
Microbiology. — *On the influence of mono iodo acetic acid on the respiration and the fermentation of yeast.* By A. J. KLUYVER and J. C. HOOGERHEIDE.

(Communicated at the meeting of June 24, 1933).

§ 1. *Introduction.*

According to the insight of many investigators, amongst whom PFLÜGER and PFEFFER may be considered to be the pioneers, the respiration process, in which sugars act as a respiration substrate, is characterized bij an initial anaerobic phase and the oxygen only reacts with products of the anaerobic breakdown of the sugar. This initial phase would then be identical with the initial phase occurring in the completely anaerobic breakdown of the sugar, i.e. in the fermentation process.

Contrary to this unitarian theory many investigators hold that from the chemical point of view respiration and fermentation are quite independent processes. Although they are willing to accept a physiological relation between the two, they reject the idea of a common phase in both.

In recent years this view has gained many fresh supporters chiefly by the important observations of LUNDSGAARD regarding the influence of mono iodo acetic acid on the living cell [1]). The experiments of LUNDSGAARD which have been fully corroborated by many other authors leave no doubt that the addition of the said acid in a suitable concentration to the medium of the cells may fully inhibit the fermentation whilst the respiration is maintained at its normal intensity.

At first sight this result seems to be absolutely incompatible with the view that the first phase of respiration is identical with that of fermentation, since in this case the suppression of the anaerobic breakdown of the sugar would also prevent the respiration.

It is especially BOYSEN JENSEN who of late has emphasized this aspect of LUNDSGAARD's work [2]).

To appreciate fully the weight of this argument it is perhaps not superfluous to remark that the reverse, i. e. the specific prevention of respiration with the preservation of fermentation, had already be attained in several ways a.o. by addition of *HCN*, *CO* and *NO* to the medium of the

---

1) E. LUNDSGAARD, Biochem. Zeitschr. **217**, 162, 1930; Ibid. **220**, 1 und 8, 1930; Ibid. **227**, 51, 1930; Ibid. **250**, 61, 1932.

2) P. BOYSEN JENSEN, Biochem. Zeitschr. **236**, 211, 1931.

cells. This fact, however, could easily be explained by assuming that the oxygen which in respiration reacts with the products of the initial anaerobic breakdown of the substrate asks for a special activating apparatus. The destruction or temporary inactivation of the latter would then lead to the mentioned effect.

The same line of argument cannot be very well applied for the explanation of the action of the mono iodo acetic acid, since one would expect that the inhibition of the fermentation will involve also the suppression of the first phase of respiration.

Since at the other hand so many facts point in favour of the unitarian theory of respiration and fermentation, it was the aim of the experiments reported in this communication to make a closer study of the action of mono iodo acetic acid in the hope to find data which allowed to reconcile the observations made with the said theory.

§ 2. *Present views regarding the influence of mono iodo acetic acid on respiration and fermentation.*

LUNDSGAARD (l. c.) himself has concluded that his experiments have brought decisive evidence that respiration and fermentation are fully independent from the chemical point of view. In proof of this contention the following summary of one of his papers may be cited: [1])

„In der Monojodessigsäurevergiftung verfügen wir über eine Methode, die uns die Trennung des Spaltungs- und des Oxydations-Stoffwechsels ermöglicht, in dem die Monojodessigsäure den ersteren aufzuheben vermag ohne den letzteren nennenswert zu beeinflussen."

Already in his first paper LUNDSGAARD gives as his opinion that the inhibition of the anoxybiotic breakdown occurs in one of the first steps of the reaction chain. In this connection he points to the fact that in fermentation experiments with poisoned yeast no disappearance of the sugar could be observed. Moreover he states that with poisoned zymase preparations no phosphorylation takes place. YAMASAKI [2]) also concludes that the effect of mono iodo acetic acid on the fermentation must be ascribed to its destructive action on the phosphorylating agent.

BARRENSCHEEN and BRAUN [3]) derive from the results of their experiments that it is chiefly the more or less complete destruction of the coenzyme which is responsible for the disappearance of the glycolytic activity of muscle under the influence of the mono iodo acetic acid. This view is rejected by LOHMANN [4]) since the latter could prove that the weakening effect of mono iodo acetic acid on the lactic acid formation by muscle

[1]) E. LUNDSGAARD, Biochem. Zeitschr. **220**, 18, 1930.
[2]) I. YAMASAKI, Biochem. Zeitschr. **228**, 123, 1930.
[3]) H. K. BARRENSCHEEN und K. BRAUN, Biochem. Zeitschr. **232**, 165, 1931.
[4]) K. LOHMANN, Biochem. Zeitschr. **236**, 444, 1931.

extract was also manifest when hexose biphosphoric ester or methylglyoxal were used as substrates.

However the effect of the poison in the case of the methylglyoxal conversion was much less pronounced than that on the glycolytic activity. For this reason LOHMANN also critisizes the view of DUDLEY [1]) who concluded from his experiments that the checking of the glycolysis would be due to the inactivation of the methylglyoxalase. LOHMANN himself thinks it probable that the action of the mono iodo acetic acid is caused by a general weakening of the glycolytic ferment system as a whole.

At the other hand NILSSON, ZEILE and VON EULER [2]) are inclined to accept the view that the action of mono iodo acetic acid is restricted to a final phase of fermentation not essential to respiration. Their chief argument is rather of an indirect nature and is based on the fact that a clear cut separation of respiration and fermentation in yeast can only be brought about by applying a special concentration of the halogenic acid. They show that somewhat higher concentrations of the acid also affect respiration, which proves that the difference between the two dissimilation processes is not as fundamental as it seems at first sight.

BOYSEN JENSEN [3]) then proved that no accumulation of any product of anaerobic sugar breakdown in experiments, in which yeast cells had been poisoned by a suitable concentration of mono iodo acetic acid, could be detected. This result may be considered incompatible with the theory of NILSSON and co-workers.

In a study on the autoxidation of thiol compounds BERSIN [4]) made the interesting suggestion that the poisoning of glycolysis in muscle would be due to the removal of the reduced component of the glutathione system by the mono iodo acetic acid.

In a recent paper QUASTEL and WHEATLEY [5]) have given special attention to this point. These authors have indeed shown that mono iodo acetic acid reacts with the physiological thiol compounds cysteine and reduced glutathione. In agreement herewith the addition of the said compounds diminished the toxic action of mono iodo acetic acid on brain tissue. The importance of this observation is now considerably increased by the demonstration that cysteine and glutathione cause a very marked increase in the rate of aerobic fermentation by yeast cells, whilst glutathione has practically no influence on the respiration. QUASTEL concludes that thiol compounds, normally present in the yeast cells, play some controling part in the relationship between respiration and fermentation.

This conclusion is materially supported by the earlier observations of

---

1) H. W. DUDLEY, Biochem. Journ. **25**, 439, 1931.

2) R. NILSSON, K. ZEILE und H. VON EULER, Zeitschr. f. physiol. Chemie **194**, 53, 1931.

3) P. BOYSEN JENSEN, Biochem. Zeitschr. **236**, 211. 1931.

4) TH. BERSIN, Biochem. Zeitschr. **248**, 3, 1932.

5) J. H. QUASTEL and A. H. M. WHEATLEY, Biochem. Journ. **26**, 2169, 1932.

BUMM and APPEL [1]) who clearly showed that glutathione, whilst having no effect on respiration and anaerobic glycolysis by tumour cells, markedly increases the aerobic glycolysis.

With these facts in mind it indeed does not seem excluded that the destruction of reduced glutathione, normally occurring in the living cell, by mono iodo acetic acid plays an important part in the specific toxic action of this acid on fermentation.

§ 3. *Hypothesis regarding the nature of the action of mono iodo acetic acid based on the unitarian theory of respiration and fermentation.*

As was stated in the introduction many facts point in favour of the view that respiration and fermentation are characterized by the identity of the first steps of their respective reaction chains. Elsewhere one of us (Kl.) has expressed this situation in this way, that fermentation may be considered as a process in which the function of the oxygen in respiration has been taken over by other hydrogen acceptors and inversely that respiration is a fermentation process which has been led in other channels by the intervention of oxygen after activation of this element by WARBURG's respiration ferment [2]).

This view is materially supported by the results of an analysis of the mechanism of fermentation and respiration, since the most probable representation of this mechanism is that both processes consist of primary reactions which are all of the oxidoreduction type. This result is especially important since it makes acceptable that all these primary reactions occur under the influence of one and the same catalyst of the nature of an oxidoreductase [3]).

Is there now any possibility to reconcile the behaviour of mono iodo acetic acid with regard to the yeast cell with this unitarian theory?

The facts reported in the foregoing paragraph seem to make this rather difficult. For it is not easy to understand that a poison can suppress fermentation completely without influencing respiration in the least. At first sight one would expect that both processes would be injured approximately to the same degree.

However the definition of respiration as a fermentation process led into new channels by the intervention of the activated oxygen seems to open a possibility for another explanation. For we may conceive that the rate of respiration is determined by the velocity with which oxygen is made available by the ironcontaining activating apparatus. If we then add the supposition that the activated oxygen is always preferent as a hydrogen acceptor in the dehydrogenation of methylglyoxal, it is clear that a decrease

[1]) E. BUMM und H. APPEL, Zeitschr. f. physiol. Chemie, **210**, 79, 1932.

[2]) A. J. KLUYVER, Archiv f. Mikrobiologie **1**, 181, 1930.

[3]) A. J. KLUYVER, Chemie der Zelle und Gewebe **13**, 134, 1926.

of the rate of anaerobic breakdown of the sugar will only affect respiration at the moment, that the reduction is so large that the demand of the oxygen can no longer be satisfied. From this point of view fermentation under aerobic conditions is only the consequence of the excess of sugar breakdown as compared with the need for dehydrogenation substrate of the respiration process.

We may in this line of thought assume that the action of increasing concentrations of mono iodo acetic acid present in the medium of the yeast cells will lead to an inactivation of an increasing part of the oxidoreduction catalyst. At first this inactivation will only affect the surplus dissimilation, i.e. the aerobic fermentation. A gradual increase of the concentration of the poison will lead at last to a complete suppression of the aerobic fermentation, the respiration maintaining its normal intensity. However as soon as this critical concentration has been surpassed the capacity of the still remaining sugar breakdown will fall below the level that is necessary for the maintenance of the normal respiration rate. In other words respiration too will become affected.

Although in the paper of NILSSON, ZEILE und VON EULER indications are found that the experimental facts are in agreement with this mode of view, it did not seem to be superfluous to reinvestigate this point in more detail, the more so because the Swedish investigators draw quite different conclusions from their experiments.

§ 4. *Experiments on the influence of various concentrations of mono iodo acetic acid on respiration and fermentation.*

As outlined above it was the aim of the experiments to study the effect of the mono iodo acetic acid in concentrations in the neighbourhood of the critical concentration which just suppresses aerobic fermentation. For this purpose it was essential to study the various factors which influence the toxic action of the acid.

In all these experiments the wellknown manometric method of WARBURG has been applied. For the measurement of the respiration and the aerobic fermentation always two parallel experiments were made in such a way, that in one of these experiments caustic potash was brought in the inner tube of the vessel for the absorption of the carbon dioxide formed. At the same time a third experiment was made in which the air was replaced by an atmosphere of oxygenfree nitrogen to which 5 % of carbon dioxide had been added, in order to saturate the medium with this gas. From this experiment the rate of anaerobic fermentation could be derived.

In all cases a suspension was made of about 400 mgrs baker's yeast in 100 ccm of a 2.5 % phosphate buffer solution to which also 1 gram of glucose and the mono iodo acetic acid in varying quantities were added. A measured quantity of this mixture was brought in the WARBURG apparatus and this was shaken in a waterbath of 30° C for about 20 minutes before readings were made.

The experiment itself lasted for one hour and from the observed changes in pressure the values of respiration (Q_{O_2}), of aerobic fermentation ($Q^{O_2}_{CO_2}$) and of anaerobic fermentation ($Q^{N_2}_{CO_2}$) were calculated in the usual way.

In all cases baker's yeast ("Koningsgist" kindly supplied by the "Nederlandsche Gist- en Spiritusfabriek") has been used. All comparative experiments were made with one and the same sample of yeast, which if necessary was kept in a refrigerator. Special experiments showed that under these conditions the intensity of respiration and fermentation remained practically constant during several days.

Preliminary experiments showed, in agreement with the results of LUNDSGAARD and of EHRENFEST [1]), that the toxic action of the mono iodo acetic acid markedly increased with increasing acidity of the medium. It was then decided to carry out the experiments at a $pH = 4.7$, since a separate set of experiments had shown that this acidity was practically optimal for both fermentation and respiration of the variety of yeast employed.

The influence of mono iodo acetic acid in several concentrations varying between $\frac{1}{100.000}$ and $\frac{1}{7000}$ was then studied.

The results of these experiments are given in Table I.

TABLE I.

The effect of increasing concentrations of mono iodo acetic acid on respiration, aerobic and anaerobic fermentation of *Saccharomyces cerevisiae* in a medium with $p_H = 4.7$.

Concentration of CH_2ICOOH	Q_{O_2}	$Q^{N_2}_{CO_2}$	$Q^{O_2}_{CO_2}$
0	123.2	279.7	88.1
1 : 100.000	127.2	121.1	68.6
1 : 65.000	120.1	81.6	...
1 : 50.000	122.4	63.9	30.5
1 : 40.000	127.0	0.9	11.8
1 : 30.000	104.9	—	1.6
1 : 20.000	70.8	—	—
1 : 15.000	43.7	—	—
1 : 10.000	27.3	—	—
1 ; 7.000	12.0	—	—

[1]) E. EHRENFEST, Journ. of Biol. Chem. **97**, 1932; Proc. Am. Soc. of Biol. Chem. 26th Ann. Meeting p. LXXVI.

In Fig. 1 a graphical representation of these results is given. In this graph the quantities of sugar consumed in respiration, aerobic and anaerobic fermentation are given. These quantities were calculated from

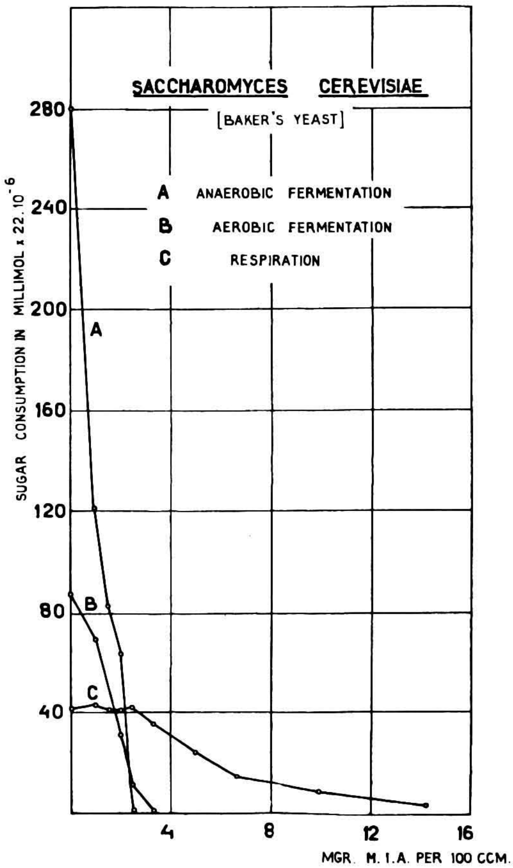

Fig. 1.

the figures for oxygen consumption and carbon dioxide evolution, on the basis of 1 molecule of sugar consuming 6 molecules of O_2 in respiration and one molecule of sugar yielding 2 molecules of carbon dioxide in fermentation.

The figures on the ordinate are of an arbitrary character (100 representing 0.0022 millimol sugar).

When we look upon the graph, we find in the first place a confirmation of LUNDSGAARD's fundamental observation, viz. that on raising the concentration of the mono iodo acetic acid anaerobic fermentation is rather quickly inhibited, whilst at first respiration is maintained at its original level. At a concentration of 2.5 mgr. per 100 ccm (1 : 40.000) anaerobic fermentation is altogether checked, the respiration still remaining constant. As for the aerobic fermentation we see that this process too is quickly inhibited by increasing concentrations of the poison and at the critical concentration already mentioned its value has practically been reduced to zero. However the remarkable fact presents itself that as soon as this critical concentration of the poison has been surpassed also the intensity of respiration is affected. It is obvious that this result is in excellent agreement with the „surplus-theory" of aerobic fermentation. As soon as the surplus breakdown of the sugar has practically disappeared and therefore aerobic fermentation has dropped out, a further increase in concentration of the poison should also lower the rate of respiration.

This situation throws a special sidelight on LUNDSGAARD's pronouncement that mono iodo acetic acid offers a specific means of bringing about a clear cut separation between oxybiotic and anoxybiotic metabolism. For it is clear that this behaviour of the yeast cells can be very well explained on the basis of the unitarian theory, viz. by assuming an inactivation of increasing parts of the oxidoreductase by increasing concentrations of the acid, the respiration always being preferent for the conversion of the remaining part of the supply of anaerobic breakdown products.

Against this mode of view the objection can be raised that at the critical concentration of the acid the undiminished intensity of respiration proves that still a fraction of the oxidoreductase is active, so that one would expect that under these conditions also a fraction of the anaerobic fermentation would be maintained. It is however tempting to connect this apparent deviation with the observations of QUASTEL and WHEATLEY that at the one hand mono iodo acetic acid reacts with the reduced form of glutathione and that at the other hand the latter compound is a more or less essential condition for the normal course of the fermentation process. QUASTEL is inclined to ascribe the function of the glutathione to its property to establish a suitable oxidation reduction potential in the cell and we should like to point out in addition that the earlier experiments of BOYLAND [1]) give rather conclusive proof that the establishment of such a suitable reduction potential is an essential condition for the fermentation process.

This might well be the cause that at the critical concentration of the acid under anaerobic conditions the still remaining part of the oxidoreductase does not make itself manifest by evolution of fermentation carbon dioxide.

It seemed possible to give a still more convincing demonstration of the correctness of the „surplus-theory" of aerobic fermentation by using other yeast species in which the intensity of this fermentation was lower. If a yeast species could be found in which under aerobic conditions the fermentation would be completely suppressed, one should expect that any addition of the mono iodo acetic acid large enough to affect the anaerobic fermentation would also diminish at once the rate of respiration. It is obvious that such a demonstration would greatly derogate LUNDSGAARD's postulate of the independence of oxybiotic and anoxybiotic metabolism.

---

[1]) E. BOYLAND, Biochem. Journ. 24, 703, 1930.

It may be stated immediately that until now we did not succeed in finding a yeast species which under the aerobic conditions, as realized in

TABLE II.
The effect of increasing concentrations of mono iodo acetic acid on respiration, aerobic and anaerobic fermentation of *Saccharomyces Marxianus* in a medium with $p_H = 4.7$.

Concentration of CH_2ICOOH	Q_{O_2}	$Q^{N_2}_{CO_2}$	$Q^{O_2}_{CO_2}$
0	92.3	122.5	9.7
1 : 100.000	74.2	61.5	...
1 : 50.000	68.5	21.7	4.4
1 : 30.000	53.7	10.7	2.9
1 : 20.000	48.4	—	—
1 : 10.000	13.8	—	—

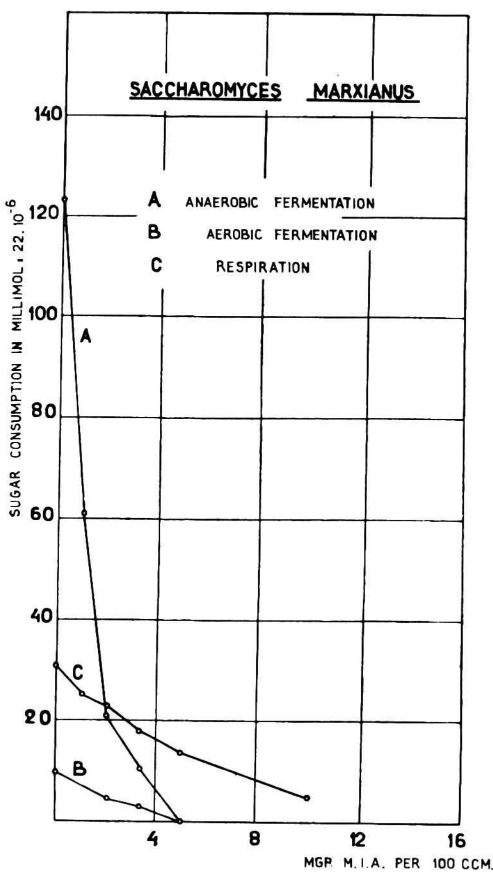

Fig. 2.

the WARBURG apparatus, was fully devoid of aerobic fermentation, whilst showing a good anaerobic fermentation. However in the course of this investigation we met with some species which approximately answered the said requirements viz. in which the aerobic fermentation was very low.

We will give here only the results of the investigation of one of these yeast species: *Saccharomyces Marxianus* Hansen.

The experiments were carried out in exactly the same way as has been described before for the baker's yeast with the understanding that in these cases three days old cultures on yeast extract glucose plates were used.

Table II gives the figures for oxygen consumption and carbon dioxide production by *Saccharomyces Marxianus* expressed in the usual way. (See table II, pag. 604.)

The results are also graphically reproduced in Figure 2 in the same way as has been done before in Figure 1.

As may be derived from Fig. 2 we meet in *Saccharomyces Marxianus* with a yeast with a very low aerobic fermentation. Even very small concentrations of the mono iodo acetic acid will practically reduce the „surplus breakdown" to nihil and therefore it should be expected that in this case respiration will be almost immediately affected. As will be seen from the graph the experimental results are in perfect agreement with this assumption.

It seems to us that the foregoing exposition justifies the conclusion that LUNDSGAARD's discovery of the action of mono iodo acetic acid on yeast cells does not suffice to reject the unitarian theory of respiration and fermentation.

Microbiology. — *On the presumed suitability of maltose as a respiration substrate for non-maltose fermenting yeasts.* By A. J. KLUYVER and J. C. HOOGERHEIDE.

(Communicated at the meeting of June 24, 1933).

In a paper published about two years ago by TRAUTWEIN and WEIGAND [1]) a new argument against the unitarian theory of respiration and fermentation was raised. In this paper the authors report about experiments which tend to show that for some yeast species which lacked the ability to ferment the disaccharide maltose this sugar still was a suitable substrate for the respiration process.

It is clear that this demonstration would imply that the chemistry of the maltose respiration proceeds by a direct oxidation of this sugar, since a preliminary hydrolysis of the disaccharide to glucose and a subsequent

[1]) K. TRAUTWEIN und K. WEIGAND, Biochem. Zeitschr. **240**, 423, 1931.