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.

anaerobic breakdown to the C_3-stage would be incompatible with the lack of ability of the said yeast species to ferment maltose. The evidence given might be considered as a serious objection against the idea that respiration and fermentation have a common introductory phase. It seemed therefore worthwile to investigate whether the results of TRAUTWEIN and WEIGAND could be corroborated.

A closer study of their paper showed that there was some reason to doubt the correctness of the interpretation of their observations. In the first place our attention was drawn to the fact that the German authors had applied highly concentrated solutions of the respiration substrates. As a matter of fact they had worked with 10 % solutions of the various sugars. Now it had already been shown by MEYERHOF [1]) for a *Torula* species that the rate of respiration is highly independent of the concentration of the respiration substrate. MEYERHOF found for instance (c.f. Tabelle VIII) that the respiration value for this yeast was the same in a 0.5 % and in a 5 % glucose medium. We learn from this result that already low concentrations of suitable substrates raise the rate of respiration to its optimal value.

If it could be shown that this effect was of quite general occurrence it might be deemed to be of far reaching importance, also for the problem under consideration. For this would mean that special attention should be given to impurities eventually present in substrates in those cases in which these were applied in high concentrations.

Our first aim was therefore to make a study of the influence of sugar concentration on the rate of respiration.

The technique used was again the manometric method of WARBURG. For particulars regarding the arrangement of the experiments we refer to our preceding paper [2]). Ordinary baker's yeast was used.

The influence of varying the glucose concentration from 10 % to 0.05 % was studied. The results are given in Table 1.

We learn from these results that indeed the rate of respiration is highly independent of the glucose concentration, in so far as only a reduction of the concentration of the sugar below 0.2 % gives a rate which is markedly reduced as compared with that in 10 % sugar. It even should be remarked, that a slight optimum in the rate of respiration is present in the region between 0.5—0.2 % glucose.

With a view to the above, it is obvious that it is not recommendable to study the suitability of some respiration substrate in high concentrations since then the danger, that positive results are due to some impurity possibly present, is markedly increased.

It was decided therefore to study the respiration of some non-maltose fermenting yeasts in media with varying concentrations of glucose and

[1]) O. MEYERHOF, Biochem. Zeitschr. **162**, 43, 1925.

[2]) These Proceedings **36**, p. 596, 1933.

TABLE 1.

The effect of various concentrations of glucose on the respiration of Saccharomyces cerevisiae (baker's yeast) in a medium with $p_H = 4{,}7$.

% glucose	Q_{O_2}
10	107.5
5	109.5
3	108.9
1	107.5
0.5	118.7
0.35	128.5
0.20	115.0
0.10	80.0
0.05	33.7
—	15.0

maltose respectivily. For these experiments use was made of pure cultures of *Saccharomyces Marxianus* HANSEN and of *Saccharomyces exiguus* REESS, these being the same species as employed by TRAUTWEIN and WEIGAND.

The results are given in Table 2.

TABLE 2.

The effect of various concentrations of glucose and maltose on the respiration of Saccharomyces Marxianus and of Saccharomyces exiguus.

	Saccharomyces Marxianus	Saccharomyces exiguus
	Q_{O_2}	Q_{O_2}
Without sugar	29.1	27.0
Glucose 1%	91.9	76.1
Glucose 10%	89.9	67.4
Maltose 1%	50.7	36.2
Maltose 10%	89.4	90.2

These figures give a confirmation of the results of TRAUTWEIN and WEIGAND in so far that indeed the respiration in a 10 % solution of maltose is equal to or even higher than that in a 10 % solution of glucose.

Moreover we learn from these figures that here also the rate of respiration is practically independent of the concentration of the glucose. In contrast herewith, however, the rate of respiration in maltose is markedly decreased by lowering the concentration of this sugar.

This latter result may be considered to be a strong indication that indeed the high rate of respiration in the maltose medium is due to some impurity present in the sugar preparation employed. If this assumption was correct it might be expected that on continuing the respiration experiments for a longer period this impurity would be consumed with a corresponding decrease of the respiration rate.

To test this theory the experiments reported in Table 3 were made.

TABLE 3.

The respiration of Saccharomyces Marxianus and of Saccharomyces exiguus in 10% glucose and in 10% maltose solutions for four successive hours.

Period	Saccharomyces Marxianus		Saccharomyces exiguus	
	Q_{O_2}		Q_{O_2}	
	10% glucose	10% maltose	10% glucose	10% maltose
1st hour	89.9	89.4	67.4	90.2
2nd „	93.1	54.6	70.7	83.0
3rd „	85.1	24.0	67.7	63.5
4th „	91.4	20.6	68.1	27.3

It is obvious that the results reported in Table 3 give an excellent confirmation of the view that the respiration in maltose containing media is due to some impurity. Whilst the rate of respiration in the glucose media remains practically constant during the whole course of the experiment, the respiration in the maltose media shows a continuous decline. In the last hour of the experiment the respiration has fallen down to the same level as in the experiments without sugar (c.f. Table 2). Since the sugar consumption in experiments like these is negligible we may conclude that the yeast cells do not show any increase in respiration even in the presence of high concentrations of maltose.

In all the foregoing experiments a commercial brand of maltose had been used, i.e. a preparation of Merck, as had been also employed by TRAUTWEIN and WEIGAND. It seemed therefore worthwhile to investigate the behaviour of other maltose preparations.

Although a chemical pure maltose of the firm of Pfanstiehl gave a much lower respiration value than the maltose of Merck and some other commercial brands, there was still a clear increase in respiration with all these products as compared with the respiration in the absence of any sugar.

Since the same was found to apply for the fermentation it became

probable that the impurity which was present in small quantities in the maltose preparations would be a fermentable sugar. It was therefore decided to purify the maltose by adding to a 10 % solution a suspension of a glucose — but not maltose — fermenting yeast. As such *Saccharomyces Marxianus* was used. After four hours incubation at 30° C. the yeast cells were separated from the maltose solution by filtration through a Seitz filter. The sterile filtrate was then used for a new series of respiration experiments.

TABLE 4.

Purified maltose against crude maltose as a substrate for the respiration of Saccharomyces Marxianus and of Saccharomyces exiguus.

	Saccharomyces Marxianus	Saccharomyces exiguus
	Q_{O_2}	Q_{O_2}
Without sugar	29.1	27.0
10 % maltose (Merck)	89.4	90.2
10 % maltose purified	30.8	36.4

As will be seen from Table 4 the rate of respiration of both species in the purified maltose solution is practically identical with that in the absence of sugar.

We may therefore conclude that pure maltose is unsuitable as a substrate for the respiration of *Saccharomyces Marxianus* and *Saccharomyces exiguus*.

Herewith the arguments given by TRAUTWEIN and WEIGAND in favour of the dualistic theory of respiration and fermentation have been refuted.

Physics. — *Preliminary note on some experiments concerning isotopes of some of the noble gases and hydrogen by means of* J. J. THOMSON's *mass spectrograph.* By P. ZEEMAN and J. DE GIER.

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

Sir JOSEPH THOMSON's beautiful parabola method, the original method of positive ray analysis depending on the use of parallel magnetic and electric fields has been much refined by ASTON but has still some advantages of its own.

We have built a mass spectrograph according to the original THOMSON pattern with some improvements according to CONRAD[1]) and some slight modifications of our own necessitated by our purpose in view: the determination of the different kinds of atoms and molecules in the discharge tube with only short expositions.

---

[1]) R. CONRAD. Phys. Z. S. **31**, 888. 1930.