results thus confirm those of Bolomey (1939), and it is our opinion that the large amounts of oxidized GSH found by some authors in yeast may be the result of inaccurate analytical procedures (cf. Fink & Just, 1940).

SUMMARY

1. Different denaturing agents (trichloroacetic acid, sulphosalicylic acid, metaphosphoric acid)

release different amounts of GSH and phosphorus from yeast cells.

2. This must be taken into consideration in the estimation of substances contained in yeast cells. Most probably the cell membrane is the cause of the difficulties.

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Preliminary Experiments in the Study of the Respiratory Activity of Micro-organisms Suspended in Thin Films of Fluid Adhering to Solid Surfaces

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It is obvious that the metabolic events taking place in soil occur, under normal field conditions, in the films of water adhering to the soil surfaces. These films vary greatly in extent and thickness, according to soil conditions, e.g. soil structure and moisture content. Evidence secured recently by Lees & Quastel (1946) in their investigations of nitrification in soil show that, even in a soil which is water-saturated but not waterlogged as in their soil perfusion apparatus (Lees & Quastel, 1944), the process of conversion of ammonia into nitrate is brought about by bacteria proliferating at the soil surfaces. It is therefore evident that, for quantitative studies of the biochemical changes taking place in soil, techniques are required which will allow investigations to be made of the metabolism of micro-organisms when these are suspended in films of fluid adhering to solid surfaces, such as those presented by soil.

The well-known Warburg or Barcroft manometric apparatus may be very conveniently adapted for the measurement of the respiration of micro-organisms, when a suspension of these is spread over suitable solid surfaces.

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Usually, for estimations of the respiration of micro-organisms, a suspension of the cells in 3 or 4 ml. fluid is placed in the manometer vessel of the Warburg or Barcroft apparatus. The oxygen consumption of the cells, when the apparatus is not shaken, proceeds slowly, the rate being determined by the surface of the fluid in the vessel and the rate of diffusion of oxygen into the fluid. When the apparatus is vigorously shaken new fluid surfaces are constantly exposed to the air in the manometer vessel with the result that the fluid becomes saturated with oxygen. There results an immediate rise in the rate of oxygen consumption by the micro-organisms. The effect of shaking the apparatus is the same as that of spreading the volume of fluid in the vessel over an area so large that the thickness of the film of fluid no longer makes diffusion of oxygen into the fluid a limiting factor. When, therefore, a suspension of the micro-organisms is spread over the surfaces of suitable substances, placed in a vessel attached to a manometer, so that the area of spread is sufficiently great to ensure that diffusion of oxygen into the fluid is not a limiting factor, the resulting rate of oxygen uptake is precisely the same as

that obtained when the suspension of cells is agitated in the usual way in the Warburg or Barcroft apparatus.

EXPERIMENTAL AND RESULTS

We have demonstrated this fact experimentally by placing in a Warburg manometer vessel various substances offering a large surface, over which a suspension of micro-organisms containing excess nutrient is spread, and by showing that the rate of oxygen uptake is the same, when the apparatus is kept quite steady, as when the suspension is placed in a vessel, in the absence of solid adsorbents, and shaken in the normal way.

As examples of solid adsorbents suitable for the spread of suspensions of micro-organisms for the examination of their respiratory activities in the Warburg respirometer we have used filter paper, pumice, Permutit, Decalso, and air-dried soil. As a test organism we have used a 2% saline suspension of fresh baker's yeast.

Filter paper. A sheet of suitable size Whatman no. 1 filter paper is folded and placed in a Warburg manometer vessel. 1 ml. of a suspension of baker's yeast composed of 0.5 ml. water, 0.2 ml. 10% (w/v) glucose solution and 0.3 ml. of a 2% saline suspension of yeast, is spread drop by drop evenly over the filter paper. 0.3 ml. 20% (w/v) KOH is placed in the inner cylindrical tube of the vessel to absorb CO₂, care being taken that no potash touches the filter paper on which the yeast suspension is spread. In each of two other vessels 1 ml. of the yeast suspension, made up as indicated above, is placed and KOH is put in the inner tubes. No filter paper or other adsorbent is placed in these vessels. All three vessels are attached to Warburg manometers and placed in water baths at 37° and allowed 35 min. to achieve thermal equilibrium. The taps of the vessels are closed after this period. Two of the manometers, one containing the filter paper on which the yeast suspension has been spread and the other containing only the yeast suspension, are not shaken. The third manometer is shaken in the usual way to ensure optimal conditions for aeration of the yeast suspension. The rates of oxygen uptake are recorded in Table 1.

It is necessary, in estimating oxygen uptakes from the manometer readings, to use vessel constants which have been calculated from the expression

$$K_{O_2} = \frac{(V - V_s - V_f) \frac{273}{T} + V_f \alpha_{O_2}}{P_0}$$

where

V = total vol. of vessel,

V_s = vol. of solid adsorbent (mass/density),

V_f = vol. of fluid in vessel,

α_{O_2} = sol. of oxygen in fluid (μ l. gas at N.T.P. dissolved in 1 μ l. fluid at partial pressure of gas equal to P),

P_0 = normal pressure = $(760 \times 13.6)/D$ (expressed in mm. of manometer fluid where D = density of manometer fluid),

T = absolute temperature.

It is assumed that the solubility of oxygen in the solid adsorbent is nil or so small as to be insignificant.

Table 1. *Respiration of yeast; shaken and unshaken, in the presence and absence of filter paper*

Manometer ...	1	2	3
	Static 1 ml. yeast suspension in glucose solu- tion spread over filter paper	Static 1 ml. yeast suspension in glucose solu- tion	Shaken 1 ml. yeast suspension in glucose solu- tion
	Oxygen uptake (μ l.)		
Time (min.)			
15	65	13	64
30	130	26	139
45	193	38	198
60	265	51	268

It will be seen (Table 1) that under the given experimental conditions the velocity of oxygen uptake by the shaken yeast suspension in presence of the excess glucose, is about five times that of the unshaken yeast suspension. The velocity of oxygen uptake of the yeast suspension spread over the filter paper and not shaken is constant and almost identical with that of the shaken yeast suspension.

These results indicate that the film of fluid in which the yeast is suspended, and which has been spread over the filter paper, is sufficiently thin to allow saturation of the fluid with oxygen under the given experimental conditions.

Fig. 1. Rates of oxygen uptake of yeast suspensions (in glucose solution) alone and when spread over pumice. Manometers not shaken. 37°. Air. (A) Yeast suspension (in glucose solution) alone. (B) Yeast suspension (in glucose solution) spread over pumice.

Pumice. 2 g. of broken pumice (British Drug Houses Ltd. preparation, 10–14 mesh) are placed in a Warburg manometer flask and 1 ml. of the yeast suspension in glucose solution is spread as evenly as possible over the crumbs of the pumice. KOH is placed in the inner tube, and after thermal equilibrium (30–40 min. at 37°) the rate of oxygen uptake is recorded in the usual way, the manometric apparatus, however, being unshaken.

A typical result is given in Fig. 1, which shows also the rate of oxygen uptake of the same volume of yeast suspension not spread over any adsorbent. The rate of oxygen uptake of the yeast suspension spread over the pumice is constant and is identical with that obtained by the suspension when this is shaken in the absence of any solid adsorbent.

These preliminary results would indicate that pumice is a convenient material for the study of the respiration of micro-organisms whose metabolism in films of fluid adhering to a solid surface is the subject of investigation. Air in the manometer vessels may, of course, be replaced by oxygen, if it is thought desirable to test whether the rate of diffusion of oxygen into the pores is a limiting factor.

Permutit and Decalco. The solid adsorbents, Permutit (Permutit Co. Ltd.) (useful as a base exchange material) and Decalco (Permutit Co. Ltd.), may also be used in the Warburg manometric apparatus for the spread of a yeast suspension. Constant rates of oxygen uptake are obtained approximating to that found with a shaken yeast suspension. Results are shown in Table 2.

Table 2. *Respiration of yeast on Decalco and Permutit*

(Warburg manometers static. 37°. Air. Yeast suspension (1 ml. containing 0.5 ml. water, 0.2 ml. 10% glucose solution, 0.3 ml. 2% yeast suspension) placed in a vessel or spread over adsorbents.)

Solid adsorbents...	Manometers not shaken			Manometer shaken
	Nil	Decalco (6 g.)	Permutit (10 g.)	
O ₂ uptake (μl. in 30 min.)	31	148	118	139
O ₂ uptake (μl. in 60 min.)	63	292	238	280

Sand. Little or no spread of a yeast suspension takes place over sand, with the result that relatively little increase in the rate of oxygen uptake is obtained in the static manometric apparatus when the cell suspension is distributed over the sand. In one experiment the oxygen uptake in 1 hr. in a static manometer at 37° by 1 ml. yeast suspension (in 2% glucose) spread over Permutit was 240 μl., and by the yeast suspension distributed over 12 g. sand was 99 μl. Thus sand cannot be used as a solid adsorbent for the study of the respiration of yeast, and probably other micro-organisms, suspended in thin films.

Coke. It was thought possible that ground and sieved coke would provide a suitable material for the spread of a suspension of micro-organisms. The specimens of coke we have used have proved to be most unsuitable. When 1 ml. yeast suspension in 2% glucose solution was distributed over 3 g. coke

particles, of approximately the same dimensions as the pumice referred to earlier in this paper, the oxygen uptake in 1 hr. at 37° was only 22 μl., less than one tenth of that obtained when the suspension was spread over filter paper. In another experiment, using coke extracted with benzene, acetone and ether, and finally well washed and dried, the oxygen uptake in 1 hr. under the same experimental conditions was 51 μl. Coke, like sand, therefore, may be excluded for the time being from the list of solid adsorbents suitable for the study of respiration of micro-organisms in thin films.

Soil. Air-dried garden soil (4 g.), crumbed and sieved (passing a 2 mm. mesh and retained by a 1 mm. mesh) is placed in each of two Warburg manometer vessels. Yeast suspension (1 ml. composed of 0.5 ml. water, 0.3 ml. 2% yeast suspension in saline and 0.2 ml. 10% glucose solution) is spread drop by drop over the soil crumbs, slowly rotating the manometer vessel to obtain as even a distribution as possible. Glucose solution (1 ml. of a 2%) is spread over the crumbs of the soil in the second manometer flask. KOH is placed in the tubes of the vessels, which are now attached to the manometers and placed in a water bath at 37° to establish thermal equilibrium. After 30–40 min. the taps of the vessels are closed and the rates of oxygen uptake are recorded, the manometers not being shaken. Constant rates of oxygen uptake are obtained and these are recorded in Fig. 2. The rate of oxygen uptake of the 1 ml. yeast suspension in 2% glucose solution spread over 2 g. pumice is also shown in Fig. 2.

There is a fairly considerable oxygen consumption by the soil over which glucose solution alone has been spread, this obviously being due to the micro-organisms present in the soil. The rate of oxygen uptake, secured by the yeast suspension in glucose solution spread over the soil, is much greater than that obtained when the glucose solution alone is added to the soil, and is appreciably greater than that obtained by the yeast suspension in glucose solution spread over the pumice. Examination of the figures recorded in Fig. 2 shows that the difference between the rate of oxygen consumption, in soil; of the yeast suspension in glucose solution, and of that of the glucose solution alone spread over the soil, is almost identical with that obtained by the yeast suspension in glucose solution spread over the pumice. This conclusion would be expected if the yeast suspension spreads as a thin film over the soil crumbs, as has been shown in the case of pumice, and if the oxygen uptake of the yeast in the film of fluid adhering to the soil crumbs is unaffected by the respiration of the soil's own microflora. Other experiments, similar to that whose results are recorded in Fig. 2, have been carried out with yeast suspensions and yield similar results. Since there is no reason at present to believe that in an experiment of short duration the respiration of yeast, in presence of excess of nutrient (glucose) and free

access of oxygen, would be affected by that of the soil's microflora, it follows that soil crumbs may conveniently be used, like those of pumice, for the study of the respiration of micro-organisms in thin films.

Fig. 2. Rates of oxygen uptake of yeast suspensions (in glucose solution) spread over air-dried soil crumbs and pumice, and of glucose solution spread over soil. Manometers not shaken. 37°. Air. (A) 1 ml. 2% glucose solution spread over 4 g. air-dried garden soil. (B) 1 ml. yeast suspension (in 2% glucose solution) spread over 2 g. granular pumice. (C) 1 ml. yeast suspension (in 2% glucose solution) spread over 4 g. air-dried garden soil.

The fact that the soil has a highly mixed microflora with a variable respiratory activity, depending on the availability of organic substrates, oxygen and water, is of course a complicating factor when studies are being made of the respiration of suspensions of micro-organisms added to soil crumbs. Use, however, may be made of the fact that many micro-organisms have very high respiratory activities, and small quantities of such organisms may secure rates of oxygen uptake far greater than that obtained by the soil alone, on which the suspensions of the organisms are spread.

The adsorbent and the nutrient medium. If an adsorbent alters the constitution of a nutrient medium, by removal of one or more constituents, an effect on the respiration of the micro-organisms under investigation might be expected to take place. So far, in our preliminary experiments, we have not noticed effects of this character. It is clear, however, that our experimental technique should make

it possible to explore such effects of adsorbents on the respiratory metabolism of micro-organisms.

Effect of water addition to the solid adsorbents

It is obvious that, as the water content of the adsorbents increases, so will the films of fluid on the adsorbent surfaces increase in thickness. If micro-organisms are suspended in these films, the rate of oxygen uptake of the cells will not be affected by the changed water content until the films assume a thickness where diffusion of oxygen into the fluid becomes a limiting factor. The velocity of oxygen uptake will then fall, the rate of fall being dependent on the rate of waterlogging of the adsorbent. Studies, therefore, of the rates of oxygen uptake of micro-organisms suspended in various volumes of fluid added to a constant quantity of a solid adsorbent will indicate the point at which waterlogging occurs, for at this point there will be a sudden drop in the rate of oxygen consumption. This point, at which waterlogging occurs, is clearly a measure of the water-holding power of the adsorbent; the greater its water-holding power, the greater the amount of fluid which can be added to the adsorbent before waterlogging occurs.

Consequently a greater volume of fluid containing a standard quantity of micro-organisms may be added to an adsorbent of high water-holding power, before a drop in respiration takes place, than can be added to an adsorbent of lesser water-holding power before a similar drop in respiration occurs.

Fig. 3. Variations of oxygen uptakes of yeast suspensions (in presence of glucose) in varying volumes of fluid spread over air-dried soil. Abscissa: Vol. fluid (containing 0.3 ml. 2% yeast suspension and 0.2 ml. 10% glucose) added to 4 g. air-dried allotment soil. Manometers static.

Typical results showing the effects of increased moisture content of soil on the respiration of an added suspension of yeast are shown in Fig. 3.

Respiration is greatest at a moisture content corresponding to the addition of 1 ml. fluid to 4 g. air-dried soil crumbs, i.e. to a water content of 20 % (wet soil). Under the given experimental conditions, greater moisture contents than this lead to the filling up of soil pores with resultant fall in respiration as access of oxygen to the yeast cells becomes more and more restricted. It is noteworthy that under the conditions employed in the experiment, whose results are given in Fig. 3, the highest rate of respiration of the yeast in presence of the soil was 367 μ l. O_2 uptake in 1 hr.; that of the soil in presence of glucose solution (1 ml. fluid to 4 g. air-dried soil) was 91 μ l. The difference between these two figures is 276 μ l. which would be expected to be the oxygen uptake in 1 hr. of the added yeast suspension independent of that of the soil's own microflora. The actual oxygen uptake of the yeast suspension under optimal aeration conditions (shaken Warburg apparatus in absence of soil) was found to be 253 μ l. in 1 hr. Our colleague Dr D. M. Webley has investigated the rates of respiration of standard quantities of bacteria of high respiratory activities, suspended in various volumes of fluid, when these are spread over soils (Webley, 1947). Using this technique he has shown how bacterial suspensions may be used for studying problems of aeration into soils. Quastel & Webley (1947) have used this manometric method of assessment of soil aeration for estimating the effects of incorporation with soil of salts of alginic acid and of other organic materials on soil air-water relationships. The addition of sodium alginate to a soil increases its water-holding power; this is shown by the fact that bacterial suspensions spread over soils containing alginate respire at much greater rates at high water contents, than when they are spread over soils containing the same amount of water but with no added alginate. The alginate does not serve as a nutrient source or stimulant to the bacteria, as shown by control experiments; its effect is to afford increased oxygen access to the soil crumbs at the high water contents. The effects of a variety of other substances, e.g. straw, farmyard manure, sawdust, cellulose derivatives, which are all of agricultural importance as organic fertilisers, on the water-holding power of soils may be compared in this way.

SUMMARY

1. If a small volume of a yeast suspension in glucose solution is spread over a suitable solid adsorbent, placed in a Warburg or Barcroft manometer apparatus which is kept unshaken throughout the experiment, its rate of respiration is constant, and is identical with that of the yeast suspension in glucose solution when this is shaken in the normal way in the absence of solid adsorbents.

2. Suitable materials for the spread of suspensions of yeast, and presumably other micro-organisms, so that their respiratory activities in thin films of fluid can be examined, are pumice, Permutit and Decalso. Filter paper also offers an excellent medium for the spread of a suspension of organisms for the study of their respiratory activities.

3. The rate of respiration of a small volume of yeast suspension in glucose solution spread over soil crumbs is constant and greater than that obtained when the suspension is spread over pumice or filter paper. If the rate of respiration due to the soil's own microflora in presence of glucose is subtracted from the rate of respiration of the yeast suspension in presence of glucose spread over the soil, a value is found which is identical with that obtained by the yeast suspension spread over pumice.

It is concluded that soil crumbs, like pumice, form a suitable medium for the spread, as thin films, of small volumes of suspensions of micro-organisms. Soil crumbs may therefore be used for investigations of respiratory activities of micro-organisms suspended in thin films of fluid.

4. Sand and coke are unsuitable materials for the spread of yeast suspensions.

5. The effects of altering the water content of a solid adsorbent on the respiratory activity of micro-organisms spread over the adsorbent are pointed out. A means is indicated of utilizing measures of rates of respiration of organisms suspended in varying volumes of fluid and spread over a standard quantity of solid adsorbent for the study of the water-holding power of the adsorbent, and the aeration conditions within it at various moisture contents. The applicability of this to soil is discussed.

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