practically gives off no CO_2 but dies

in a few hours. It does not recover, for after remaining in air for 24 hours no CO_2 has yet been given off.

On bread, contrary to expectation, the respiratory quotient does not change in smaller percentages of oxygen. Fig. 17 (table 36) gives the facts for a mixture containing 8½ % of oxygen, fig. 18 (table 37) for a mixture containing 3 % of oxygen.

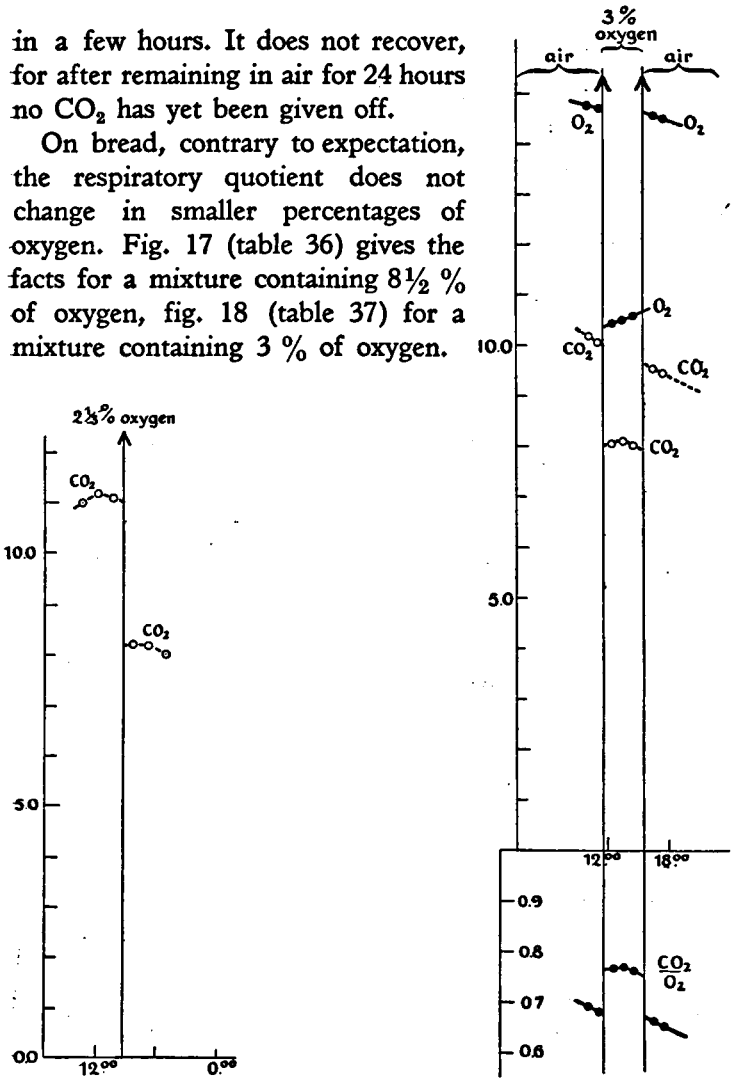


FIG. 19. Influence of an Atmosphere containing 2⅓ per cent. of Oxygen on the Respiration on Oil-medium, see Table 40.

FIG. 20. Influence of an Atmosphere containing 3 per cent. of Oxygen on the Respiratory Quotient on Oil-medium, see Table 42.

These experiments will be discussed after the description of the behaviour of the cultures on ground linseed.

B. Oil-medium. 8 % of oxygen has not yet the slightest influence on the respiration of cultures on ground linseed. At reduction to about 6 % of oxygen, the CO_2 evolution goes down to about 92 % (table 39) of the normal amount. At 2⅓ % of oxygen to about 74 % (fig. 19 table 40). On oil-media, apparently, Phycomyces can stand low oxygen tensions better than on starch-media, at least as far as the evolution of CO_2 is concerned.

In contradistinction to the starch-medium, the respiratory quotient changes on oil-media, as has been ascertained for 4½ (table 41) and 3% oxygenmixtures (table 42, fig. 20; table 43). The CO_2-evolution diminished in these cases to 85 % and 81 % respectively, the O_2-consumption to 77 % and 71 % respectively. The respiratory quotient therefore has increased.

At very low oxygentensions respiration stops, as was the case on bread. In 1 % oxygen the CO_2-evolution has dropped to about 30 % (table 44), in hydrogen Phycomyces dies (table 45).

§ 4. Discussion.

The experiments reveal many peculiarities about the respiration of Phycomyces. It cannot give off CO_2 anaerobically, neither on oil-media nor on carbohydrates. In respect to the latter fact Phycomyces differs from what has been found for fungi and higher plants. The greater part of the bread indeed consists of starch. But it is probable that Phycomyces consumes this starch by changing it first into sugars. Moreover, bread contains already some sugars.

Phycomyces evidently behaves altogether differently from Mucor species, which ferment sugars in a hydrogen atmosphere, and consequentely the less oxygen the atmosphere contains, the larger their respiratory quotient will be.

The respiratory quotient of Phycomyces remains unchanged on carbohydrates at lower oxygentensions, because Phycomyces cannot give off CO_2 intramolecularly formed. If the fact that it is a little higher than 1.00 in air was not the result of fat manufacture but of sugar fermentation, it would rise at lower oxygentensions. It is true that in pure oxygen the quotient is lowered, but probably certain substances in the medium are oxidized, independently of the plant, the consumption of oxygen on ground linseed being also raised.

The quotient on oil-media increases at low oxygentensions. At first sight it might be supposed that in this case a carbohydrate respiration partly takes the place of the oil respiration, in consequence of the latter requiring more oxygen. But then respiration on starch-media ought to be higher, whereas it is higher on oil-media at low oxygentensions.

As facts in literature would rather lead one to expect the reverse, it is very curious that on oil-media respiration is the least affected by small quantities of oxygen. There is nothing similar to be found in literature.

By many investigators, in accordance with Pfeffer's views, respiration is considered as consisting of two processes, at first a splitting of sugars into alcohol and CO_2, secondly an oxidation of the alcohol to CO_2 and H_2O. The CO_2 formed anaerobically is due to the first process, which is of course less sensitive to a lack of oxygen than the second one. On fat, anaerobic CO_2-evolution only takes place when the fungus itself has manufactured carbohydrates from the fat (Flieg).

The phenomena found for Phycomyces, both on starch- and oil-media do not fit in this scheme.

Apart from the mutual differences both on oil-and starch-media, a decrease in oxygen will affect Phycomyces much sooner than is ordinarily the case with other plants, as may

be seen from § 1. In chapter I it has often been remarked that Phycomyces appeared to be very aerophilous. For all that the mycelium will grow well so long as the oxygen percentage does not drop below 8 or 9 %.

I described the small respiration on liquid media on p. 128. Is it due to the water as such, or to a small diffusion of oxygen? In the latter case respiration will increase when the fungus is brought into an oxygen atmosphere. An experiment was carried out (table 46) showing that on media mixed with much water respiration remains low in pure oxygen.

The fact, therefore, that Phycomyces grows badly in a liquid culture medium is for the greater part due to the liquid as such and is probably only to a slight degree the result of a small oxygen diffusion.

CHAPTER IV.

THE RESPIRATION VELOCITY AND THE MAGNITUDE OF THE RESPIRATORY QUOTIENT OF PHYCOMYCES AS A FUNCTION OF THE TEMPERATURE.

The influence of temperature on respiration has often been studied. The discussion of the literature on the subject, however, will show that there are still various problems which have not been solved. This was the reason why I decided to investigate the question more in detail, especially as it gave me an experimental object with which I was well acquainted.

§ 1. Discussion of the Literature.

Sachs in 1860(83), in investigating the influence of the temperature on the growth of seedlings, introduced the concept of the three cardinal points: minimum, optimum and maximum; these three points were in later years also determined for other processes for instance the photosynthesis.

As regards respiration, it struck the older investigators that the optimum, if there is one, is always much higher than with other processes (cf. e.g. Ad. Mayer, 62). A question of much discussion was how respiration reached this high optimum. According to Wolkoff and Mayer (96), Ad. Mayer (62) and Bonnier and Mangin (11) the intensity of respiration was nearly a linear function of the temperature. Rischawi (79), instead of a straight line, found a curve convex towards the temperature axis, the curve given by Dehérain and Moissan (23) and also the one of Pedersen (68) was even convex to a higher degree. Both ascend rapidly.

The contradictions may be the consequence of a difference

in objects or in methods, but generally the respiration curve was found more or less convex towards the temperature axis. The curve was much steeper than those for other processes, such as photosynthesis (Kreusler 50); the optimum was found near the lethal temperature.

Contrary to this opinion Clausen (20) and Ziegenbein (98) found that optimum and lethal temperature do not lie close together. At higher temperatures above the optimum the respiration may be lower than at the optimum, and for all that constant

If in these experiments the temperature was lowered, the respiration appeared to be smaller than it was before at the same temperature. Pfeffer (71) remarks that apparently part of the plants had died. Pfeffer therefore holds the view that an optimum never can be spoken of with respiration, because in this case the curve would bend at a temperature not yet noxious to the plant, as happens according to him with photosynthesis and the growth of plants.

Another explanation of the optimum is suggested by Tammann (92) and Duclaux (27) for enzymes. According to them, it is the result of the enzymes being destroyed at higher temperatures, and the higher the temperature, the more of them there are destroyed. The optimum therefore is the result of the noxious effect of the higher temperatures. The process itself would not be impeded by a rise in temperature. Without this harmful effect the enzyme action would increase with the temperature in a continuously ascending curve (Duclaux).

It has been the great merit of F. F. Blackman (9) that he proposed a similar theory, independent of Tammann and Duclaux for all physiological processes, in connection with a research by one of his pupils Miss Matthaei. Miss Matthaei (59) studied the CO_2 assimilation of the leaves of Prunus Laurocerasus. When care is

taken that there is always sufficient CO_2 and light, no optimumcurve is found, but the rate of assimilation increases rapidly with the temperature, according to a curve, convex towards the temperature axis. The reaction velocities at these high temperatures are however not constant. A leaf cannot keep up this maximal assimilation, and the higher the temperature, the more rapidly it decreases. The temperature curves of assimilation values will therefore vary in proportion to the time the plant is kept at the high injurious temperatures.

From the data obtained by Miss Matthaei, F. F. Blackman claims that:

1o. Physiological reactions are influenced by temperature in a similar way as chemical reactions, if only the organism is not injured. According to Van 't Hoff's law (or rather according to the interpretation the biologists of that time gave of Van 't Hoff's law) the reaction velocity is doubled or trebled for a rise of ten degrees in temperature (also expressed by the formula: temperature coefficient $Q_{10} = 2$ or $Q_{10} = 3$).

2o. The optimum curve is the result of the time factor. The shorter the time of observation, the less the injury and the higher the optimum. If it were possible to observe after a "time o" a "Van 't Hoff's curve" would be found.

3o. This "Van 't Hoff's curve" can therefore be constructed in two ways by extrapolation. After the first method the Q_{10} is used, determined at lower temperatures where no injury takes place. By means of this Q_{10} the curve is constructed for higher temperatures. After the second method the values of the theoretical curve at higher temperatures are extrapolated from the values obtained after 1, 2, 3 etc. hours of observation.

It is now obvious that Ziegenbein (98) would have found the optimum at another temperature if he had observed

either sooner or later. The experiments of Chudiakow (19) who found the optimum of fermentation of yeast at 40°, 35°, 30° or 25°, according to the time elapsed, fit in with the above theory.

Kuyper (52) was the first who tried to apply Blackman's theory to respiration. He experimented with green peas and other seeds on the Pettenkofer-Pfeffer method. According to him the "Van 't Hoff's" rule holds good up to 20°. $Q_{10} = \pm 2.8$. By means of this coefficient it was possible to construct by extrapolation the exponential curve for higher temperatures. The values of this theoretical curve are much higher than those, which would be found by means of the second extrapolation method of Blackman. Kuyper however still believes that Blackman's theory in its general lines is correct.

In my opinion the different result with the two methods of extrapolation may have been caused by the following facts.

When Kuyper moves his plants from a low to a higher, thought not injurious, temperature, the objects take on the new temperature after about 10 minutes. He has, however, to wait an hour before starting his experiments. Otherwise he will get too low an amount of CO_2 at the beginning. According to Kuyper the respiration apparently does not adapt itself directly to the new temperature. The same thing must happen on the objects being brought to a higher injurious temperature. Here there are two tendencies, an increase in the CO_2-evolution owing to the adaptation to the new higher temperature and a decrease owing to injury. Kuyper now experimentally determines the time at which the first measurement must be taken. Before as well as after this time the amount is lower.

In my opinion the reason for this so-called adaptation to the new temperature, is that during the first half hour after the temperature has been raised, gas is collected which was still formed at the lower temperature (see p. 126).

Further, it has lately become evident that there are many objections against the materials used in Kuyper's experiments. Stålfelt (90) namely has shown that the seed coat offers an obstacle to the diffusion of gas. From the experiments of Sierp (88) it also appears that peas are, physiologically, very complex structures (See also the recent publication of Frietinger, 34).

Van Amstel and Van Iterson (2, 3) object against Kuyper that in the course of long periods of observation, say of several hours, adaptation and growth processes may appear. The authors used yeast and so always worked with cells which are small and will therefore quickly assume the new temperature. They used a definite quantity of the yeast, of which the velocity of fermentation, respiration etc. at the lower uninjurious temperatures is known. The velocity was determined after 5, 10, 15 and 20 min. pre-heating. After the measurement had been taken the temperature was at once changed to a harmless one and the fermentation velocity determined. This fermentation velocity is only a part of the velocity the yeast would have at the same temperature if it had not been injured at the high temperature. This proportion supplies a certain value with which the velocity at the high temperature has to be multiplied, to get the velocity at high temperatures if nothing had been injured.

For all the examined functions the theoretical "zero-hour" curve does not show Blackman's exponential curve, but an optimum curve, so that according to Van Amstel and Van Iterson the theory of Duclaux-Blackman must be rejected for these processes. This also follows from the behaviour at harmless temperatures. Up to 45° namely, the reaction velocity is independent of the preliminary heating, but yet the Q_{10} already decreases.

Rutgers (80, 81) determined the influence of temperature on the geotropic presentation time. He concludes

from his experiments that the "zero-hour" curve never can be extrapolated from the values found after 1, 2, 3 hours etc., because the reaction-velocities only adapt themselves gradually to a new temperature. The greatest support for this theory Rutgers finds in Kuyper's publication. I have shown that in this case the adaptation is probably due to experimental errors. I therefore do not agree with Rutgers when he suggests that this gradual adaptation has influenced the results of Van Amstel and Van Iterson, who moreover have refuted (2, 4) this and other criticisms from Rutgers (80, 81) and Kuyper (53).

It was already mentioned by Rutgers (81) that with chemical reactions Q_{10} may diminish at higher temperatures. Cohen Stuart (21) studied this subject more in detail and pointed out that Van 't Hoff's law is often wrongly interpreted by biologists. Especially in physiological processes when there is a heterogeneous system, Q_{10} as a rule will decrease at higher temperatures. The "zero-hour" line therefore need not be an exponential curve, as Blackman supposed, and cannot be constructed by means of the Q_{10} found at low harmless temperatures.

In the following pages the results are stated of investigations as to whether or not respiration will directly adapt itself to a new temperature. As this proved to be the case it was tried to construct the "zero-hour" line according to the second method of Blackman, namely by an exact extrapolation from the points on the "injury"-curves.

The present writer also investigated the influence of temperature on the respiratory quotient $\frac{CO_2}{O_2}$ of Phycomyces growing on fat-and starch-media.

Very little has been done about studying the influence of temperature on respiratory quotients. The older inves-

tigators were divided in their views. Bonnier and Mangin (11, 12) found that the respiratory quotient is independent of the temperature. According to Dehérain and Moissan (23), Moissan (63) and Dehérain and Maquenne (22) it increases with the temperature. Puriewitsch (75) confirmed their experiments. The younger the objects, the greater is the increase of the quotient at a higher temperature. He found something similar for fungi. The influence of temperature here is namely smaller, in proportion as the lack of food is greater.

§ 2. The Respiration of Phycomyces on Oil-media at different Temperatures.

A. Experiments.

The experiments described in the preceding chapters were mostly carried out at 25° C. In this chapter the experiments are given at different temperatures.

In the figures of § 2 A and § 3 A the ordinate-axis represents the rate of respiration in ccm. per hour, the abscissa-axis has been taken as the time-axis, graduated into hours. The solid lines are again the "O_2-lines", the broken ones the "CO_2-lines". In the same figures the respiratory quotients are indicated by a solid line. Here the ordinates represent the magnitude of the respiratory quotients.

The hatched part represents the time required by the water in the basin to take on the new temperature. The vertical line in front of it indicates the time at which the experiments at the initial temperature were finished, the vertical line behind indicates the time at which the experiments started at the new temperature. The "O_2-and CO_2-curves" are extended as far as the hatched part (cf. page 197).

At higher injurious temperatures, where respiration decreases, no delay is allowed in starting the new experiment

in order to get as much as possible of the first part of the "injury-curve".

It does not do to start the new experiment before every thing has assumed the new temperature. Blank experiments were carried out in order to test this. Nearly the whole apparatus very rapidly takes on the temperature of the water in the basin. Only in the culture medium (see § 2 B) and in the alkaline solution in the glass-vessel M (fig. 1) the lag of the temperature is greater. The experiments were therefore usually started 22½ minutes after

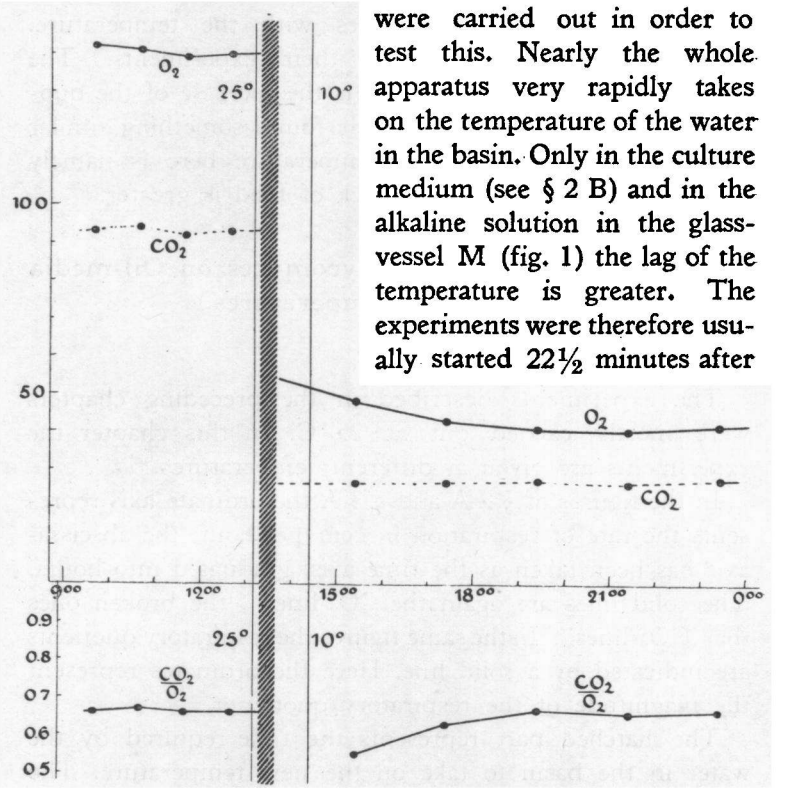


FIG. 21. The Transition from 25° to 10° C. on Oil-media (see table 47).

the water in the basin had taken on the new temperature. In the meantime some manipulations have to be carried out, such as the sucking of air of the required temperature through the Pettenkofer tubes (see p. 123).

At the average temperatures the measurements are taken every hour, at the lower temperatures where respiration is smaller even after longer periods. At higher temperatures, however, the initial measurements are taken every half hour, because respiration decreases rapidly and its exact progress has to be found; the oxygen consumption is even determined every quarter of an hour. In the last case the values obtained are indicated in the figures by means of a small circle instead of a dark dot. Shorter periods of observation are not desirable as errors will then become relatively too large.

All experiments were carried out with two thin layers of culture medium of 1¼ grm. each on a set of frames. The respiration was always measured in the "constant" part of the grand period.

In fig. 21 (table 47) the respiration is given at 25° and at 10° C. The respiration is less at the lower temperature. As regards the evolution of CO_2 nothing is seen of a gradual adaptation to the new temperature. It immediately becomes about 28.5 % of what it was at 25° C. and remains so during the following hours.

The amount of O_2 taken in, however, becomes constant only after some hours. At first it only decreases to 38.5 % and then gradually drops to 28.5 %, which with the CO_2-evolution is immediately the case.

As mentioned above, blank experiments showed that the air in the respiration vessel in every case assumed the new temperature within 20 min. A contraction of the air can therefore not be the cause of the higher initial oxygen consumption.

At a transition from a higher to a lower temperature the amount of oxygen absorbed is at first apparently too high as compared with the CO_2 given off. Consequently the respiratory quotient is lower at first and reaches its original value only when the respiration becomes constant.

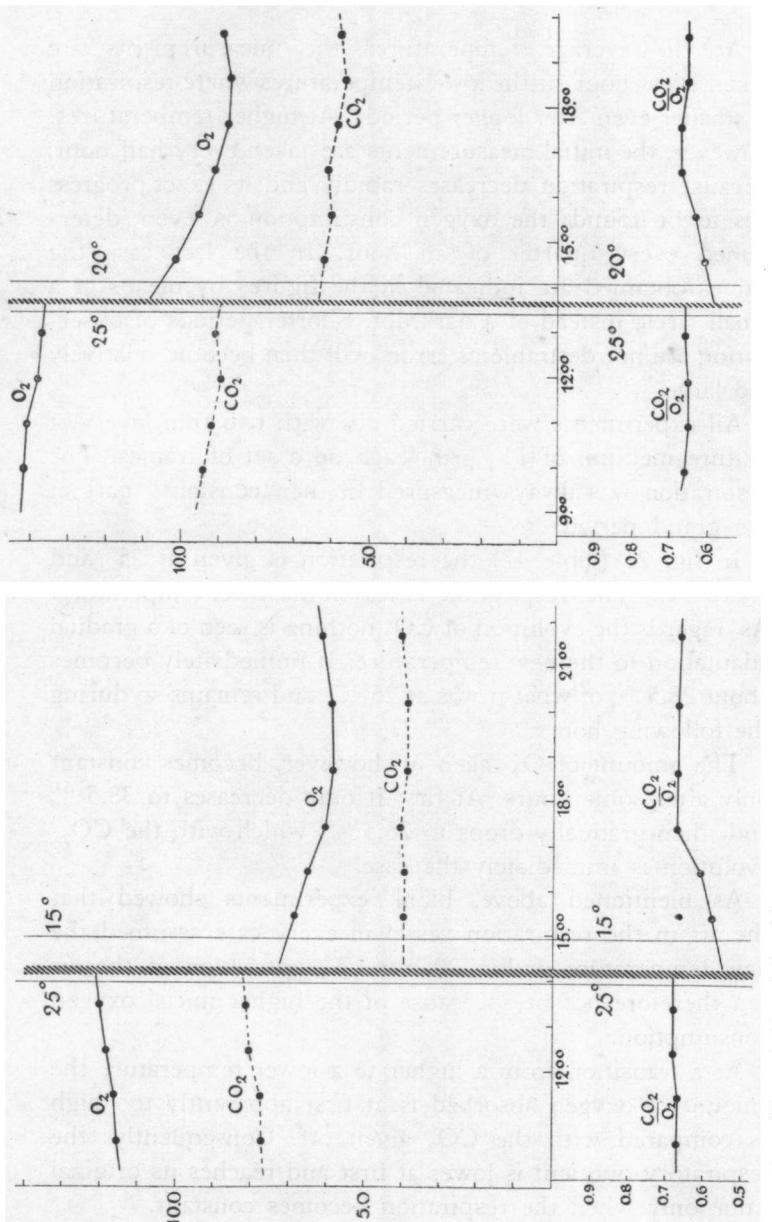


FIG. 22 and 23. The Transitions from 25° to 15° and 20° C. on Oil-media (see table 48 and 49).

The same thing happens when the transition is to 15° and 20° C. The values of the respiration at new temperatures will not be given separately for each temperature but are summarized in table 59 (p. 185). Fig. 22 (table 48) shows the effect of the transition from 25° to 15°, fig. 23 (table 49) shows it from 25° to 20°. The deviation of the initial oxygen respiration becomes smaller the less the difference is in temperature; the same may be said about the initial respiratory quotients.

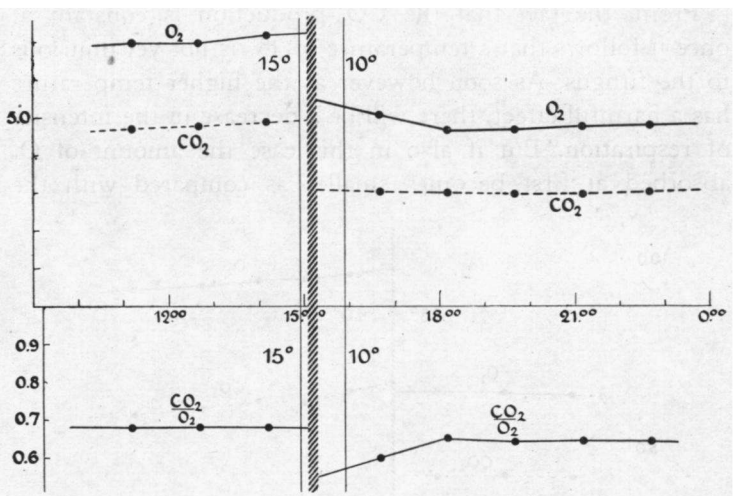


FIG. 24. The Transition from 15° to 10° C. on Oil-media (see table 50).

I also determined the respiration velocities at 10°, 15°, 20° and 25° C. by starting the experiments at 15°. Fig. 24 (table 50) gives the situation at the transition from 15° to 10°. The oxygenconsumption is again a little higher at first and the respiratory quotient smaller.

In the following experiments we for the first time see the transitions from a lower to a higher temperature. At the change from 15° to 20°, as is shown by fig. 25 (table 51),

both the CO_2-evolution and the O_2-absorption are constant almost immediately. The respiratory quotient is not smaller at first but perhaps even larger. This is more obvious in the next fig. 26 (table 52), giving the situation at the transition from 15° to 25°. The respiratory quotient again only reassumes its original value after some time, but this time because it is higher at first. In contrast with the former experiments, the initial O_2-consumption is namely too low when the culture is brought to a higher temperature.

From the fact that the CO_2-production is constant at once it follows that a temperature of 25° is not yet injurious to the fungus. As soon however as the higher temperature has a harmful effect, there will be a decrease in the intensity of respiration. But if also in this case the amount of O_2 absorbed at first becomes smaller as compared with the

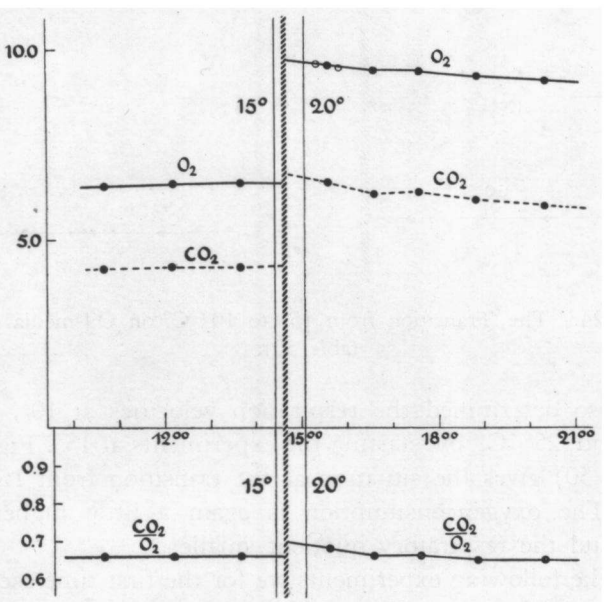


FIG. 25. The Transition from 15° to 20° C. on Oil-media (see table 51).

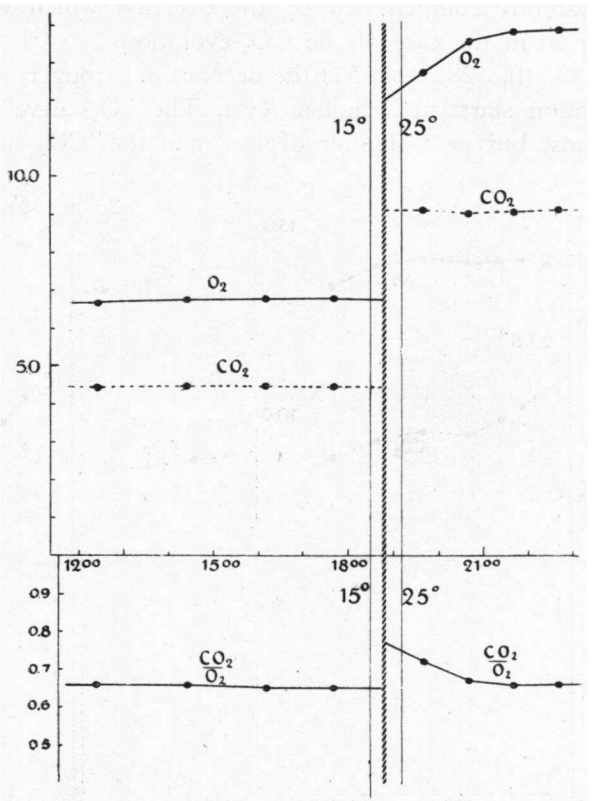


FIG. 26. The Transition from 15° to 25° C. on Oil-media (see table 52).

CO_2 given off, the "O_2-curve" will start too low and descend less rapidly than the "CO_2-curve".

This is indeed the case at higher temperatures. At 27°5 the injurious effect has begun. It is obvious from the CO_2-evolution; for the first time we see an initial decrease: fig. 27 (table 53). The O_2-consumption however is constant. The increase of it, which might be expected in view of its behaviour at a transition to a higher temperature from fig. 26,

is apparently compensated by the decrease which would appear as in the case of the CO_2-evolution.

At 30° (fig. 28, table 54) the decrease is stronger, as the respiration starts at a higher level. The "O_2-curve" also descends, but in a slighter degree than the "CO_2-curve",

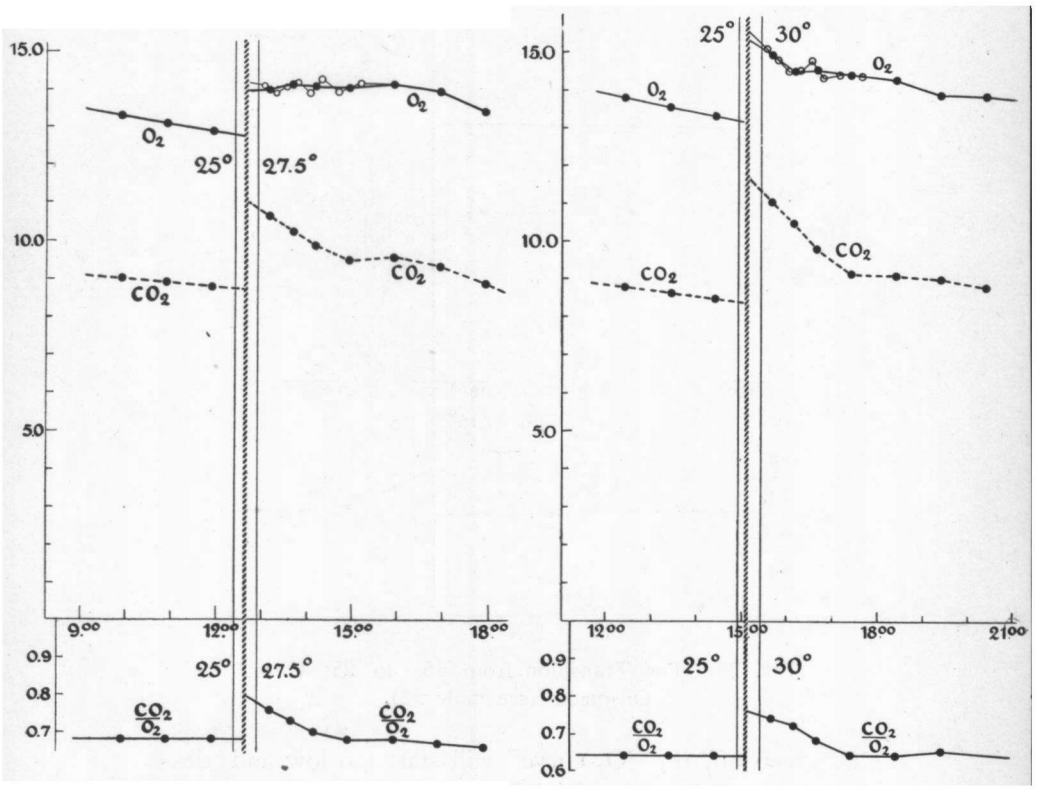


FIG. 27 and 28. The Transitions from 25° to 27°5 and 30° C. on Oil-media (see table 53 and 54).

as the O_2-consumption again does not attain its value at once. In consequence of these facts, in fig. 27 and 28 the respiratory quotients are too high at first just as was the case in fig. 26. When the downward movement ceases and

the respiration again becomes constant, the respiratory quotient reassumes its original value.

At 32°5 the respiration becomes constant no more: fig. 29 (table 55). The noxious influence of the temperature

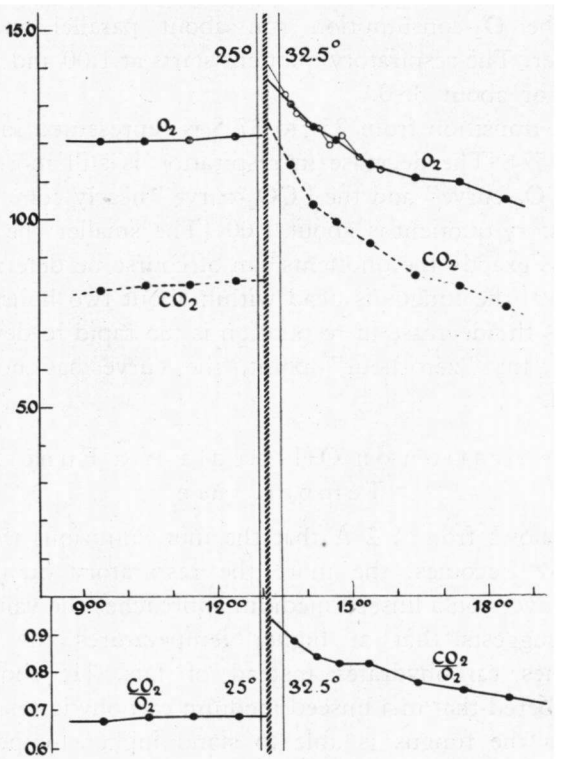


FIG. 29. The Transition from 25° to 32°5 C. on Oil-media (see table 55).

continues. The "O_2-curve" and the "CO_2-curve" start closer together and also remain so more or less. The respiratory quotient at first already approaches the value of 1.00 and never again reassumes its original value of about 0.67.

The fact that the values of the O_2 taken in and the CO_2 evolved tend to approach each other, becomes more apparent at higher temperatures.

At 35°, fig. 30 (table 56), the respiration decreases rapidly and the fungus dies in the long run. The CO_2-production and the O_2-consumption run about parallel and close together. The respiratory quotient starts at 1.00 and remains above or about 0.90.

The transition from 25° to 37°5 is represented in fig. 31 (table 57). The decrease in respiration is still more rapid. The "O_2-curve" and the "CO_2-curve" nearly coincide, the respiratory quotient is about 1.00 (The smaller the values, the less exactly the quotients can of course be determined).

At 40° the fungus is dead within about two hours (table 58). As the decrease in respiration is too rapid to determine exactly the "zero-hour" point, the curve has not been plotted.

B. Respiration on Oil-media as a Function of Temperature.

It follows from § 2 A.that the more injurious the temperature becomes, the more the respiratory quotient of Phycomyces on a linseed medium approaches the value 1.00. This suggests that at higher temperatures the fungus consumes carbohydrates instead of fats. (It should be remembered that in a linseed medium carbohydrates occur). Perhaps the fungus is able to stand higher temperatures better on starch-media than on oil ones. In § 3 therefore the influence of the temperature on starch-media cultures will be investigated.

The change in the respiratory quotient gives a peculiar shape to the "zero-hour"-line. In extrapolating the points of the "zero-hour"-line from the values found it is necessary to trace back the different "injury"-curves to the zero time i.e. the time at which the culture medium reached

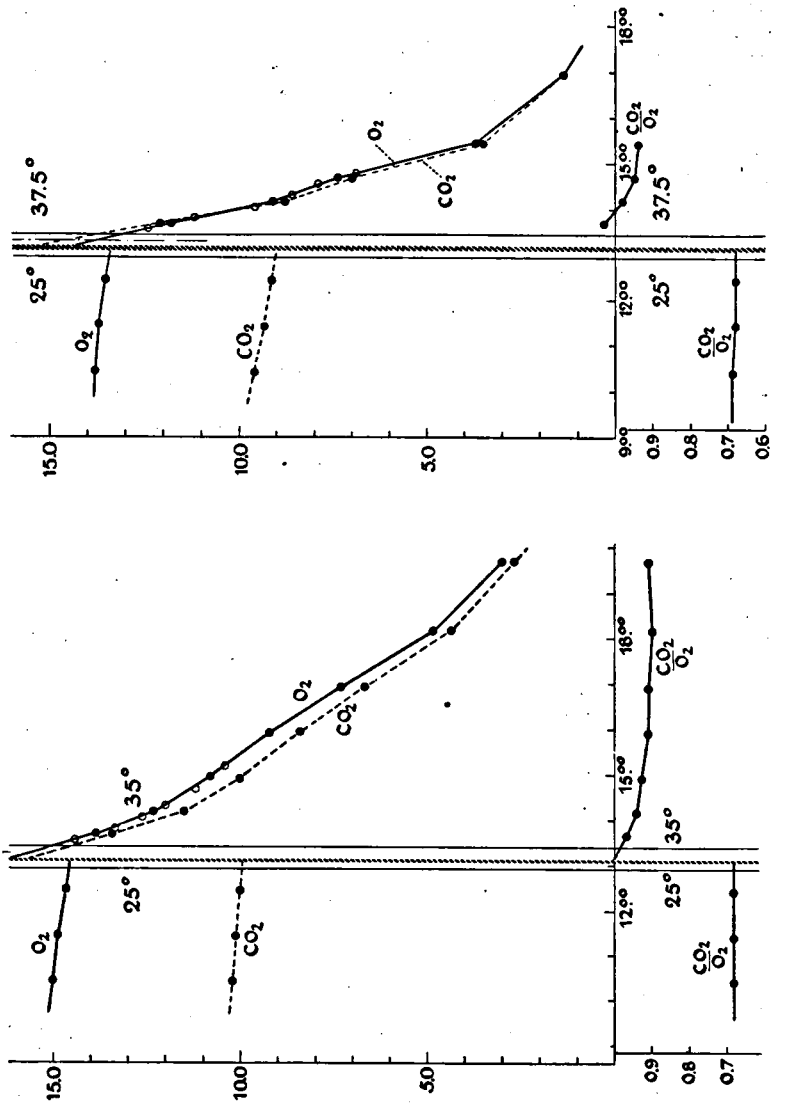


FIG. 30 and 31. The Transitions from 25° to 35° and 37°5 C. on Oil-media (see table 56 and 57).

the new temperature. This extrapolation is difficult at high injurious temperatures, as a slight deviation in the extrapolated line will cause a great mistake in the position of the "zero-hour" point. As many experiments as possible were therefore carried out.

The time passing between the zero time and the beginning of the first experiment must be known exactly.

The experiments were all carried out with a suction velocity of 3 to 3½ l. per hour and in the respiration vessel of 325 to 350 ccm cubic contents. From table 1 it follows that in this case the CO_2 given off at a certain moment by the plant needs 4½ minutes on an average to arrive at the Pettenkofer tube. In the curves the values measured are therefore indicated 4½ minutes before the average time of observation.

I was unable to measure the temperature of the mycelium, I think however that the fungus and the culture medium may be supposed to assume temperatures at the same time. How much time will elapse between the moment that the water of the basin in which the apparatus is fixed attains the new temperature, and the moment at which the culture medium assumes it? As mentioned on page 172 the temperature lag in the culture medium is rather large. In the Physical Laboratory I determined it thermoelectrically [1]). When the water in the basin is brought to a higher temperature the temperature of the culture medium increases very rapidly at first and then very gradually till the desired temperature is reached.

Now it would be wrong to choose as the zero-time the time at which the culture medium finally reached the new temperature, as at higher temperatures the noxious action has already begun. I therefore took one half of the time necessary for the medium to assume the new temperature.

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[1]) I have to thank Prof. Ornstein for his kindness in assisting me.

In this case the zero time at 37°5 is 7½ to 10 min., at 35° about 7½ min., at 32°5 5 to 7½ min. and at 30° about 5 min. after the basin reached the new temperature.

In table 59 the respiration-velocities are given for the

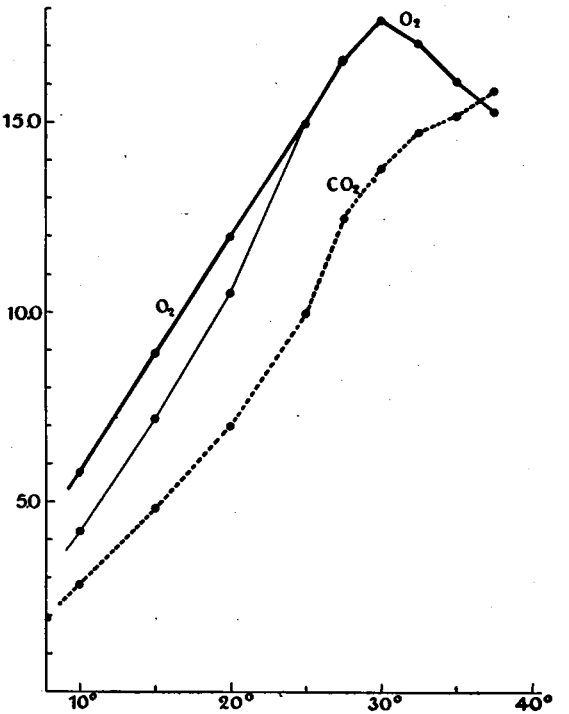

FIG. 32. Respiration on Oil-media (Linseed) at Different Temperatures.

The ordinate-axis represents the rate of respiration in ccm. per hour, the abscissa-axis the temperature in Centigrade degrees. The broad lines represent the course of the "zero-hour" line. The constant values of the O_2 absorbed below 25°, attained after some time (as explained in the text) are indicated by a thin line. For further explanation see text.

harmless temperatures 10°, 15°, 20° and 25° C., calculated by means of the figures 21, 22 and 23. They are given in percentages of the amount at 25° and also in ccm, when

the CO_2-production at 25° is assumed to be 10 ccm. per hour and the O_2-consumption 15 ccm. per hour.

(See table 59).

In table 60 the respiration velocities are given for temperatures above 25° C. Fig. 32 represents the "zero-hour"-line. (See table 60).

The temperaturequotients apparently decrease rapidly. As there is a small temperature interval I give the Q_5.

$$Q\frac{15^0}{10^0} = \frac{4.95}{3.1} = 1.60 \text{ (fig. 24)}. \quad Q\frac{20^0}{15^0} = \frac{6.5}{4.3} = 1.51 \text{ (fig. 25)}.$$

$$Q\frac{25^0}{20^0} = \frac{9.0}{6.35} = 1.41 \text{ (fig. 23)}.$$

The "O_2-curve" is even an optimum curve because at higher temperatures the O_2 values approach the CO_2 values and therefore decrease.

Fig. 33 gives the respiration-velocities at different temperatures after different periods. Besides, the thin lines at the top give the "zero-hour"-line when the time at which the basin assumed the new temperature is taken as the zero time.

§ 3. The Respiration of Phycomyces on Starch-media at different Temperatures.

A. Experiments.

If on linseed-media the change in the respiratory quotients, as found in § 2, is really the result of a more or less intensive consumption of carbohydrates, on bread-media, where the consumption of carbohydrates will always be the chief feature, these changes in the respiratory quotients will not occur at the transitions to other temperatures.

Fig. 34 (table 61) renders the transition from 25° to 10°. The respiration-velocity is much smaller on bread than on linseed, the values, moreover, are small in consequence of the low temperature. A relatively larger error is therefore

TABLE 59.

	CO_2-production.	Initial O_2-consumption.	O_2-consumption, become constant.
10°	$\frac{2.65}{9.3} \times 100 = 28.5\ \% = 2.85$ ccm.	$\frac{5.4}{14.0} \times 100 = 38.5\ \% = 5.8$ ccm.	$\frac{\pm 4.0}{14.0} \times 100 = 28.5\ \% = 4.3$ ccm.
15°	$\frac{4.0}{8.3} \times 100 = 48\ \% = 4.8$ ccm.	$\frac{7.3}{12.25} \times 100 = 59.5\ \% = 8.9$ ccm.	$\frac{\pm 5.85}{12.25} \times 100 = 47.5\ \% = 7.15$ ccm.
20°	$\frac{6.35}{9.0} \times 100 = 71\ \% = 7.1$ ccm.	$\frac{10.85}{13.6} \times 100 = 80\ \% = 12.0$ ccm.	$\frac{\pm 9.6}{13.6} \times 100 = 70\ \% = 10.6$ ccm.
25°	$100\ \% = 10.0$ ccm.	$100\ \% = 15.0$ ccm.	$100\ \% = 15.0$ ccm.

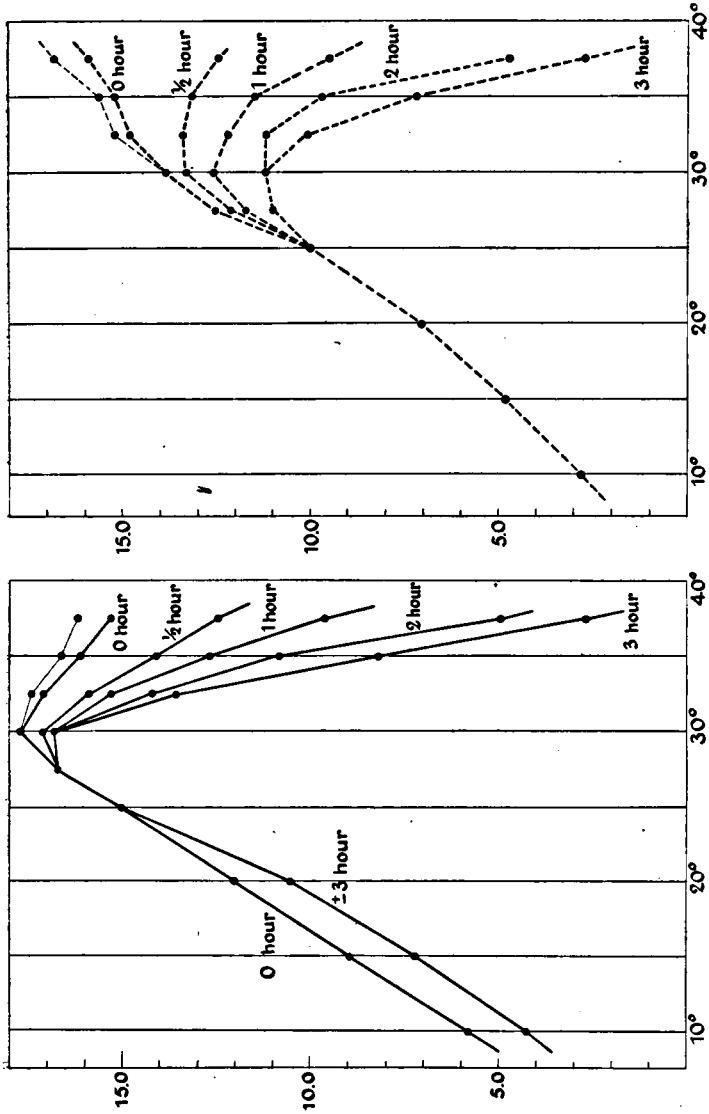

FIG. 33. Velocities of Respiration on Oil-media (Linseed) at Different Temperatures after Different Periods. A. The Consumption of Oxygen. B. The Production of Carbondioxide.

TABLE 60.

	CO_2-production.	O_2-consumption.	
25°	100 % = 10.0 ccm.	100 % = 15.0 ccm.	
27°5	$\frac{11.0}{8.75} \times 100 = 125\ \% = 12.5$ ccm.	$\frac{14.15}{12.75} \times 100 = 111\ \% = 16.65$ ccm.	Fig. 27.
30°	$\frac{11.6}{8.35} \times 100 = 138\ \% = 13.8$ ccm.	$\frac{15.5}{13.1} \times 100 = 118\ \% = 17.7$ ccm.	Fig. 28.
32°5	$\frac{12.4}{8.4} \times 100 = 148\ \% = 14.8$ ccm.	$\frac{13.9}{12.2} \times 100 = 114\ \% = 17.1$ ccm.	Fig. 29.
35°	$\frac{15.05}{9.9} \times 100 = 152\ \% = 15.2$ ccm.	$\frac{15.6}{14.5} \times 100 = 108\ \% = 16.1$ ccm.	Fig. 30.
37°5	$\frac{14.2}{8.95} \times 100 = 159\ \% = 15.9$ ccm.	$\frac{13.55}{13.4} \times 100 = 102\ \% = 15.3$ ccm.	Fig. 31.

made in determining the respiratory quotient. But at first the quotient apparently is a little higher. The O_2-consumption immediately becomes constant at the new temperature. It seems that the CO_2-production starts a little too high. The same is to be seen from the transitions from 25° to 15° or 20°, represented in fig. 35 (table 62) and 36 (table 63).

From the transition from a lower to a higher harmless temperature e.g. from 15° to 25° (fig. 37, table 64) it is again evident that in contradistinction to the respiration on oil-media, the O_2 absorbed directly becomes constant and that the respiratory quotient remains constant.

At 27°5 and 30° the respiration also immediately becomes constant, fig. 38 (table 65) and fig. 39 (table 66). The CO_2-evolution is at best a bit higher at first. The respiratory quotient remains nearly the same.

At 32°5 the noxious action of the temperature has begun, fig. 40 (table 67). The respiration still becomes constant in the long run.

At 32°5 the respiratory quotient at last approaches the value of 1.00, which is still better seen at the other high injurious temperatures.

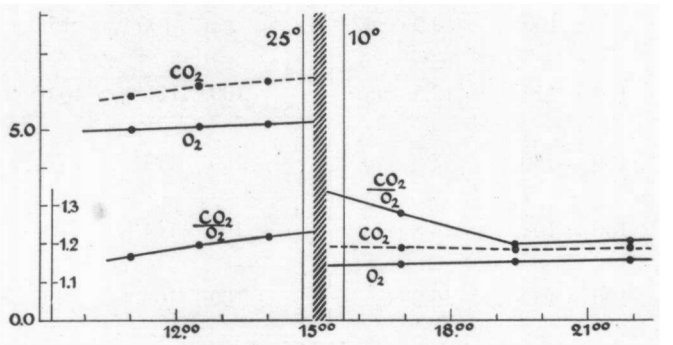

FIG. 34. The Transition from 25° to 10° C. on Starch-media (see table 61).

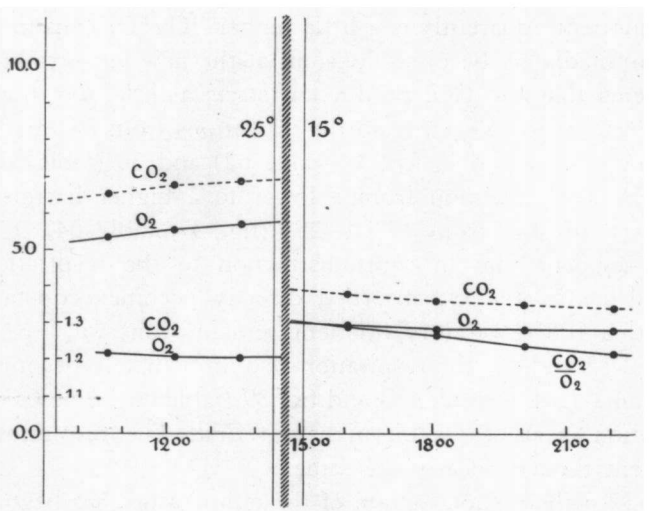

FIG. 35. The Transition from 25° to 15° C. on Starch-media (see table 62).

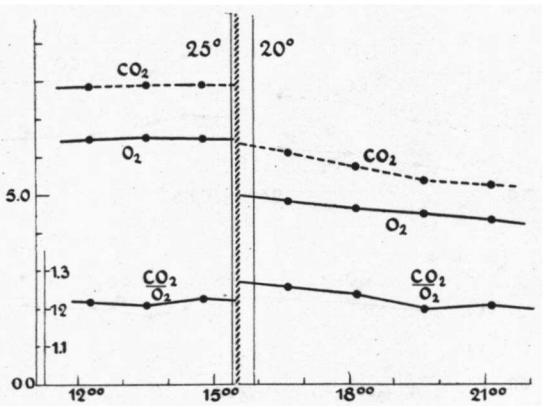

FIG. 36. The Transition from 25° to 20° C. on Starch-media (see table 63).

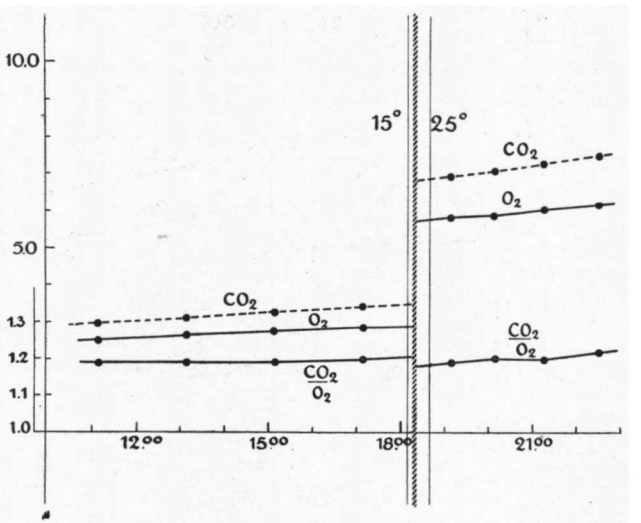

FIG. 37. The Transition from 15° to 25° C. on Starch-media (see table 64).

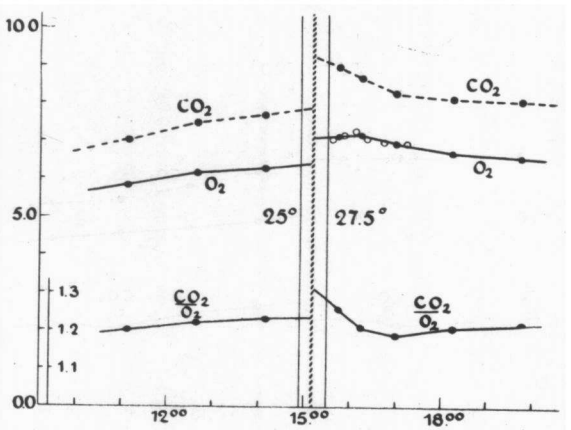

FIG. 38. The Transition from 25° to 27°5 C. on Starch-media (see table 65).

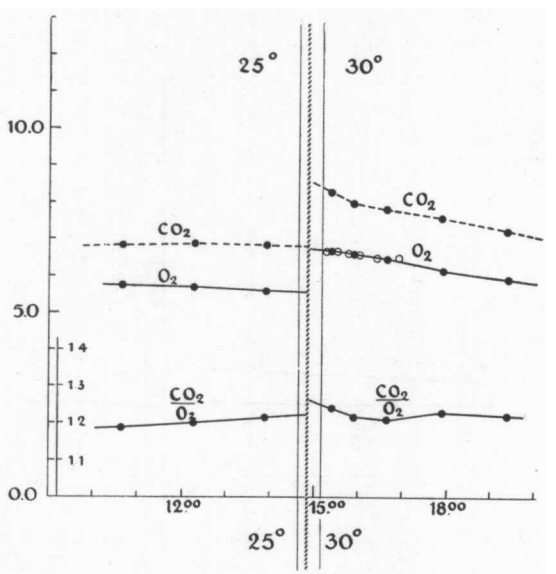

FIG. 39. The Transition from 25° to 30° C. on Starch-media (see table 66).

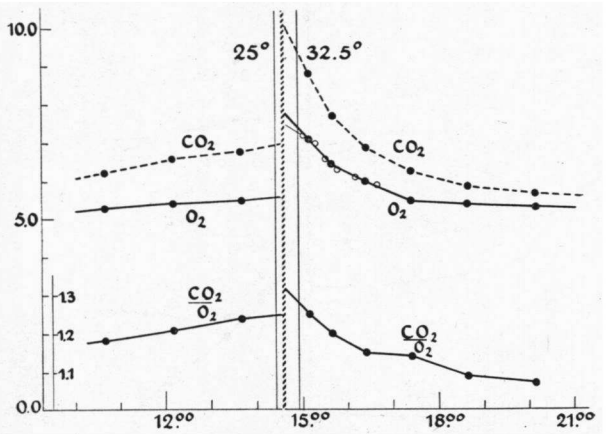

FIG. 40. The Transition from 25° to 32°5 C. on Starch-media (see table 67).

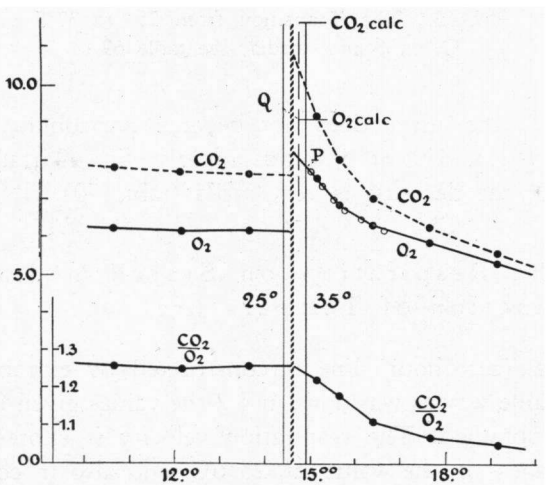

FIG. 41. The Transition from 25° to 35° C. on Starch-media (see table 68).

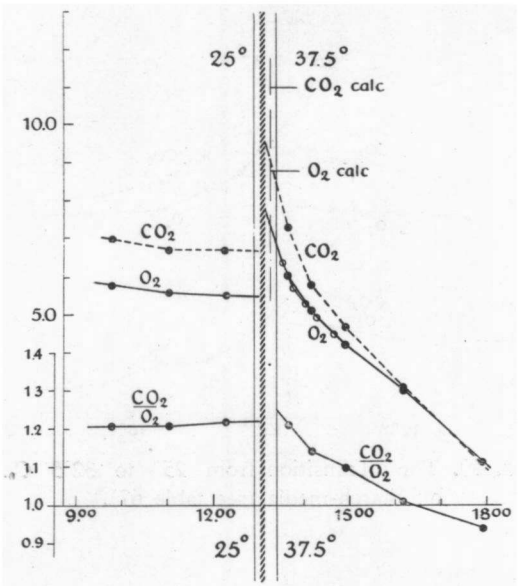

FIG. 42. The Transition from 25° to 37°5 C. on Starch-media (see table 69.)

At 35° the curve does not become horizontal, fig. 41 (table 68); at 37°5 it descends rapidly, fig. 42 (table 69). At 40° the decrease is very rapid (table 70).

B. The Respiration on Starch-media as a Function of Temperature.

If the "zero-hour"-line is constructed by extrapolation in the same way as was done in § 2 the values given in table 71 are obtained. The respiration velocity is expressed in percentages of the value at 25° C. and also in ccm. per hour if the evolution of CO_2 at 25° is 7.0 ccm. per hour and the absorption of O_2 is 5.75 ccm. per hour.

(See table 71).

TABLE 71.

	CO_2-production.	O_2-consumption.	
10°	$\frac{1.95}{6.4} \times 100 = 30.5\ \% = 2.15$ ccm.	$\frac{1.45}{5.2} \times 100 = 28\ \% = 1.6$ ccm.	Fig. 34.
15°	$\frac{3.95}{7.0} \times 100 = 56\ \% = 3.9$ ccm.	$\frac{3.05}{5.85} \times 100 = 52\ \% = 3.0$ ccm.	Fig. 35.
20°	$\frac{6.4}{7.95} \times 100 = 80.5\ \% = 5.65$ ccm.	$\frac{5.0}{6.5} \times 100 = 77\ \% = 4.45$ ccm.	Fig. 36.
25°	$100\ \% = 7.0$ ccm.	$100\ \% = 5.75$ ccm.	
27°5	$\frac{9.2}{7.9} \times 100 = 116\ \% = 8.1$ ccm.	$\frac{7.1}{6.4} \times 100 = 111\ \% = 6.4$ ccm.	Fig. 38.
30°	$\frac{8.6}{6.85} \times 100 = 126\ \% = 8.8$ ccm.	$\frac{6.8}{5.6} \times 100 = 122\ \% = 7.0$ ccm.	Fig. 39.
32°5	$\frac{10.0}{7.0} \times 100 = 143\ \% = 10.0$ ccm.	$\frac{7.55}{5.6} \times 100 = 135\ \% = 7.75$ ccm.	Fig. 40.
35°	$\frac{10.6}{7.65} \times 100 = 138\ \% = 9.65$ ccm.	$\frac{8.35}{6.15} \times 100 = 136\ \% = 7.8$ ccm.	Fig. 41.
37°5	$\frac{9.0}{6.65} \times 100 = 136\ \% = 9.5$ ccm.	$\frac{7.4}{5.5} \times 100 = 134\ \% = 7.7$ ccm.	Fig. 42.

Fig. 43 shows the "zero-hour"-line. The curve reveals the remarkable fact that the intensity of respiration, as measured by gas-exchange, is an almost linear function of the temperature. As the CO_2-production in nearly all figures was at first a little too high, the "CO_2-line" is not quite straight.

Fig. 43 seems to suggest that the "zero-hour"-line deviates from this straight line at higher temperatures.

The temperature-quotients decrease even more than on the oil-media.

$$Q\frac{15°}{10°} = \frac{5.2}{2.8} = 1.85. \qquad Q\frac{20°}{15°} = \frac{7.7}{5.2} = 1.48.$$

$Q\frac{25°}{20°} = \frac{100}{77} = 1.30$ $\quad$ $Q\frac{30°}{25°} = \frac{122}{100} = 1.22.$ (fig. 43).

As in fig. 33 for the oil-media, the respiration-velocities on starch-media are given in fig. 44 for different temperatures after different periods.

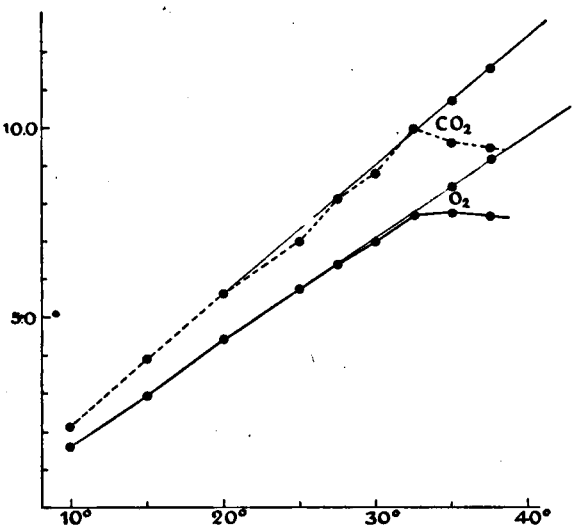

FIG. 43. Respiration on Starch-media (Bread) at Different Temperatures. (See fig. 32 and text).

§ 4. Discussion.

It follows from the experiments in this chapter that the range in temperature between which Phycomyces can live is smaller than is usually the case with other plants. At 40° Phycomyces already dies in a few hours. Bartetzko (6) and Lindner (55) moreover found that it will be frozen to death at a relatively high temperature.

All the experiments were repeated several times, especially as the greater part of the results were contrary to expec-

tation. The results were exactly the same, only sometimes the O_2-consumption at 30° on a linseed medium remained higher for a longer period, so that the respiratory quotients did not reassume their original value until after 4 or 5 hours, instead of 2 or 3 hours.

Though the differences are not large, the respiration-velocity in different experiments on the same culture medium is not always the same. This is chiefly due to the amount of water in the culture medium (p. 145). It may be asked whether this fact has no influence on the ratio of the respiration-velocities at different temperatures. This is not the case. Several experiments were carried out with very wet bread or linseed e.g. at the transition from 15° to 25° and the temperaturequotients were always found to be the same as with those cultures, where the quantity of water was normal.

In § 2 it was suggested that on linseed media the consumption of oil changed into a consumption of carbohydrates at high temperatures. An examination of the figures in § 2 might lead one to think that the initial values of the O_2-absorption were caused by some obstacle to the diffusion of oxygen. From chapter III, however, it may be seen that the oxygentension can be reduced to less than half the amount without affecting the respiration. It is therefore very improbable that the decrease in the oxygenconsumption on linseed at higher temperatures should be the result of a lack of oxygen.

In § 3 A it was moreover found that the O_2-absorption had directly adapted itself to the new temperature. If in § 2 the diffusion of oxygen had been limiting the respiration, this would also have been the case in § 3.

The supposition that Phycomyces is able to withstand high temperatures better on starch-media than on oil is also supported by the facts found in § 3 A. The respiration at equal temperatures does indeed decrease more rapidly

on oil-media than on starch-media. (compare fig. 31 and 42 for 37°5; fig. 30 and 41 for 35°). Further, in the long run respiration at 32°5 again becomes constant on starch-media, whereas on oil-media the decrease continues. On

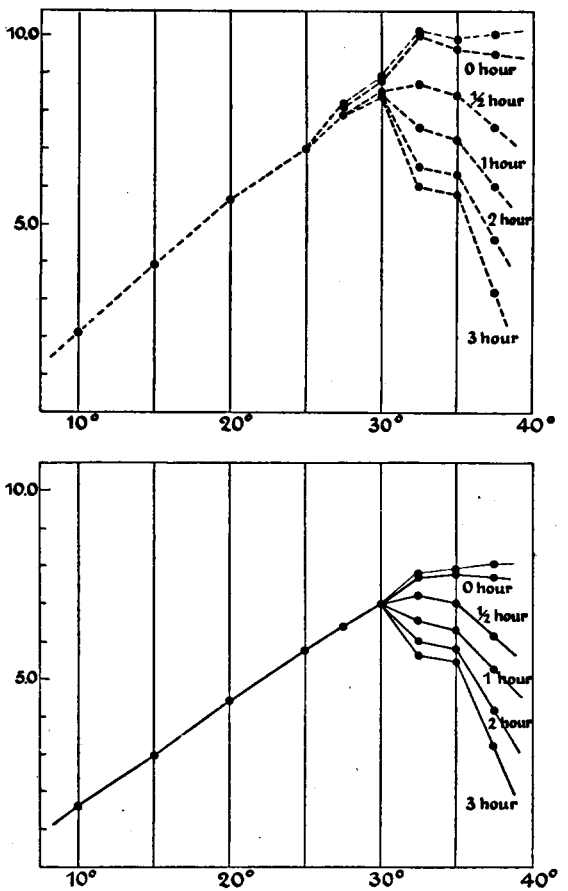

FIG. 44. Velocities of Respiration on Starch-media (Bread) at Different Temperatures after Different Periods. A. The Production of Carbondioxide. B. The Consumption of Oxygen.

bread the respiration is almost immediately constant at 30°, and hence, in contrast with the linseed media, a harmful influence cannot yet be spoken of.

It might be argued that as the respiration on linseed passed into a consumption of carbohydrates at higher temperatures, there would be no further reason for the respiration to decrease more rapidly than on the other carbohydrate medium bread. It must be borne in mind however, that at the initial temperature 25°, the respiration on linseed originally was more intensive than it can ever be on the same quantity of bread. Besides, in linseed there is a very small amount of carbohydrates.

In fig. 40, 41 and 42 the respiratory quotient approaches the value 1.00. At high temperatures where the fungus cannot consume fats, it is probably also unable to manufacture fat from carbohydrates.

In § 3 it has been mentioned that, in the case of the carbohydrate consumption, the "zero-hour"-line found by means of extrapolation seems to deviate at high temperatures from the straight line found for lower temperatures. The question might be asked whether this is not due to the method of extrapolation. I do not think it is. If the respiration proceeded along a straight line also at higher temperatures, the "zero-hour" values of CO_2-production and O_2- consumption would be higher. In fig. 41 and fig. 42 these calculated values are represented on the line indicating the zero-time i.e. the time at which the culture medium on an average assumed the new temperature (p. 183). For the O_2-absorption this value in fig. 41 at 35° is at the point Q. Of course it is possible that in the 10 minutes between the zero time and the beginning of the new experiments the curve may descend very rapidly. As far as the CO_2-production is concerned this cannot be denied. For the "O_2-curve" however, it seems to me impossible because in this case

twice the number of observations were carried out. From the very beginning of the experiment, moreover, the consumption of oxygen may be gathered from the rate of electrolyzation (galvanometer, fig. 1). So in fig. 41 the "O_2-curve" really runs to point P as has been plotted. It seems improbable that the curve should continue along the line P Q, as in this case there would be a sharp bend in the curve.

Apart from the behaviour at harmful temperatures, the unexpected fact is revealed that the intensity of respiration on starch-media (as measured by gas-exchange) at harmless temperatures is an almost linear function of the temperature.

Hille Ris Lambers (39) found the same function for the influence of temperature on protoplasmic streaming. Here the explanation is obvious as this process is chiefly a matter of viscosity of the protoplasm, a physical process, the velocity of which is a linear function of the temperature.

In the case of respiration various complicated processes take place, ending in a process of combustion. As the intensities of chemical processes are ordinarily exponential functions of the temperature, a curve convex to the temperature-axis was to be expected, at least for the lower temperatures, where probably diffusion processes not yet can be limiting factors. An explanation of the straight line is therefore difficult.

On the other hand it has been proved that the respiration adapts itself at once to new temperatures, and in my case not gradually as Kuyper and Rutgers supposed. Whenever the adaptation seems to be gradual (the absorption of O_2 on linseed media) there is a definite reason for this behaviour.

And finally oscillations in the respiration velocity, such as Kuyper (52) and Fernandes (31) described for seeds, did not occur in my experiments. It is difficult to say whether this is the result of the simpler objects used, or the consequence of more minute measurements.

SUMMARY.

No direct influence of light on respiration could be detected. Experiments were further carried out on the influence of other external factors on the respiration of *Phycomyces Blakesleeanus*.

By means of an exact determination of the respiratory quotients it was possible to find what kind of food the fungus used from a heterogeneous culture medium.

The grand period of respiration was determined at 25° C. on different quantities and different kinds of culture medium. The length of the sporangiophores is an index of their stage of development, corresponding to a specific point in the grand period.

As a medium rich in oil I used ground linseed, as a starch-medium bread. The respiratory quotient varies in different parts of the grand period of respiration. On linseed it rises from about 0.65 to about 0.75. On bread it becomes 1.00 in the long run but at the maximum of respiration it is about 1.20. It was made probable that this is due to the manufacture of fat from carbohydrates.

Phycomyces by preference takes fat when this is to be had; on fatty media the respiration is more intense than on starch-media. Proteins do not essentially participate in the respiration.

The fungus cannot live anaerobically, neither on oil-media nor on carbohydrates. A decrease in oxygen tension affects Phycomyces very soon. On starch-media some effect becomes noticeable in about 9 per cent. of oxygen, in 2 per cent. of oxygen the respiration is reduced to half the normal amount. Phycomyces can stand low oxygen tensions on oil-media better than on carbohydrate media, at least in so far as the CO_2-evolution is concerned. On oil-media there is a visible

influence in about 7 per cent. of oxygen, in 2 per cent. of oxygen the CO_2-evolution is reduced to about 70 per cent. of the normal amount.

On bread the respiratory quotient does not change in smaller percentages of oxygen, it increases on linseed media.

Pure oxygen has no influence on the CO_2-respiration, the O_2-consumption only increases by a small amount.

It was proved that respiration adapts itself at once to new temperatures and that a gradual adaptation to new temperatures as found by former investigators may be caused by experimental errors.

At all temperatures the respiration can be represented by flowing lines, oscillations in the respiration did not occur.

At harmful temperatures the consumption of fat changes into a consumption of carbohydrates. On linseed the "zero-hour"-line has therefore a peculiar course. Both the "O_2-curve" and the "CO_2-curve" are slightly convex towards the temperature axis, at high harmful temperatures, the "O_2-curve" becomes an optimum curve because the O_2 values approach the CO_2 values and therefore decrease.

The respiration on carbohydrate media is an almost linear function of the temperature. The "zero-hour" line deviates from the straight line at high temperatures.

The foregoing investigations were carried out in the Botanical Laboratory of the University of Utrecht.

This is the place to express my appreciation to Prof. Dr. F. A. F. C. Went for his kindly help, interest and criticism.

TABLES.

TABLE 3. (Fig. 2).

Small earthenware trough with bread.
Date: 29-4-'26; Time 23.00; Temp. in the vessel 25° C.

Date.	Time.	Number of hours	CO_2 evolved in ccm.	O_2 absorbed in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
30-4-'26	9.30-12.30	3	14.1	12.4	1.14	4.7	4.15	9.00 thin sp. ph. 1½ cm.
	12.30-15.30	3	17.5	15.3	1.14	5.8	5.1	
	15.30-18.30	3	21.15	18.4	1.15	7.05	6.1	
	18.30-21.30	3	24.45	21.2	1.15	8.15	7.1	21.00 thin sp. ph. 3 to 3½ cm.
30/4-1/5-'26	21.30- 9.30	12	—	—	—	—	—	thick sp. ph. 1 cm.
1-5-'26	9.30-11.30	2	22.55	19.8	1.14	11.3	9.9	9.00 few thin sp. ph. 4 cm.
	11.30-13.30	2	21.8	19.2	1.14	10.9	9.6	many thick sp. ph. 1½ to 2 cm.
	13.30-15.30	2	21.9	19.2	1.14	10.95	9.6	
	15.30-17.30	2	21.7	19.0	1.14	10.85	9.5	
	17.30-19.30	2	21.5	18.25	1.18	10.8	9.1	15.00 thick sp. ph. 2½ to 3 cm.
	19.30-21.30	2	21.5	18.45	1.17	10.8	9.2	
1/2-5-'26	21.30-11.30	14	—	—	—	—	—	22.00 some new thick sp. ph.
2-5-'26	11.30-13.30	2	18.5	16.75	1.10	9.25	8.4	
	13.30-18.30	5	—	—	—	—	—	
	18.30-20.30	2	16.6	15.0	1.11	8.3	7.5	11.00 thick sp. ph. of different
2/3-5-'26	20.30- 9.30	13	—	—	—	—	—	length.
3-5-'26	9.30-12.30	3	20.15	18.75	1.07	6.7	6.25	
	12.30-15.30	3	18.8	18.05	1.04	6.3	6.0	
	15.30-18.00	2½	15.6	14.5	1.08	6.2	5.8	
	18.00-21.00	3	17.2	16.5	1.04	5.7	5.5	
3/4-5-'26	21.00- 9.30	12½	—	—	—	—	—	
4-5-'26	9.30-11.30	2	11.0	10.65	1.04	5.5	5.3	
	11.30-14.30	3	16.0	—	—	5.3	—	
	14.30-17.30	3	15.5	15.5	1.00	5.2	5.2	
	17.30-21.30	4	19.75	19.4	1.02	4.9	4.85	
4/5-5-'26	21.30- 9.30	12	—	—	—	—	—	
5-5-'26	9.30-13.30	4	18.4	18.35	1.00	4.6	4.6	

TABLE 4.

Old culture on bread.
Placed in the respiration vessel in the evening of 11-5-'26.

Date.	Time.	Number of hours	CO_2 evolved in ccm.	O_2 absorbed in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
12-5-'26	9.00-15.00	6	12.4	12.2	1.02	2.1	2.0	
	15.00-21.00	6	16.35	16.25	1.01	2.7	2.7	
12/13-5-'26	21.00-10.00	37	—	—	—	—	—	
13-5-'26	10.00-16.30	6½	13.35	13.15	1.02	2.05	2.0	

TABLE 5 (Fig. 3).

2½ grm. bread in two thin layers.
Placed in the respiration vessel in the evening of 17-5-'26.

Date.	Time.	Number of hours	CO_2 evolved in ccm.	O_2 absorbed in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
18-5-'26	14.30-16.00	1½	8.7	7.3	1.20	5.8	4.85	10.00 sp. ph. 1 cm.
	16.00-17.30	1½	9.1	7.5	1.21	6.05	5.0	
	17.30-21.00	3½	—	—	—	—	—	
	21.00-22.30	1½	9.55	7.95	1.20	6.35	5.3	
	22.30-24.00	1½	9.3	7.7	1.21	6.2	5.15	
18/19-5-'26	24.00- 9.30	9½	—	—	—	—	—	
19-5-'26	9.30-11.00	1½	7.95	7.0	1.15	5.3	4.65	10.00 sp. ph. 3 cm.
	11.00-12.30	1½	7.7	6.75	1.14	5.15	4.5	
	12.30-16.00	3½	—	—	—	—	—	
	16.00-17.30	1½	6.85	6.3	1.10	4.55	4.15	
	17.30-19.00	1½	6.7	6.05	1.10	4.45	4.0	
19/20-5-'26	19.00- 9.30	14½	—	—	—	—	—	
20-5-'26	9.30-12.00	2½	8.9	8.4	1.06	3.55	3.35	10.00 sp. ph. 4 to 5 cm.
	12.00-14.30	2½	8.45	8.0	1.06	3.4	3.2	
	14.30-21.00	6½	—	—	—	—	—	
	21.00-23.30	2½	7.1	6.8	1.04	2.85	2.7	
20/21-5-'26	23.30- 9.30	10	—	—	—	—	—	
21-5-'26	9.30-12.00	2½	6.0	6.05	0.99	2.4	2.4	

TABLE 6 (Fig. 4).

Small earthenware trough with ground linseed.
Placed in the respiration vessel in the morning of 24-6-'26.

Date.	Time.	Number of hours	CO_2 evolved in ccm.	O_2 absorbed in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
24-6-'26	20.00-22.45	2¾	8.05	10.1	0.80	2.9	3.7	20.00 mycelium developed.
24/25-6-'26	22.45-10.00	11¼	—	—	—	—	—	
25-6-'26	10.00-11.45	1¾	14.6	22.25	0.66	8.35	12.7	10.00 sp. ph. ½ cm.
	11.45-13.30	1¾	15.4	23.45	0.65	8.8	13.4	
	13.30-15.00	1½	14.0	21.5	0.65	9.3	14.3	
	15.00-16.30	1½	14.75	22.1	0.66	9.8	14.75	
	16.30-22.00	5½	—	—	—	—	—	17.00 sp. ph. 1½ cm.
	22.00-23.30	1½	15.6	23.8	0.66	10.4	15.85	20.30 sp. ph. about 2 cm.
25/26-6-'26	23.30-10.00	10½	—	—	—	—	—	
26-6-'26	10.00-11.20	1⅓	14.6	22.1	0.66	10.95	16.6	10.00 many thick sp. ph. of about 3½ cm.
	11.20-12.40	1⅓	14.0	21.5	0.65	10.5	16.0	
	12.40-15.40	3	—	—	—	—	—	
	15.40-17.00	1⅓	13.8	20.4	0.67	10.35	15.3	
	17.00-18.20	1⅓	13.6	19.7	0.69	10.2	14.8	
	18.20-21.00	2⅔	—	—	—	—	—	18.30 sp. ph. 5 cm.
	21.00-22.20	1⅓	—	19.35	—	—	14.5	
26/27-6-'26	22.20-18.00	19⅔	—	—	—	—	—	
27-6-'26	18.00-19.30	1½	11.2	15.7	0.71	7.45	10.5	
	19.30-21.00	1½	10.8	15.05	0.72	7.2	10.0	
27/28-6-'26	21.00-14.30	17½	—	—	—	—	—	
28-6-'26	14.30-16.00	1½	8.75	11.75	0.74	5.8	7.8	
	16.00-17.30	1½	9.1	11.5	0.79	6.05	7.65	
	17.30-21.00	3½	—	—	—	—	—	
	21.00-22.30	1½	8.8	11.5	0.76	5.85	7.65	
	22.30-24.00	1½	8.8	11.5	0.76	5.85	7.65	
28/29-6-'26	24.00-10.15	10¼	—	—	—	—	—	
29-6-'26	10.15-12.15	2	11.35	14.75	0.77	5.65	7.4	
	12.15-14.15	2	11.55	14.85	0.78	5.8	7.45	

TABLE 7 (Fig. 5).

Small earthenware trough with linseed meal.
Placed in the respiration vessel at 14.00, 9-6-'26.

Date.	Time.	Number of hours	CO_2 evolved in ccm.	O_2 absorbed in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
9-6-'26	19.30-21.30	2	12.65	14.7	0.86	6.3	7.35	14.00 sp. ph. ¼ cm.
	21.30-23.30	2	13.15	16.05	0.82	6.55	8.0	23.00 many thick sp. ph. 1 cm.
9/10-6-'26	23.30- 9.45	10¼	—	—	—	—	—	
10-6-'26	9.45-11.35	1⅚	15.8	20.1	0.79	8.6	11.0	9.30 sp. ph. 2 cm.
	11.35-13.25	1⅚	15.9	20.6	0.77	8.65	11.2	
	13.25-15.15	1⅚	16.2	21.0	0.77	8.8	11.45	
	15.15-16.00	¾	—	—	—	—	—	
	16.00-17.30	1½	14.3	18.9	0.76	9.6	12.6	
	17.30-19.00	1½	14.9	19.25	0.77	9.9	12.8	20.00 sp. ph. 3½ cm.
10/11-6-'26	19.00-10.15	15¼	—	—	—	—	—	
11-6-'26	10.15-11.45	1½	16.0	20.75	0.77	10.7	13.8	10.00 sp. ph. 5 cm.
	11.45-13.15	1½	15.5	20.55	0.76	10.3	13.7	
	13.15-15.30	2¼	—	—	—	—	—	
	15.30-17.00	1½	15.5	20.1	0.77	10.3	13.4	
	17.00-18.30	1½	15.4	20.0	0.77	10.3	13.3	
11/12-6-'26	18.30- 9.30	15	—	—	—	—	—	
12-6-'26	9.30-11.00	1½	12.7	15.9	0.80	8.5	10.6	
	11.00-12.30	1½	12.2	15.1	0.80	8.1	10.1	
	12.30-16.45	4¼	—	—	—	—	—	
	16.45-18.15	1½	11.8	14.15	0.83	7.9	9.4	
	18.15-19.45	1½	11.35	13.75	0.83	7.6	9.2	
12/14-6-'26	19.45-11.30	39¾	—	—	—	—	—	
14-6-'26	11.30-14.30	3	13.1	15.4	0.85	4.4	5.1	
	14.30-17.30	3	12.65	15.1	0.84	4.2	5.0	
	17.30-20.30	3	12.2	14.75	0.82	4.1	4.9	
14/15-6-'26	20.30-10.15	13¾	—	—	—	—	—	
15-6-'26	10.15-13.15	3	11.0	13.3	0.83	3.65	4.4	
	13.15-16.30	3¼	11.8	13.85	0.85	3.6	4.3	

TABLE 8 (Fig. 6).

Small earthenware trough with linseed-meal containing about 2½ % fat. Placed in the respiration vessel at 11.00, 5-7-'26.

Date.	Time.	Number of hours	CO_2 evolved in ccm.	O_2 absorbed in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
5-7-'26	21.00-22.30	1½	8.35	9.1	0.92	5.6	6.1	
	22.30- 0.00	1½	8.8	9.4	0.94	5.8	6.25	21.00 sp. ph. ¾ cm.
5/6-7-'26	0.00-10.00	10	—	—	—	—	—	
6-7-'26	10.00-11.30	1½	10.8	11.1	0.97	7.2	7.4	
	11.30-13.00	1½	11.2	11.4	0.98	7.45	7.6	9.30 sp. ph. rather thin 3 cm.
	13.00-14.00	1	—	—	—	—	—	
	14.00-15.30	1½	11.2	11.4	0.98	7.45	7.6	
	15.30-17.00	1½	10.8	11.35	0.95	7.2	7.55	
6/7-7-'26	17.00-10.30	17½	—	—	—	—	—	21.00 sp. ph. 5 cm. rather thin.
7-7-'26	10.30-12.30	2	9.5	10.45	0.91	4.75	5.25	
	12.30-14.30	2	8.95	9.75	0.92	4.5	4.9	
	14.30-16.30	2	8.5	9.2	0.92	4.25	4.6	

TABLE 9 (Fig. 7).

Small earthenware trough with linseed-meal totally deprived of fat. Placed in the respiration vessel at 9.00, 12-7-'26.

Date.	Time.	Number of hours	CO_2 evolved in ccm.	O_2 absorbed in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks
12-7-'26	13.30-16.00	2½	11.1	11.15	1.00	4.45	4.5	10.00 sp. ph. ½ cm.
	16.00-18.30	2½	11.8	11.25	1.05	4.7	4.5	14.00 sp. ph. about 1 cm. thinner ones longer.
	18.30-21.00	2½	11.7	11.4	1.03	4.7	4.55	21.00 thick sp. ph. nearly 2 cm., thin sp. ph. 3 cm.
12/13-7-'26	21.00- 9.45	12¾	—	—	—	—	—	9.00 thick sp. ph. about 3 cm.,
13-7-'26	9.45-11.45	2	9.0	9.15	0.99	4.5	4.6	thin sp. ph. 5 cm.
	11.45-14.45	3	12.35	12.75	0.97	4.1	4.25	
	14.45-16.45	2	7.9	8.0	0.99	4.0	4.0	
13/14-7-'26	16.45-10.45	18	—	—	—	—	—	
14-7-'26	10.45-14.45	4	9.35	9.6	0.97	2.35	2.4	
	14.45-18.45	4	8.55	8.8	0.97	2.1	2.2	
	18.45-22.45	4	8.25	8.4	0.98	2.05	2.1	
14/15-7-'26	22.45- 9.45	11	—	—	—	—	—	
15-7-'26	9.45-13.45	4	7.5	7.7	0.97	1.9	1.9	

TABLE 10.

Small earthenware trough with "milkbread".
Placed in the respiration vessel at 11.00, 5-8-'26.

Date.	Time.	Number of hours	CO_2 evolved in ccm.	O_2 absorbed in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
5-8-'26	14.45-19.45	5	10.8	10.5	1.03	2.2	2.1	
	19.45-23.15	3½	11.7	11.15	1.05	3.35	3.2	
5/6-8-'26	23.15- 9.45	10½	—	—	—	—	—	
6-8-'26	9.45-12.15	2½	18.8	17.1	1.10	7.5	6.8	9.30 thin sp. ph. 2½ cm.
	12.15-14.45	2½	19.5	18.0	1.08	7.8	7.2	thick sp. ph. ½ cm.?
	14.45-19.00	4¼	—	—	—	—	—	
	19.00-21.00	2	18.0	16.5	1.09	9.0	8.3	19.00 thin sp. ph. 3 to 3½ cm.
	21.00-23.00	2	18.3	16.85	1.09	9.1	8.4	thick sp. ph. 1 to 1½ cm.
6/7-8-'26	23.00-12.00	13	—	—	—	—	—	
7-8-'26	12.00-14.00	2	18.8	17.5	1.07	9.4	8.8	11.30 thick sp. ph. about 2½ cm.
	14.00-16.00	2	18.65	17.5	1.06	9.3	8.8	
	16.00-20.30	4½	—	—	—	—	—	
	20.30-22.30	2	18.55	17.8	1.05	9.3	8.9	
7/8-8-'26	22.30-18.30	20	—	—	—	—	—	
8-8-'26	18.30-20.30	2	14.5	14.7	0.99	7.3	7.4	
	20.30-22.30	2	14.5	14.7	0.99	7.3	7.4	

TABLE 11. (Fig. 8).

2½ grm. ground linseed in two thin layers.
Placed in the respiration vessel at 9.00, 13-12-'26.

Date.	Time.	Number of hours	CO_2 evolved in ccm.	O_2 absorbed in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
13-12-'26	12.00-14.00	2	13.25	17.2	0.77	6.6	8.6	12.00 mycelium grown over the whole surface; no sp. ph.
	14.00-16.00	2	14.65	19.75	0.74	7.3	9.9	
	16.00-17.30	1½	12.0	16.4	0.73	8.0	10.9	
	17.30-20.30	3	—	—	—	—	—	
	20.30-22.00	1½	13.95	20.15	0.69	9.3	13.4	20.00 no sp. ph.; (only at the 4 inoculated places thin ones.)
	22.00-23.30	1½	13.8	20.3	0.68	9.2	13.5	
13/14-12-'26	23.30- 9.00	9½	—	—	—	—	—	
14-12-'26	9.00-10.30	1½	12.8	19.25	0.67	8.5	12.8	9.00 thick sp. ph. ½ cm.?
	10.30-12.00	1½	12.5	18.3	0.68	8.3	12.3	
	12.00-13.30	1½	—	17.9	—	—	11.9	13.00 sp. ph. about 1¼ cm.
	13.30-16.30	3	—	—	—	—	—	
	16.30-18.00	1½	11.55	17.2	0.67	7.7	11.5	
	18.00-19.30	1½	10.8	16.05	0.67	7.2	10.7	
	19.30-23.30	4	—	—	—	—	—	19.30 sp. ph. about 2 cm.
	23.30- 1.00	1½	9.1	13.9	0.66	6.1	9.3	
14/15-12-'26	1.00- 9.30	8½	—	—	—	—	—	1.00 sp. ph. 2½ to 3 cm.
15-12-'26	9.30-11.30	2	9.65	14.65	0.66	4.8	7.3	9.00 sp. ph. nearly 4 cm.
	11.30-14.00	2½	11.75	17.9	0.66	4.7	7.15	
	14.00-15.30	1½	—	—	—	—	—	
	15.30-18.00	2½	10.4	15.75	0.66	4.15	6.3	
	18.00-20.00	2	8.05	11.65	0.69	4.0	5.8	
	20.00-22.00	2	7.7	10.6	0.72	3.85	5.3	
15/16-12-'26	22.00-10.00	12	—	—	—	—	—	
16-12-'26	10.00-12.00	2	7.4	10.1	0.73	3.7	5.05	

TABLE 12 (Fig. 9).

2½ grm. bread plus 10 % peptone in two layers.
In the respiration vessel at 10.00, 31-5-'26.

Date.	Time.	Number of hours	CO_2 evolved in ccm.	O_2 absorbed in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
31-5-'26	15.30-20.00	4½	18.1	15.75	1.15	4.0	3.5	11.00 sp. ph. visible?
	20.00-23.00	3	15.4	13.0	1.19	5.1	4.3	23.00 sp. ph. 1 cm., thick.
31/5-1/6-'26	23.00-10.00	11	—	—	—	—	—	
1-6-'26	10.00-12.30	2½	15.9	13.1	1.22	6.35	5.2	9.00 sp. ph. 1¾ cm.
	12.30-15.00	2½	15.25	12.45	1.22	6.1	5.0	
	15.00-17.45	2¾	15.9	13.15	1.21	5.8	4.8	
	17.45-20.30	2¾	14.3	12.05	1.19	5.2	4.4	20.30 sp. ph. 2 to 2½ cm.
1/2-6-'26	20.30-10.30	14	—	—	—	—	—	
2-6-'26	10.30-14.30	4	16.2	15.2	1.07	4.05	3.8	10.00 sp. ph. fully 3 cm.
	14.30-18.00	3½	13.25	12.8	1.04	3.8	3.7	
	18.00-22.00	4	13.4	12.9	1.04	3.35	3.2	
2/3-6-'26	22.00-10.00	12	—	—	—	—	—	
3-6-'26	10.00-14.00	4	13.05	13.0	1.00	3.25	3.25	
	14.00-18.00	4	12.6	12.1	1.04	3.15	3.0	
	18.00-22.15	4¼	12.0	11.6	1.03	2.8	2.7	

TABLE 13.

2½ grm. bread, mixed with much water, in two thin layers.
In the respiration vessel at 9.00, 2-5-'27.

Date	Time.	Number of hours	CO_2 evolved in ccm.	O_2 absorbed in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
2-5-'27	13.30-18.00	4½	7.5	7.15	1.05	1.65	1.6	
	18.00-21.00	3	7.45	6.95	1.07	2.5	2.3	
	21.00-23.00	2	6.0	5.4	1.10	3.0	2.7	
	23.00- 0.30	1½	5.1	4.55	1.12	3.4	3.0	0.00 sp. ph. 1 cm., thin.
2/3-5-'27	0.30- 9.00	8½	—	—	—	—	—	
3-5-'27	9.00-11.00	2	9.0	7.65	1.18	4.5	3.8	9.00 thick sp. ph. ½ cm.
	11.00-13.00	2	8.9	7.65	1.17	4.45	3.8	
	13.00-15.00	2	8.75	7.5	1.17	4.35	3.75	
	15.00-19.00	4	—	—	—	—	—	16.00 thick sp. ph. 1 cm.
	19.00-21.00	2	8.05	7.05	1.14	4.0	3.5	
	21.00-23.00	2	7.7	6.8	1.14	3.85	3.4	
3/4-5-'27	23.00- 9.00	10	—	—	—	—	—	23.00 sp. ph. nearly 2 cm.
4-5-'27	9.00-11.30	2½	8.05	7.35	1.10	3.2	2.95	
	11.30-14.00	2½	7.75	7.25	1.07	3.1	2.9	
	14.00-19.00	5	—	—	—	—	—	
	19.00-21.30	2½	7.0	6.75	1.04	2.8	2.7	

TABLE 14.

Culture of *Phycomyces* on a small trough with bread. Sporangiophores about 6 c.m.

Respiration vessel 325 ccm. Suction velocity 2 l. per hour.

From	10.00 to 11.00	→ 6.2	ccm.	CO_2	
„	11.00 „ 12.00	→ 6.5	„	„	
„	12.00 „ 13.00	→ 6.5	„	„	
„	13.00 „ 14.00	→ 6.5	„	„	← daylight.
„	14.00 „ 15.00	→ 6.8	„	„	
„	15.00 „ 16.00	→ 6.8	„	„	← light of 800 M.C.
„	16.00 „ 17.00	→ 6.4	„	„	
„	17.00 „ 18.00	→ 6.8	„	„	
„	18.00 „ 19.00	→ 6.7	„	„	
„	19.00 „ 20.00	→ 6.4	„	„	
„	20.00 „ 21.00	→ 6.4	„	„	← „ „ 800 „
„	21.00 „ 22.00	→ 6.4	„	„	← „ „ 800 „
„	22.00 „ 23.00	→ 6.4	„	„	

TABLE 15.

Culture of *Phycomyces* on a small trough with bread. The second generation of sporangiophores has stopped growing.

Respiration vessel 325 ccm. Suction velocity 2 l. per hour.

From	13.00 to 14.00	→ 4.9	ccm.	CO_2	
„	14.00 „ 15.00	→ 5.1	„	„	← light of 6000 M.C.
„	15.00 „ 16.00	→ 5.1	„	„	
„	16.00 „ 17.00	→ 5.1	„	„	
„	17.00 „ 18.00	→ 5.1	„	„	
„	18.20 „ 19.20	→ 5.0	„	„	
„	19.20 „ 20.20	→ 5.2	„	„	
					Sp. ph. removed.
„	20.40 „ 21.40	→ 4.6	„	„	
„	21.40 „ 22.40	→ 4.5	„	„	

TABLE 16.

Culture of *Phycomyces* on a small trough with bread. Sporangiophores of different lengths.

Respiration vessel 325 ccm. Suction velocity 2 l. per hour.

From	11.00 to 12.00	⟶ 4.8	ccm.	CO_2		
„	12.00 „ 13.00	⟶ 4.6	„	„		
„	13.00 „ 14.00	⟶ 4.4	„	„		
„	14.00 „ 15.00	⟶ 4.6	„	„		
„	15.00 „ 16.00	⟶ 4.8	„	„	⟵ light of	800 M.C.
„	16.00 „ 17.00	⟶ 4.7	„	„	⟵ „ „	800 „
„	17.00 „ 18.00	⟶ 4.6	„	„	⟵ „ „	800 „
„	18.00 „ 19.00	⟶ 4.8	„	„		
					Sp. ph. removed.	
„	19.20 „ 20.20	⟶ 3.9	„	„		
„	20.20 „ 21.20	⟶ 3.9	„	„		
„	21.20 „ 22.20	⟶ 3.9	„	„		
„	22.20 „ 23.20	⟶ 3.8	„	„		

TABLE 17.

6 pieces of carrot, lenght 5 ccm., overgrown with mycelium of *Polyporus destructor*.

Respiration vessel 600 ccm. Suction velocity 5 l. per hour.

From	12.00 to 14.00	⟶ 8.8	ccm.	CO_2	⟵ daylight.	
„	14.00 „ 16.00	⟶ 8.8	„	„	⟵ „	
„	16.00 „ 18.00	⟶ 8.4	„	„		
„	18.00 „ 20.00	⟶ 8.6	„	„		
„	20.00 „ 22.00	⟶ 8.4	„	„	⟵ light of	800 M.C.

TABLE 18.

6 pieces of carrot, length 5 c.m., overgrown with mycelium of *Polyporus destructor*.

Respiration vessel 600 ccm. Suction velocity $5^1/_2$ per hour.

From	9.00 to 11.00	⟶ 7.7	ccm.	CO_2		
„	11.00 „ 13.00	⟶ 8.0	„	„		
„	13.00 „ 15.00	⟶ 8.2	„	„		
„	15.00 „ 17.00	⟶ 7.8	„	„	⟵ light of	800 M.C.
„	17.00 „ 19.00	⟶ 8.0	„	„	⟵ „ „	800 „
„	19.00 „ 21.00	⟶ 7.8	„	„		
„	21.00 „ 23.00	⟶ 8.1	„	„		

TABLE 19.

6 pieces of carrot, length 5 c.m., overgrown with mycelium of *Polyporus destructor*.

Respiration vessel 600 ccm. Suction velocity 4½ l. per hour.

From	8.00 to 10.00	⟶	8.6	ccm.	CO_2		
„	10.00 „ 12.00	⟶	9.0	„	„		
„	12.00 „ 14.00	⟶	9.1	„	„	⟵	daylight.
„	14.00 „ 16.00	⟶	8.6	„	„		
„	16.00 „ 18.00	⟶	8.6	„	„		
„	18.00 „ 20.00	⟶	8.4	„	„	⟵	light of 6000 M.C.
„	20.00 „ 22.00	⟶	8.5	„	„	⟵	„ „ 6000 „

TABLE 20.

6 small specimens of *Lactarius rufus*.

Respiration vessel 325 ccm. Suction velocity 2½ l. per hour.

From	10.00 to 12.00	⟶	7.6	ccm.	CO_2		
„	12.00 „ 14.00	⟶	7.5	„	„		
„	14.00 „ 16.00	⟶	7.4	„	„	⟵	light of 6000 M.C.
„	16.00 „ 18.00	⟶	7.2	„	„		
„	18.00 „ 20.00	⟶	6.8	„	„		
„	20.00 „ 22.00	⟶	6.85	„	„	⟵	„ „ 800 „
„	22.00 „ 0.00	⟶	6.85	„	„		

TABLE 21.

20 very small specimens of *Laccaria amethysta*.

Respiration vessel 325 ccm. Suction velocity 3 l. per hour.

From	10.00 to 12.00	⟶	4.5	ccm.	CO_2		
„	12.00 „ 14.00	⟶	4.35	„	„		
„	14.00 „ 16.00	⟶	4.35	„	„	⟵	light of 800 M.C.
„	16.00 „ 18.00	⟶	4.35	„	„		
„	18.00 „ 20.00	⟶	4.5	„	„	⟵	„ „ 6000 „
„	20.00 „ 22.00	⟶	4.35	„	„		

TABLE 22.

Small trough with bread.
22-10-'26 at 10.00 in the respiration vessel.
Thick sp. ph. then 1½ c.m.

Date.	Time.	Number of hours	ccm. CO_2 given off	ccm. CO_2 given off per hour	
22—10—'26	17.00—20.00	3	24.9	8.3	air
	20.00—23.00	3	26.8	8.9	
22/23—10—'26	23.00— 9.30	10½	—	—	
23—10—'26	9.30—11.30	2	21.1	10.55	
	11.30—13.30	2	19.1	9.55	
	13.30—15.30	2	18.5	9.25	
	15.30—16.00	½	—	—	oxygen
	16.00—18.00	2	16.4	8.2	
	18.00—20.00	2	15.1	7.55	
	20.00—22.00	2	14.0	7.0	
23/24—10—'26	22.00—12.00	14	—	—	
24—10—'26	12.00—14.30	2½	11.0	4.4	
	14.30—17.30	3	12.7	4.2	

TABLE 23 (Fig. 11).

Bread in two thin layers.
1-10-'26 at 16.00 in the respiration vessel.

Date.	Time.	Number of hours	ccm. CO_2 evolved	ccm. O_2 absorbed	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
1-10-'26	20.00-23.00	3	9.2	8.35	1.10	3.1	2.8	
1/2-10-'26	23.00- 9.30	10½	—	—	—	—	—	
2-10-'26	9.30-11.45	2¼	11.0	9.0	1.22	4.9	4.0	9.30 thick sp. ph. ½ cm. (thin sp. ph. 2 cm.)
	11.45-14.00	2¼	10.4	8.6	1.21	4.6	3.8	
	14.00-16.15	2¼	10.0	8.1	1.23	4.4	3.6	
	16.15-16.35	¼		Filled with oxygen.				
	16.35-18.50	2¼	9.2	8.25	1.12	4.1	3.7	
	18.50-21.05	2¼	8.6	7.75	1.11	3.8	3.4	
	21.05-23.20	2¼	8.1	7.2	1.13	3.6	3.2	

TABLE 24

Bread in two thin layers. Sp. ph. grown out.
In the respiration vessel the evening before.

Time.	Number of hours	ccm. CO_2 evolved	ccm. O_2 absorbed	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour
10.00—13.00	3	7.25	6.35	1.14	2.4	2.1
13.00—16.00	3	6.8	6.0	1.13	2.3	2.0
16.00—16.20	⅓	Filled with oxygen.				
16.20—19.20	3	6.05	5.7	1.04	2.0	1.9
19.20—22.20	3	5.2	5.1	1.02	1.75	1.7

TABLE 25.

Small trough with ground linseed.
Thick sp. ph. 2 to 2½ c.m.

Date.	Time	Number of hours	ccm. CO_2 totally evolved	ccm. CO_2 evolved per hour	
2—11—'26	13.45—15.30	1¾	16.1	9.2	air
	15.30—17.15	1¾	20.0	10.0	
	17.15—17.45	½	—	—	oxygen
	17.45—19.30	1¾	19.6	11.2	
	19.30—21.15	1¾	20.8	11.9	
	21.15—23.00	1¾	21.5	12.3	
2/3—11—'26	23.00—13.30	14½	—	—	
3—11—'26	13.30—15.30	2	22.8	11.4	
	15.30—17.30	2	22.5	11.25	
	17.30—19.30	2	21.9	10.95	
	19.30—21.30	2	21.35	10.7	
3/4—11—'26	21.30—11.30	14	—	—	
4—11—'26	11.30—13.30	2	18.2	9.1	
	13.30—15.30	2	17.8	8.9	
	15.30—17.30	2	17.3	8.65	
	17.30—19.30	2	17.0	8.5	
	19.30—21.30	2	16.65	8.3	
4/5—11—'26	21.30—13.30	16	—	—	
5—11—'26	13.30—15.30	2	14.0	7.0	
	15.30—17.30	2	13.6	6.8	
	17.30—19.30	2	13.0	6.5	

TABLE 26 (Fig. 12).
Ground linseed in two layers.
No. sp. ph.

Time.	Number of hours	ccm. CO_2 evolved	ccm. O_2 absorbed	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour
10.00—11.50	1⅝	11.5	17.25	0.67	6.3	9.4
11.50—13.40	1⅝	11.1	17.0	0.66	6.05	9.25
13.40—14.25	¾	Filled with oxygen.				
14.25—16.15	1⅝	11.0	17.2	0.64	6.0	9.4
16.15—18.05	1⅝	11.0	16.55	0.66	6.0	9.0

TABLE 27.
2½ grm. ground linseed in two layers.
No. sp. ph.

Time.	Number of hours	ccm. CO_2 per hour	ccm. O_2 per hour	$\frac{CO_2}{O_2}$
10.00—11.00	1	8.55	13.1	0.65
11.00—12.00	1	8.25	12.9	0.64
12.00—12.40	⅔	Filled with oxygen.		
12.40—13.40	1	8.15	13.15	0.62
13.40—14.40	1	7.95	12.85	0.62
14.40—15.20	⅔	Filled with air.		
15.20—16.20	1	7.55	11.9	0.64
16.20—17.20	1	7.45	11.9	0.63

TABLE 29.
2½ grm. bread in two layers.
Sp. ph. about 1½ c.m.

Time.	Number of hours	ccm. CO_2 evolved per hour	
10.00—11.00	1	8.4	air.
11.00—12.00	1	8.4	
12.00—12.30	½	—	6½ % O_2
12.30—13.30	1	6.95	
13.30—14.30	1	7.15	
14.30—15.00	½	—	air.
15.00—16.00	1	8.5	
16.00—17.00	1	8.4	

TABLE 30 (Fig. 13).

Small trough with bread.
Thick sp. ph. 2 to 2½ c.m

Date.	Time.	Number of hours	ccm. CO_2 totally evolved	ccm. CO_2 evolved per hour	
9—11—'26	10.45—12.45	2	20.5	10.25	air
	12.45—14.45	2	21.5	10.75	
	14.45—16.45	2	22.0	11.0	
	16.45—17.25	⅔	—	—	2% O_2
	17.25—19.25	2	10.3	5.15	
	19.25—21.25	2	10.3	5.15	
	21.25—23.25	2	10.8	5.4	
9/10—11—'26	23.25— 9.25	10	—	—	
10—11—'26	9.25—11.25	2	12.6	6.3	
	11.25—13.55	2½	15.9	6.4	
	13.55—16.25	2½	15.9	6.4	

TABLE 31 (Fig. 14).

Small trough with bread. Young culture.
Thick sp. ph. ½ c.m.

Time.	Number of hours	ccm. CO_2 totally evolved	ccm. CO_2 evolved per hour	
11.15—13.15	2	7.3	3.65	air.
13.15—15.30	2¼	9.6	4.3	
15.30—17.30	2	9.9	4.95	
17.30—17.45	¼	—	—	2% O_2
17.45—19.45	2	6.7	3.35	
19.45—21.45	2	7.0	3.5	
21.45—23.45	2	7.0	3.5	

TABLE 32. (Fig. 15).
Small trough with bread. Young culture.
Thick sp. ph. 1 c.m.

Time.	Number of hours	ccm. CO_2 totally evolved	ccm. CO_2 evolved per hour	
10.30—12.30	2	11.4	5.7	air.
12.30—14.30	2	12.75	6.375	
14.30—16.30	2	14.5	7.25	
16.30—17.00	½	—	—	$1\frac{1}{2}$ % O_2
17.00—19.00	2	7.25	3.6	
19.00—21.00	2	7.1	3.55	

TABLE 33 (Fig. 16).
Small trough with bread.
Thick sp. ph. 2 to 2½ c.m.

Time.	Number of hours	ccm. CO_2 totally evolved	ccm. CO_2 evolved per hour	
9.00—11.00	2	21.2	10.6	air.
11.00—13.00	2	22.25	11.1	
13.00—15.00	2	23.3	11.65	
15.00—15.30	½	—	—	trace of oxygen.
15.30—17.30	2	6.95	3.5	
17.30—19.30	2	5.0	2.5	
19.30—22.00	2½	3.4	1.35	

TABLE 34.
Small trough with bread.
Sp. ph. 3 c.m.

Time.	Number of hours	ccm. CO_2 totally evolved	ccm. CO_2 evolved per hour	
13.30—15.30	2	21.7	10.85	air.
15.30—17.30	2	22.1	11.05	
17.30—19.00	1½	—	—	hydrogen.
19.00—21.00	2	0.75	0.4	
21.00—23.00	2	0.05	0.025	

The next day no CO_2 was given off.

TABLE 35.

Small trough with bread.
Sp. ph. 2½ c.m.

Time.	Number of hours	ccm. CO_2 totally evolved	ccm. CO_2 evolved per hour	
10.00—12.00	2	17.8	8.9	air.
12.00—14.00	2	17.8	8.9	
14.00—14.30	½	—	—	hydrogen.
14.30—16.30	2	1.85	0.9	
16.30—18.30	2	0.25	0.1	

TABLE 36 (Fig. 17).

Bread in 2 thin layers

Time.	Number of hours	ccm. CO_2 evolved	ccm. O_2 absorbed	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.00—12.00	2	8.6	7.15	1.20	4.3	3.6	
12.00—12.40	⅔	Filled with gas containing 8.5 % oxygen.					
12.40—14.40	2	8.4	7.05	1.19	4.2	3.5	
14.40—16.40	2	8.85	7.5	1.18	4.4	3.75	
16.40—17.10	½	Filled with air.					
17.10—19.10	2	9.8	8.4	1.17	4.9	4.2	19.00 thick sp. ph. ½ to 1 cm.

TABLE 37 (Fig. 18).

Bread in 2 thin layers.

Time.	Number of hours	ccm. CO_2 evolved	ccm. O_2 absorbed	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
12.00—14.00	2	8.9	7.5	1.19	4.5	3.8	
14.00—15.15	1¼	Filled with gas containing 3 % oxygen.					
15.15—17.15	2	6.0	4.85	1.23	3.0	2.4	
17.15—19.15	2	6.25	5.0	1.25	3.1	2.5	
19.15—20.15	1	Filled with air.					
20.15—22.15	2	10.05	8.25	1.21	5.0	4.1	20.30 thick sp. ph. 1 cm.

TABLE 38.

Bread in 2 thin layers.

Time.	Number of hours	ccm. CO_2 evolved	ccm. O_2 absorbed	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour.	ccm. O_2 per hour	Remarks.
10.00—12.00	2	8.8	6.9	1.26	4.4	3.45	
12.00—14.00	2	9.3	7.25	1.28	4.7	3.6	
14.00—14.45	¾	Filled with gas containing 3 % oxygen.					
14.45—16.45	2	6.65	5.2	1.28	3.3	2.6	
16.45—18.45	2	7.4	5.9	1.26	3.7	2.95	
18.45—19.15	½	Filled with air.					
19.15—21.00	1¾	9.2	7.4	1.24	5.25	4.25	21.00 thick sp. ph. 1 cm.

TABLE 39.

2½ grm. ground linseed in 2 layers.

No. sp. ph.

Time.	Number of hours	ccm. CO_2 per hour	
10.00—11.00	1	12.8	air.
11.00—12.00	1	13.0	
12.00—12.30	½	—	6 % oxygen.
12.30—13.30	1	12.0	
13.30—14.30	1	12.0	
14.30—15.00	½	—	air.
15.00—16.00	1	12.8	
16.00—17.00	1	13.0	

TABLE 40 (Fig. 19).

2½ grm. ground linseed in 2 layers.

No. sp. ph.

Time.	Number of hours	ccm. CO_2 evolved	ccm. CO_2 per hour	
10.00—11.30	1½	16.4	11.0	air.
11.30—13.00	1½	16.8	11.2	
13.00—14.30	1½	16.7	11.1	
14.30—15.00	½	—	—	2½ % oxygen.
15.00—16.30	1½	12.3	8.2	
16.30—18.00	1½	12.3	8.2	
18.00—19.30	1½	12.0	8.0	

TABLE 41.

2½ grm. ground linseed in 2 layers.
No. sp. ph.

Time.	Number of hours	ccm. CO_2 evoled	ccm O_2 absorbed	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.00—11.00	1	—	—	0.70	9.3	13.25	
11.00—12.00	1	—	—	0.69	9.05	13.1	
12.00—12.45	¾	Filled with gas containing 4½ % oxygen.					
12.45—14.30	1¾	13.4	17.3	0.77	7.65	9.9	
14.30—15.30	1	—	—	0.75	7.7	10.25	
15.30—16.00	½	Filled with air.					
16.00—17.00	1	—	—	0.66	8.65	13.05	
17.00—18.00	1	—	—	0.65	8.3	12.8	

TABLE 42 (Fig. 20).

2½ grm. ground linseed in 2 layers.
No. sp. ph.

Time.	Number of hours	ccm. CO_2 per hour	ccm. O_2 per hour	$\frac{CO_2}{O_2}$	Remarks.
10.00—11.00	1	10.2	14.75	0.69	
11.00—12.00	1	10.05	14.7	0.68	
12.00—12.40	⅔	Filled with gas containing 3 % oxygen.			
12.40—13.40	1	8.05	10.45	0.77	
13.40—14.40	1	8.15	10.55	0.77	
14.40—15.40	1	8.05	10.65	0.76	
15.40—16.20	⅔	Filled with air.			
16.20—17.20	1	9.55	14.6	0.66	
17.20—18.20	1	9.45	14.55	0.65	

TABLE 43.

2½ grm. ground linseed in 2 layers.

No sp. ph.

Time.	Number of hours	ccm. CO_2 per hour	ccm. O_2 per hour	$\frac{CO_2}{O_2}$	Remarks.
10.00—11.00	1	9.25	13.8	0.67	
11.00—12.00	1	9.0	13.5	0.67	
12.00—12.30	½	Filled with gas containing 3 % oxygen.			
12.30—13.30	1	7.3	9.7	0.75	
13.30—14.30	1	7.1	9.6	0.74	
14.30—15.00	½	Filled with air.			
15.00—16.00	1	8.2	12.55	0.65	
16.00—17.00	1	8.05	12.3	0.65	

TABLE 44.

Small trough with ground linseed.

Sp. ph. 2 c.m.

Time.	Number of hours	ccm. CO_2 evolved	ccm. CO_2 per hour	
10.30—12.30	2	14.15	7.1	air.
12.30—14.30	2	16.0	8.0	
14.30—16.30	2	16.9	8.45	
16.30—17.00	½	—	—	about 1% oxygen.
17.00—19.00	2	5.9	2.95	
19.00—21.00	2	4.75	2.35	
21.00—23.00	2	4.15	2.05	

TABLE 45.

Small trough with ground linseed.

Sp. ph. 3½ c.m.

Time.	Number of hours	ccm. CO_2 evolved	ccm. CO_2 per hour	
15.00—16.15	1¼	14.5	11.6	air.
16.15—17.30	1¼	15.0	12.0	
17.30—18.00	½	—	—	hydrogen.
18.00—20.00	2	2.5	1.25	
20.00—22.00	2	0.3	0.15	

TABLE 46.

2½ gr. bread, mixed with much water, in 2 layers. Sp. ph. 2 c.m.

Time.	Number of hours	ccm. CO_2 evolved	ccm. CO_2 per hour	
10.00—12.00	2	8.9	4.45	air.
12.00—14.00	2	8.75	4.35	
14.00—14.30	½	—	—	oxygen.
14.30—16.00	1½	6.15	4.1	
16.00—17.30	1½	5.6	3.75	

TABLE 47 (Fig. 21).

From 25° to 10° C. Linseed medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
9.15—10.15	1	—	—	0.66	9.3	14.2	
10.15—11.15	1	—	—	0.67	9.4	14.1	
11.15—12.15	1	—	—	0.66	9.2	13.9	
12.15—13.15	1	—	—	0.66	9.3	14.0	
13.15—13.18	3′	—	—	—	—	—	ventilated at 25° C.
13.18—13.40	22′	—	—	—	—	—	from 25° to 10° C.
13.40—14.30	50′	—	—	—	—	—	ventilated at 10° C.
14.30—16.30	2	5.3	9.6	0.55	2.65	4.8	
16.30—18.30	2	5.4	8.65	0.63	2.7	4.3	
18.30—20.30	2	5.5	8.2	0.67	2.75	4.1	
20.30—22.30	2	5.3	8.1	0.66	2.65	4.05	
22.30— 0.30	2	5.6	8.4	0.67	2.8	4.2	0.30 sp. ph. ½ cm?

TABLE 48 (Fig. 22).

From 25° to 15° C. Linseed medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.00—11.00	1	—	—	0.67	7.75	11.6	
11.00—12.00	1	—	—	0.67	7.8	11.7	
12.00—13.00	1	—	—	0.68	8.1	11.9	
13.00—14.00	1	—	—	0.68	8.2	12.1	
14.00—14.05	5′	—	—	—	—	—	ventilated at 25° C.
14.05—14.15	10′	—	—	—	—	—	from 25° to 15° C.
14.15—15.00	45′	—	—	—	—	—	ventilated at 15° C.
15.00—16.00	1	—	—	0.58	4.0	6.9	
16.00—17.00	1	—	—	0.61	4.0	6.55	
17.00—18.00	1	—	—	0.67	4.1	6.1	
18.00—19.30	1½	5.9	8.8	0.67	3.9	5.9	
19.30—21.00	1½	5.9	8.85	0.67	3.9	5.9	
21.00—22.30	1½	6.2	9.3	0.66	4.1	6.2	23.00 sp. ph. visible?

TABLE 49 (Fig. 23).

From 25° to 20° C. Linseed medium.

Time.	Number of hours	ccm. CO_2 per hour	ccm. O_2 per hour	$\frac{CO_2}{O_2}$	Remarks.
9.30—10.30	1	9.4	14.15	0.66	
10.30—11.30	1	9.2	14.1	0.65	
11.30—12.30	1	8.95	13.8	0.65	
12.30—13.30	1	9.1	13.7	0.66	
13.30—13.35	5′	—	—	—	ventilated at 25° C.
13.35—13.40	5′	—	—	—	from 25° to 20° C.
13.40—14.10	30′	—	—	—	ventilated at 20° C.
14.10—15.10	1	6.2	10.15	0.61	
15.10—16.10	1	6.0	9.55	0.63	
16.10—17.10	1	6.1	9.1	0.67	
17.10—18.10	1	5.85	8.75	0.67	
18.10—19.10	1	5.65	8.7	0.65	
19.10—20.10	1	5.8	8.85	0.65	20.30 sp. ph. 1 cm.

TABLE 50 (Fig. 24).

From 15° to 10° C. Linseed medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.30—12.00	1½	7.1	10.5	0.68	4.7	7.0	
12.00—13.30	1½	7.2	10.55	0.68	4.8	7.0	
13.00—15.00	1½	7.35	10.75	0.68	4.9	7.2	
15.00—15.05	5′	—	—	—	—	—	ventilated at 25° C.
15.05—15.15	10′	—	—	—	—	—	from 15° to 10° C.
15.15—16.00	45′	—	—	—	—	—	ventilated at 10° C.
16.00—17.30	1½	4.7	7.6	0.60	3.05	5.1	
17.30—19.00	1½	4.55	7.0	0.65	3.0	4.65	
19.00—20.30	1½	4.5	7.0	0.64	3.0	4.7	
20.30—22.00	1½	4.55	7.15	0.64	3.	4.75	
22.00—23.30	1½	4.6	7.25	0.64	3.05	4.8	0.00 sp. ph. 1 cm.

TABLE 51 (Fig. 25).

From 15° to 20° C. Linseed medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.00—11.30	1½	6.35	9.65	0.66	4.25	6.4	
11.30—13.00	1½	6.4	9.7	0.66	4.3	6.5	
13.00—14.30	1½	6.4	9.75	0.66	4.3	6.5	
14.30—14.35	5′	—	—	—	—	—	ventilated at 15° C.
14.35—14.40	5′	—	—	—	—	—	from 15° to 20° C.
14.40—15.10	½	—	—	—	—	—	ventilated at 20° C.
[15.10—15.40	½	—	4.825	—	—	9.65]	
[15.40—16.10	½	—	4.775	—	—	9.55]	
15.10—16.10	1	—	—	0.68	6.5	9.6	
16.10—17.10	1	—	—	0.66	6.25	9.5	
17.10—18.10	1	—	—	0.66	6.25	9.45	
18.10—19.40	1½	9.05	14.05	0.65	6.05	9.35	
19.40—21.10	1½	8.9	13.8	0.65	5.9	9.2	

TABLE 52 (Fig. 26).

From 15° to 25° C. Linseed medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
11.30—13.30	2	8.9	13.45	0.66	4.45	6.7	
13.30—15.30	2	8.95	13.55	0.66	4.5	6.75	
15.30—17.00	1½	6.7	10.2	0.65	4.5	6.8	
17.00—18.30	1½	6.65	10.2	0.65	4.45	6.8	
18.30—18.46	16′	—	—	—	—	—	ventilated at 15° C.
18.46—18.50	4′	—	—	—	—	—	from 15° to 25° C.
18.50—19.15	25′	—	—	—	—	—	ventilated at 25° C.
19.15—20.15	1	—	—	0.72	9.15	12.8	
20.15—21.15	1	—	—	0.67	9.05	13.6	
21.15—22.15	1	—	—	0.66	9.1	13.85	
22.15—23.15	1	—	—	0.66	9.15	13.9	

TABLE 53 (Fig. 27).

From 25° to 27°5 C. Linseed medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
9.30—10.30	1	—	—	0.68	9.0	13.3	
10.30—11.30	1	—	—	0.68	8.95	13.1	
11.30—12.30	1	—	—	0.68	8.8	12.9	
12.30—12.37⁵	7′5	—	—	—	—	—	ventilated at 25° C.
12.37⁵—12.40	2′5	—	—	—	—	—	from 25° to 27°5 C.
12.40—13.00	20′	—	—	—	—	—	ventilated at 27°5 C.
[13.00—13.15	¼	—	3.525	—	—	14.1]	
[13.15—13.30	¼	—	3.475	—	—	13.9]	
13.00—13.30	½	5.35	7.0	0.76	10.7	14.0	
[13.30—13.45	¼	—	3.525	—	—	14.1]	
[13.45—14.00	¼	—	3.55	—	—	14.2]	
13.30—14.00	½	5.15	7.075	0.73	10.3	14.15	
[14.00—14.15	¼	—	3.475	—	—	13.9]	
[14.15—14.30	¼	—	3.575	—	—	14.3]	
14.00—14.30	½	4.95	7.05	0.70	9.9	14.1	
[14.30—15.00	½	—	6.975	—	—	13.95]	
[15.00—15.30	½	—	7.1	—	—	14.2]	
14.30—15.30	1	9.5	14.075	0.68	9.5	14.1	
15.30—16.30	1	—	—	0.68	9.6	14.2	
16.30—17.30	1	—	—	0.67	9.35	13.95	
17.30—18.30	1	—	—	0.66	8.9	13.45	

TABLE 54 (Fig. 28).

From 25° to 30° C. Linseed medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
12.00—13.00	1	—	—	0.64	8.8	13.8	
13.00—14.00	1	—	—	0.64	8.65	13.55	
14.00—15.00	1	—	—	0.64	8.5	13.3	
15.00—15.05	5′	—	—	—	—	—	ventilated at 25° C.
15.05—15.07[5]	2′5	—	—	—	—	—	from 25° to 30° C.
15.07[5]—15.30	22′5	—	—	—	—	—	ventilated at 30° C.
[15.30—15.45	¼	—	3.775	—	—	15.1]	
[15.45—16.00	¼	—	3.7	—	—	14.8]	
15.30—16.00	½	5.55	7.475	0.74	11.1	14.95	
[16.00—16.15	¼	—	3.625	—	—	14.5]	
[16.15—16.30	¼	—	3.625	—	—	14.5]	
16.00—16.30	½	5.25	7.25	0.72	10.5	14.5	
[16.30—16.45	¼	—	3.7	—	—	14.8]	
[16.45—17.00	¼	—	3.575	—	—	14.3]	
16.30—17.00	½	4.9	7.275	0.68	9.8	14.55	
[17.00—17.30	½	—	7.2	—	—	14.4]	
[17.30—18.00	½	—	7.2	—	—	14.4]	
17.00—18.00	1	9.15	14.4	0.64	9.15	14.4	
18.00—19.00	1	—	—	0.64	9.1	14.3	
19.00—20.00	1	—	—	0.65	9.0	13.9	
20.00—21.00	1	—	—	0.64	8.8	13.85	

TABLE 55 (Fig. 29).

From 25° to 32°5 C. Linseed medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
9.00 —10.00	1	—	—	0.67	8.1	12.1	
10.00 —11.00	1	—	—	0.68	8.25	12.1	
11.00 —12.00	1	—	—	0.68	8.25	12.1	
12.00 —13.00	1	—	—	0.68	8.35	12.2	
13.00 —13.03	3′	—	—	—	—	—	ventilated at 25° C.
13.03 —13.07⁵	4′5	—	—	—	—	—	from 25° to 32°5 C.
13.07⁵—13.30	22′5	—	—	—	—	—	ventilated at 32°5 C.
[13.00 —13.45	½	—	3.325	—	—	13.3]	
[13.45 —14.00	½	—	3.2	—	—	12.8]	
13.30 —14.00	½	5.725	6.525	0.88	11.45	13.05	
[14.00 —14.15	½	—	3.125	—	—	12.5]	
[14.15 —14.30	½	—	3.1	—	—	12.4]	
14.00 —14.30	½	5.2	6.225	0.84	10.4	12.45	
[14.30 —14.45	½	—	2.975	—	—	11.9]	
[14.45 —15.00	½	—	3.05	—	—	12.2]	
14.30 —15.00	½	4.95	6.025	0.82	9.9	12.05	
[15.00 —15.30	½	—	5.8	—	—	11.6]	
[15.30 —16.00	½	—	5.65	—	—	11.3]	
15.00 —16.00	1	—	—	0.82	9.35	11.45	
16.00 —17.00	1	—	—	0.77	8.5	11.1	
17.00 —18.00	1	—	—	0.75	8.25	11.0	
18.00 —19.00	1	—	—	0.73	7.65	10.5	19.00 sp. ph. ¼ cm.

TABLE 56 (Fig. 30).

From 25° to 35° C. Linseed medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.00 —11.00	1	—	—	0.68	10.2	15.0	
11.00 —12.00	1	—	—	0.68	10.1	14.85	
12.00 —13.00	1	—	—	0.68	10.0	14.65	
13.00 —13.05	5′	—	—	—	—	—	ventilated at 25° C.
13.05 —13.07⁵	2′5	—	—	—	—	—	from 25° to 35° C.
13.07⁵—13.30	22′5	—	—	—	—	—	ventilated at 35° C.
[13.30 —13.45	¼	—	3.6	—	—	14.4]	
[13.45 —14.00	¼	—	3.325	—	—	13.3]	
13.30 —14.00	½	6.7	6.925	0.97	13.4	13.85	
[14.00 —14.15	¼	—	3.15	—	—	12.6]	
[14.15 —14.30	¼	—	3.0	—	—	12.0]	
14.00 —14.30	½	5.75	6.15	0.94	11.5	12.3	
[14.30 —15.00	½	—	5.6	—	—	11.2]	
[15.00 —15.30	½	—	5.2	—	—	10.4]	
14.30 —15.30	1	10.0	10.8	0.93	10.0	10.8	
15.30 —16.30	1	—	—	0.91	8.4	9.2	
16.30 —17.30	1	—	—	0.91	6.7	7.3	
17.30 —19.00	1½	6.55	7.3	0.90	4.35	4.85	
19.00 —20.30	1½	4.25	4.65	0.91	2.7	3.05	

TABLE 57 (Fig. 31).
From 25° to 37°5 C. Linseed medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.00—11.00	1	—	—	0.69	9.6	13.85	
11.00—12.00	1	—	—	0.68	9.35	13.75	
12.00—13.00	1	—	—	0.68	9.15	13.55	
13.00—13.06	6′	—	—	—	—	—	ventilated at 25° C.
13.06—13.10	4′	—	—	—	—	—	from 25° to 37°5 C.
13.10—13.30	20′	—	—	—	—	—	ventilated at 37°5 C.
[13.30—13.45	¼	—	3.1	—	—	12.4]	
[13.45—14.00	¼	—	2.8	—	—	11.2]	
13.30—14.00	½	6.05	5.9	1.03	12.1	11.8	
[14.00—14.15	¼	—	2.4	—	—	6.6]	
[14.15—14.30	¼	—	2.15	—	—	8.6]	
14.00—14.30	½	4.4	4.55	0.97	8.8	9.1	
[14.30—14.45	¼	—	1.975	—	—	7.9]	
[14.45—15.00	¼	—	1.725	—	—	6.9]	
14.30—15.00	½	3.5	3.7	0.95	7.0	7.4	
15.00—16.00	1	—	—	0.94	3.5	3.7	
16.00—18.00	2	2.8	2.75	1.02	1.4	1.4	

TABLE 58.
From 25° to 40° C. Linseed medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
14.00—15.00	1	—	—	0.65	9.9	15.25	
15.00—16.00	1	—	—	0.65	9.45	14.5	
16.00—17.00	1	—	—	0.65	9.0	13.8	
17.00—17.02	2′	—	—	—	—	—	ventilated at 25° C.
17.02—17.05	3′	—	—	—	—	—	from 25° to 40° C.
17.05—17.30	25′	—	—	—	—	—	ventilated at 40° C.
[17.30—17.45	¼	—	3.25	—	—	13.0]	
[17.45—18.00	¼	—	2.65	—	—	10.6]	
17.30—18.00	½	5.6	5.9	0.95	11.2	11.8	
[18.00—18.15	¼	—	1.8	—	—	7.2]	
[18.15—18.30	¼	—	0.975	—	—	3.9]	
18.00—18.30	½	2.65	2.775	0.96	5.3	5.55	
18.30—19.00	½	1.00	0.775	[1.3]	2.0	1.55	

TABLE 61 (Fig. 34).

From. 25° to 10° C. Starch medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.20—11.50	1½	8.85	7.5	1.17	5.9	5.0	
11.50—13.20	1½	9.25	7.65	1.20	6.15	5.1	
13.20—14.50	1½	9.45	7.75	1.22	6.3	5.2	
14.50—15.00	10′	—	—	—	—	—	ventilated at 25° C.
15.00—15.15	15′	—	—	—	—	—	from 25° to 10° C.
15.15—15.45	½	—	—	—	—	—	ventilated at 10° C.
15.45—18.15	2½	4.75	3.7	1.28	1.9	1.5	
18.15—20.45	2½	4.7	3.9	1.20	1.9	1.55	
20.45—23.15	2½	4.8	3.95	1.21	1.9	1.6	

TABLE 62 (Fig. 35).

From. 25° to 15° C Starch medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.00—11.30	1½	9.8	8.05	1.22	6.55	5.4	
11.30—13.00	1½	10.2	8.4	1.21	6.8	5.6	
13.00—14.30	1½	10.4	8.6	1.21	6.9	5.75	
14.30—14.35	5′	—	—	—	—	—	ventilated at 25° C.
14.35—14.45	10′	—	—	—	—	—	from 25° to 15° C.
14.45—15.10	25′	—	—	—	—	—	ventilated at 15° C.
15.10—17.10	2	7.6	5.9	1.29	3.8	2.95	
17.10—19.10	2	7.25	5.7	1.27	3.65	2.85	
19.10—21.10	2	7.05	5.7	1.24	3.55	2.85	
21.10—23.10	2	6.9	5.7	1.22	3.45	2.85	

TABLE 63 (Fig. 36).

From 25° to 20° C. Starch medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
11.45—13.00	1¼	9.9	8.1	1.22	7.9	6.5	
13.00—14.15	1¼	9.95	8.2	1.21	7.95	6.55	
14.15—15.30	1¼	9.95	8.1	1.23	7.95	6.5	
15.30—15.35	5′	—	—	—	—	—	from 25° to 20° C.
15.35—16.00	25′	—	—	—	—	—	ventilated at 20° C.
16.00—17.30	1½	9.2	7.3	1.26	6.15	4.9	
17.30—19.00	1½	8.65	7.0	1.24	5.8	4.65	
19.00—20.30	1½	8.1	6.8	1.20	5.4	4.55	
20.30—22.00	1½	8.0	6.55	1.21	5.3	4.35	

TABLE 64 (Fig. 37).

From 15° to 25° C. Starch medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.15—12.15	2	6.0	5.05	1.19	3.0	2.55	
12.15—14.15	2	6.3	5.3	1.19	3.15	2.65	
14.15—16.15	2	6.6	5.55	1.19	3.3	2.8	
16.15—18.15	2	6.85	5.7	1.20	3.45	2.85	
18.15—18.17[5]	2′5	—	—	—	—	—	ventilated at 15° C.
18.17[5]—18.20	2′5	—	—	—	—	—	from 15° to 25° C.
18.20—18.45	25′	—	—	—	—	—	ventilated at 25° C.
18.45—19.45	1	—	—	1.19	6.95	5.85	
19.45—20.45	1	—	—	1.20	7.1	5.9	
20.45—22.00	1½	9.15	7.6	1.20	7.3	6.1	
22.00—23.15	1½	9.45	7.75	1.22	7.55	6.2	

TABLE 65 (Fig. 38).

From 25° to 27°5 C. Starch medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.30 —12.00	1½	10.55	8.8	1.20	7.05	5.85	
12.00 —13.30	1½	11.25	9.25	1.22	7.5	6.15	
13.30 —15.00	1½	11.6	9.45	1.23	7.75	6.3	
15.00 —15.10	10′	—	—	—	—	—	ventilated at 25° C.
15.10 —15.12⁵	2′5	—	—	—	—	—	from 25° to 27°5 C.
15.12⁵—15.35	22′5	—	—	—	—	—	ventilated at 27° C.
[15.35 —15.50	¼	—	1.775	—	—	7.1]	
[15.50 —16.05	¼	—	1.8	—	—	7.2]	
15.35 —16.05	½	4.5	3.575	1.26	9.0	7.15	
[16.05 —16.20	¼	—	1.825	—	—	7.3]	
[16.20 —16.35	¼	—	1.775	—	—	7.1]	
16.05 —16.35	½	4.35	3.6	1.21	8.7	7.2	
[16.35 —17.05	½	—	3.5	—	—	7.0]	
[17.05 —17.35	½	—	3.475	—	—	6.95]	
16.35 —17.35	1	8.3	6.975	1.19	8.3	7.0	
17.35 —19.05	1½	12.25	10.1	1.21	8.15	6.75	
19.05 —20.35	1½	12.1	9.9	1.22	8.05	6.6	

TABLE 66 (Fig. 39).

From 25° to 30° C. Starch medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.00 —11.30	1½	10.25	8.65	1.19	6.85	5.8	
11.30 —13.15	1¾	12.1	10.05	1.20	6.9	5.75	
13.15 —14.45	1½	10.25	8.4	1.22	6.85	5.6	
14.45 —14.50	5′	—	—	—	—	—	ventilated at 25° C.
14.50 —14.52⁵	2′5	—	—	—	—	—	from 25° to 30° C.
14.52⁵—15.15	22′5	—	—	—	—	—	ventilated at 30° C.
[15.15 —15.30	¼	—	1.675	—	—	6.7]	
[15.30 —15.45	¼	—	1.675	—	—	6.7]	
15.15 —15.45	½	4.15	3.35	1.24	8.3	6.7	
[15.45 —16.00	¼	—	1.65	—	—	6.6]	
[16.00 —16.15	¼	—	1.65	—	—	6.6]	
15.45 —16.15	½	4.0	3.3	1.22	8.0	6.6	
[16.15 —16.45	½	—	3.25	—	—	6.5]	
[16.45 —17.15	½	—	3.25	—	—	6.5]	
16.15 —17.15	1	—	—	1.21	7.85	6.5	
17.15 —18.45	1½	11.4	9.25	1.23	7.6	6.2	
18.45 —20.15	1½	10.9	8.9	1.22	7.3	5.95	

TABLE 67 (Fig. 40).

From 25° to 32°5 C. Starch medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.00 —11.30	1½	9.3	7.9	1.18	6.2	5.3	
11.30 —13.00	1½	9.9	8.2	1.21	6.6	5.4	
13.00 —14.30	1½	10.2	8.25	1.24	6.8	5.5	
14.30 —14.35	5′	—	—	—	—	—	ventilated at 25° C.
14.35 —14.37⁵	2′5	—	—	—	—	—	from 25° to 32°5 C.
14.37⁵—15.00	22′5	—	—	—	—	—	ventilated at 32°5 C.
[15.00 —15.15	¼	—	1.8	—	—	7.2]	
[15.15 —15.30	¼	—	1.75	—	—	7.0]	
15.00 —15.30	½	4.425	3.55	1.25	8.85	7.1	
[15.30 —15.45	¼	—	1.65	—	—	6.6]	
[15.45 —16.00	¼	—	1.575	—	—	6.3]	
15.30 —16.00	½	3.85	3.225	1.20	7.7	6.45	
[16.00 —16.30	½	—	3.05	—	—	6.1]	
[16.30 —17.00	½	—	2.95	—	—	5.9]	
16.00 —17.00	1	—	—	1.15	6.9	6.0	
17.00 —18.00	1	—	—	1.14	6.3	5.5	
18.00 —19.30	1½	8.8	8.1	1.09	5.9	5.4	
19.30 —21.00	1½	8.55	8.0	1.07	5.7	5.35	

TABLE 68 (Fig. 41).

From 25° to 35° C. Starch medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
10.00 —11.30	1½	11.8	9.4	1.26	7.85	6.25	
11.30 —13.00	1½	11.6	9.3	1.25	7.75	6.2	
13.00 —14.30	1½	11.7	9.3	1.26	7.8	6.2	
14.30 —14.35	5′	—	—	—	—	—	ventilated at 25° C.
14.35 —14.37⁵	2′5	—	—	—	—	—	from 25° to 35° C.
14.37⁵—15.00	22′5	—	—	—	—	—	ventilated at 35° C.
[15.00 —15.15	¼	—	1.925	—	—	7.7]	
[15.15 —15.30	¼	—	1.85	—	—	7.4]	
15.00 —15.30	½	4.6	3.775	1.22	9.2	7.55	
[15.30 —15.45	¼	—	1.75	—	—	7.0]	
[15.45 —16.00	¼	—	1.675	—	—	6.7]	
15.30 —16.00	½	4.025	3.425	1.18	8.05	6.85	
[16.00 —16.30	½	—	3.25	—	—	6.5]	
[16.30 —17.00	½	—	3.1	—	—	6.2]	
16.00 —17.00	1	—	—	1.11	7.05	6.35	
17.00 —18.30	1½	9.4	8.85	1.07	6.25	5.9	
18.30 —20.00	1½	8.4	8.0	1.05	5.6	5.35	

TABLE 69 (Fig. 42).

From 25° to 37°5 C. Starch medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
9.15 —10.30	1¼	8.75	7.25	1.21	7.0	5.8	
10.30 —11.45	1¼	8.45	7.0	1.21	6.75	5.6	
11.45 —13.00	1¼	8.45	6.9	1.22	6.75	5.55	
13.00 —13.02[5]	2′5	—	—	—	—	—	ventilated at 25° C.
13.02[5]—13.07[5]	5′	—	—	—	—	—	from 25° to 37°5 C.
13.07[5]—13.30	22′5	—	—	—	—	—	ventilated at 37°5 C.
[13.30 —13.45	¼	—	1.6	—	—	6.4]	
[13.45 —14.00	¼	—	1.425	—	—	5.7]	
13.30 —14.00	½	3.65	3.025	1.21	7.3	6.05	
[14.00 —14.15	¼	—	1.325	—	—	5.3]	
[14.15 —14.30	¼	—	1.225	—	—	4.9]	
14.00 —14.30	½	2.9	2.55	1.14	5.8	5.1	
[14.30 —15.00	½	—	2.25	—	—	4.5]	
[15.00 —15.30	½	—	2.0	—	—	4.0]	
14.30 —15.30	1	4.675	4.25	1.10	4.7	4.25	
15.30 —17.00	1½	4.625	4.575	1.01	3.1	3.05	
17.00 —19.00	2	2.2	2.35	[0.94]	1.1	1.175	

TABLE 70.

From 25° to 40° C. Starch medium.

Time.	Number of hours	CO_2 in ccm.	O_2 in ccm.	$\frac{CO_2}{O_2}$	ccm. CO_2 per hour	ccm. O_2 per hour	Remarks.
15.00—16.30	1½	9.35	8.0	1.17	6.2	5.35	
16.30—18.00	1½	10.15	8.55	1.18	6.75	5.7	
18.00—19.30	1½	10.6	8.95	1.19	7.1	5.95	
19.30—19.36	6′	—	—	—	—	—	ventilated at 25° C.
19.36—19.40	4′	—	—	—	—	—	from 25° to 40° C.
19.40—20.05	25′	—	—	—	—	—	ventilated at 40° C.
20.05—20.35	½	3.775	3.55	1.06	7.55	7.1	
20.35—21.35	1	3.95	4.075	0.98	3.95	4.1	
21.35—23.35	2	1.75	1.85	[0.94]	0.85	0.95	

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