PASTEUR EFFECT IN DEAD YEAST*

by

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If one restricts the term "PASTEUR Effect" to the strictly reversible and non-progressive inhibition produced in air or oxygen on the steady rate of glycolysis of fermentation of cells and tissues, as we propose to do, one is confronted with the difficulty that such a system does not lend itself easily to a more intimate analysis. On the other hand, the uncritical use of the term for every inhibitory influence of oxygen, be it reversible or not, on any phase of intermediary metabolism or enzyme action, is misleading and invites wrong generalizations and interpretations of this effect. This situation has already been discussed on several occasions[1] (see also DEAN BURK[2]) and need not to be dealt with here again.

In the former work of one of us, it was shown that the Pasteur effect in living yeast was the result not of the presence of oxygen but of respiration[3]. The quotient,

$$\frac{\text{mol } CO_2 \text{ fermentation suppressed by oxidation}}{\text{mol } O_2 \text{ consumed}}$$

was around 2. This quotient which OTTO WARBURG has called the "MEYERHOF quotient" (MQ)[4] must be distinguished from the term used by one of us (O.M.), the oxidation quotient,

$$\frac{\text{mol sugar splitting suppressed by oxidation}}{\text{mol sugar oxidized}}$$

which is three times as high. For simplifying the situation we will use here exclusively WARBURG's quotient, MQ.

One condition which must be fulfilled for a quantitative measurement of the PASTEUR effect is a completely stationary state of all three metabolic rates involved: fermentation in N_2–CO_2 (5%), fermentation in O_2–CO_2 (5%), respiration in O_2. Recently one of us described a quickly dried yeast preparation in which the ATP-ase is preserved and the HARDEN-YOUNG effect is absent[5]. This yeast, therefore, ferments sugar with

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constant speed without accumulation of phosphate esters or change in the content of inorganic phosphate.

Yeast prepared in this way shows also respiration and a typical PASTEUR effect. Unfortunately brewers' yeast cannot be used advantageously because its respiration is too small. Indeed, the respiration of the dried yeast is affected by the drying procedure in much the same way as is fermentation. If for instance, in a living yeast the Q_{O_2} is only 5% of the $Q^{N_2}_{CO_2}$, then a similar relation exists with the dried yeast. In bakers' yeast respiration may be 25 to 30% of the rate of fermentation. Our experiments were, therefore, made with various preparations of bakers' yeast.

METHODS

By applying the quick drying procedure to ordinary fresh bakers' yeast a stable preparation is obtained which in general behaves like brewers' yeast except for its high respiration. Especially, the ATP-ase is preserved so well that while most of the added sugar ferments in 60 to 80 mins at 30°, very little if any phosphate esters accumulate. However, in one important aspect, the yeast is less suited for the study of cofactors and inhibitors than is brewers' yeast. The yeast cell remains practically impermeable and phosphate as well as coenzymes cannot be washed out. Moreover, the yeast is not really killed by the drying procedure. As cultivation tests show, about 50% of the cells are still able to grow*. If this dried yeast is subjected to sonic vibration for 60 to 80 mins, it is mostly killed and, at the same time, it becomes much more permeable and a great difference in the rate of fermentation results, depending upon whether cofactors are added or not. With this treatment, some ATP-ase is inevitably destroyed and the concentration of inorganic phosphate decreases during fermentation. However, the esterification is still so slow that a constant rate of fermentation in the presence of a low concentration of phosphate can be maintained easily for one hour.

For the sonic vibration of the dried bakers' yeast, we used a magnetostriction oscillator with water cooling (Raytheon Co.). Generally 1 g dried yeast + 4 ml 0.1 *M* glutathione + 15 ml distilled water + 1 ml 5% $NaHCO_3$ were vibrated at 9 kilocycles for 60 to 80 minutes. The glutathione serves for detoxication of the traces of metal which go into solution from the vibrating disc. Bicarbonate keeps the reaction near the neutral point. Afterwards, the suspension was used without further centrifugation.

Another preparation used by us is the yeast LK 2 G 12, abbreviated K, of SPIEGELMAN *et al.*[**6]. If this yeast is cultivated according to these authors[***7] anaerobically in autolyzed yeast extract, the respiration is very low. If, however, it is very strongly aerated for three days, during which time the sugar is consumed, respiration is very much increased (see Table V), quite similar to the behaviour of various cultivated bakers' yeasts. A dried preparation of the yeast cultivated in this way shows an appreciable respiration and the PASTEUR effect can easily be measured. Here, the phosphate and the larger part of the coenzymes can easily be washed out. Also, as the plate counts show, it contains only 2% of living cells. Again, a part of the ATP-ase is destroyed, and esterification of the phosphate sets in immediately with fermentation. However, if enough phosphate is added, fermentation remains constant for from 60 to 80 minutes.

For preparation of these dried yeasts, it is necessary to suck them off on a Büchner funnel until no more water can be removed. They are squeezed through a sieve and dried in a very thin layer on filter paper at 35° for 2.5 to 3 hours as was described for brewers' yeast[5].

Fermentation and respiration were measured at 29° C with the usual WARBURG manometric technique. For fermentation we used mostly vessels of 35 ml with two side arms containing sugar and coenzymes. For respiration we used 15 ml vessels with insert cylinders. 0.1 ml *N* KOH was placed in the insert cylinder as well as in the side arm for increasing CO_2 absorption. Phosphate was determined according to the LOHMANN AND JENDRÁSSIK modification[8] of the procedure of FISKE AND SUBBAROW.

The inhibitors used were commercial preparations. However, *para*-nitrophenol as well as dinitrophenol of Eastman Kodak Co. were purified by repeated recrystallizations from hot water. Ethyl

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*** In one liter of water: 2 g autolyzed yeast extract powder, 5 g bactopeptone, 1 g ammonium sulfate, 2 g KH_2PO_4, 0.25 mg $MgSO_4$, 0.3 g $CaCl_2$, 7 ml 50% sodium lactate, 60 g dextrose.

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carbylamine was obligingly prepared for us in the Scientific Laboratory of Hoffmann La Roche Inc.* Cozymase was the preparation of Schwarz Laboratories of 50% purity. Later on we also used a preparation, which had a purity of 83%, made by the Sigma Chemical Co.** according to the directions of OHLMEYER[9]. HDP was purified from a Schwarz preparation.

In general the following amounts were used in samples of 1.0 to 1.2 ml: 8 to 15 mg dried yeast suspended in 0.5 ml of a dilute solution of KH_2PO_4 + 0.05 ml 3.8% $MgSO_4$. The final concentration of phosphate was 0.01 to 0.02 *M* and the Mg concentration, 0.005 *M*. Added to this were 0.1 to 0.2 distilled water or specific substances. The side arm was filled with 0.4 to 0.5 ml consisting of 0.2 ml cozymase solution with 150 to 300 γ pure DPN, 0.05 ml ATP with 50 γ 7′ P, 0.05 ml HDP with 15 γ P and 0.05 acetaldehyde 0.5%. In the cases where these quantities were varied special mention is made in the experimental part.

RESULTS

A. Experiments with dried bakers' yeast. MQ.

Most of our experiments were made with dried bakers' yeast from the National Yeast Corporation. The drying should not take longer than three hours. Good preparations show a $Q^{N_2}_{CO_2}$ of about 150 and nearly no phosphate uptake during the whole period of fermentation. In Table III, the change of inorganic phosphate is compared with the production of CO_2. Expressed in μmols, less than 1% as much P is phosphorylated during 40 to 80 mins. as would be required for a fullfledged HARDEN-YOUNG effect where 1 mol CO_2 produced would correspond to 1 mol esterified phosphate. Table I shows the PASTEUR effect with various batches of such a yeast. Only a very small selection is made from the numerous experiments with dried bakers' yeast. The variation in the absolute values stems from the speed of drying and the freshness of the bakers' yeast used. The MQ varies very little, not much outside the experimental error. Only that time period in which all metabolic rates were completely constant was used for calculating, generally the time from 10 to 60 mins., after the induction period is over. The values are calculated as Q according to WARBURG (cmm CO_2 or O_2 per mg dry weight and one hour).

TABLE I

INTACT DRIED BAKERS' YEAST

No. of prep.	mg dried yeast	Time in min	Q			MQ
			N_2/CO_2	O_2/CO_2	O_2	
4	10	40	157	98	39.5	1.5
7	5.6	60	143	68.5	38	1.8
21 A	10	30	159	68.2	41.6	2.2
118	10	60	166	82	48.3	1.8
66	1.5	40	98	59.7	27.5	1.4
78*	10	40	96	55	27.4	1.5

* Old preparation of dried yeast.

Although many factors were varied, no influence on the MQ was observed. However, this is easily explained by the slight permeability of the preparation. Neither the inorganic phosphate nor the coenzymes can be washed out to any appreciable degree. Addition of cofactors to this preparation increased the fermentation only in a small degree.

* We thank cordially Dr R. DUSCHINSKY for this preparation.
** We thank Dr D. BROIDA of Sigma Chemical Co. for a gift of this sample of cozymase.

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B. Reversibility

Strict reversibility of the PASTEUR effect is shown in the following experiment (Table II). Here the metabolic rates were determined in one vessel filled with N_2/CO_2 (1), in two vessels with O_2/CO_2 (2, 3), and in one with KOH in the insert (4). After 20 mins. the vessel (3) was removed and gassed with N_2/CO_2 and the reading continued. This vessel (Table II, 8c) which had given in the first 20 mins. a $Q_{CO_2}^{O_2}$ of fermentation of 57 like the vessel 2, gave for the third 20 mins. in N_2/CO_2 a Q of 134 like the vessel (1) in the second period.

TABLE II

REVERSIBILITY OF PASTEUR EFFECT IN DRIED BAKERS' YEAST

No. of prep.	mg dried yeast	Time in min	Q			MQ
			N_2/CO_2	O_2/CO_2	O_2	
8a	10	20	144 (1)	57 (2)	40.0 (4)	1.9
b	10	20	134 (1)	63 (2)	39.0 (4)	
c	10	20	134 (3) ←	57 (3)	43.5 (4)	

Number in bracket means the number of the manometric vessel.
← Means that the O_2 filling of the gas space of vessel (3) is after 20 mins replaced by N_2.

The constancy of the metabolic rates makes this preparation suitable for the study of known inhibitors of the PASTEUR reaction. Most of them were without effect or inhibited the PASTEUR effect proportionally to the inhibition of respiration, *e.g.*, azide and ethyl carbylamine.

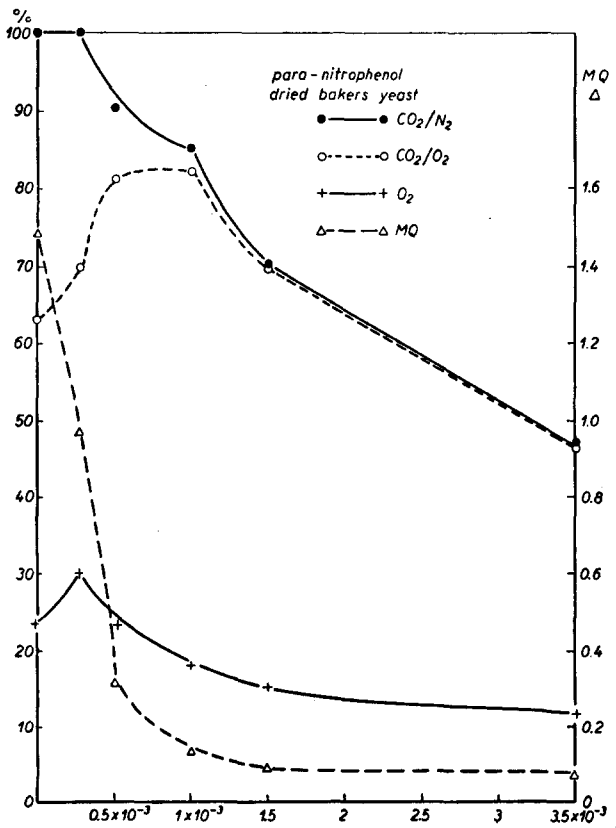

Fig. 1. Metabolic rates in the presence of *para*-nitrophenol. Abscissa: M conc. of *para*-nitrophenol. Ordinate: the metabolic rates are expressed as % of $Q_{CO_2}^{N_2}$ in the absence of nitrophenol. The MQ is expressed in absolute values according to the right ordinate.

C. Inhibition

The only substances which we found as specific inhibitors of the PASTEUR effect were *para*-nitrophenol and 2,4-dinitrophenol. The former gives more regular results and inhibits the PASTEUR effect with only a slight inhibition of the anaerobic fermentation. Fig. 1 shows the results with different concentrations and the same is shown for dinitrophenol in Fig. 2. All the experiments were done with repeat-

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edly crystallized samples. As one can see from Fig. 1, $Q_{CO_2}^{N_2}$ goes slightly down with $0.5 \cdot 10^{-3}$ *M* nitrophenol but $Q_{CO_2}^{O_2}$ goes up until both values nearly meet at $1 \cdot 10^{-3}$ *M*. The increase of fermentation in O_2 is the best proof for the inhibition of the PASTEUR effect because at the same time respiration is very little affected. From $2 \cdot 10^{-3}$ *M* on all metabolic rates are increasingly inhibited. The MQ remains about 0.1. With dinitrophenol (Fig. 2) the MQ becomes not less than 0.5 under similar conditions and becomes nearly zero only if all metabolic rates are strongly inhibited. The experiments with *p*-nitrophenol are, therefore, a better demonstration of the inhibition of the PASTEUR reaction. A still weaker effect is shown by 3,5-dinitro-*o*-cresol (Eastman), which decreases the MQ from 1.71 to 0.75 in $6 \cdot 10^{-5}$ *M* solution.

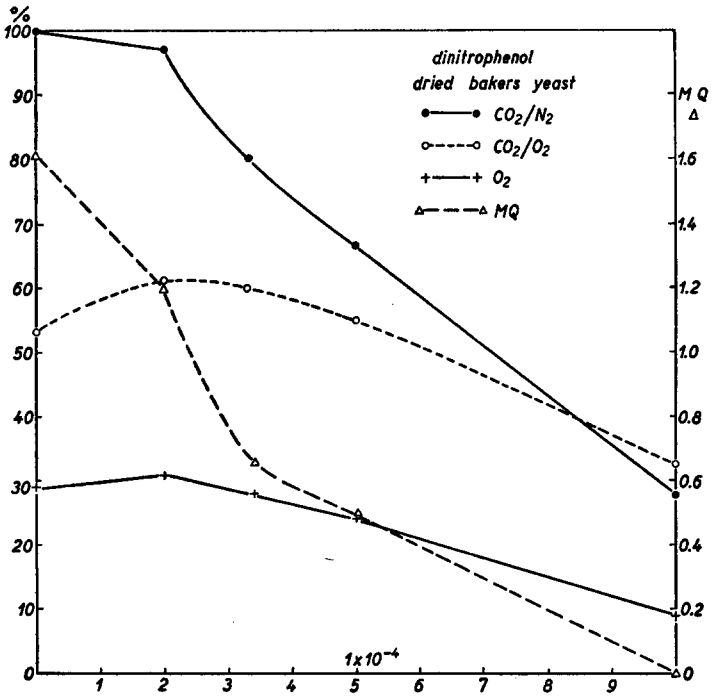

Fig. 2. Metabolic values in the presence of 2,4-dinitrophenol. Legends like Fig. 1.

Several authors have described the separation of respiration and oxidative phosphorylation in animal cells by means of dinitrophenol (see [10]). Such an effect can be demonstrated also in dried bakers' yeast, better with *p*-nitrophenol than with dinitrophenol. It occurs with the same concentration as inhibits the PASTEUR effect. This demonstration needs the complete preservation of the activity of the ATP-ase. If this enzyme remains in step with hexokinase as it does in living yeast, no phosphate ester accumulates during fermentation in N_2[5]. But quite a little phosphate is esterified in O_2. Under such conditions $1 \cdot 10^{-3}$ *M* nitrophenol inhibits completely this extra phosphorylation. This ideal case is seldom realized. Mostly there is a little esterification also in N_2, but it corresponds to only 1% or less of that expected according to the HARDEN-YOUNG equation.

$1 \cdot 10^{-3}$ *M* nitrophenol inhibits a little this anaerobic phosphorylation but this may

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be caused by the inhibition of fermentation itself. On the other hand, in O_2 the phosphorylation is larger although fermentation is smaller. Nitrophenol shows a still greater inhibition. Some such experiments are reproduced in Table III. In the last column the change of esterified P produced by nitrophenol is expressed in μmols. One can see that it is from 2 to 4 times as great in oxygen as in nitrogen. This effect is no longer observable if the esterification caused by fermentation prevails so much over that coupled with oxidation that the esterification of P goes directly parallel to the CO_2 produced.

TABLE III

PHOSPHATE UPTAKE IN THE PRESENCE OF NITROPHENOLS

No. of protocol	Time in min	Addit.	$10^4 M$ conc.	Gas including 5% CO_2	MQ	μ mol CO_2	μ mol inorg. P change	ΔP change with nitroph. μ mol
113	80	—		N_2		67	—0.45	
	80	*p*-N*	15	N_2		47	—0.22	0.23
	80	*p*-N	10	N_2		52	—0.22	0.23
	80	—	—	O_2	1.4	37	—1.0	
	80	*p*-N	15	O_2		38	—0.1	0.9
	80	*p*-N	10	O_2	0.38	42	0	1.0
125	70	—		N_2		66	—0.4	
	70	*p*-N	10	N_2		48	0	0.4
	70	—	—	O_2		37	—0.65	
	70	*p*-N	10	O_2		43	+0.32	0.97
114	80	—		N_2		65	—0.87	
	80	Din.**	3.3	N_2		53	—0.42	0.45
	80	—	—	O_2	1.43	34	—1.0	
	80	Din.	3.3	O_2	0.63	39	—0.12	0.88

* *p*-N = *para*-nitrophenol
** Din. = 2,4-dinitrophenol

We may assume that this inhibition of oxidative phosphorylation is connected with the inhibition of the PASTEUR effect. All other substances tested have no visible influence on the PASTEUR effect. Azide inhibits respiration of dried yeast from $2 \cdot 10^{-6} M$ onwards (30% inhibition). The MQ remains constant even if the respiration is 85% inhibited by $2 \cdot 10^{-5} M$. A similar situation is obtained with ethyl carbylamine. Other substances quoted as inhibitors of the PASTEUR effect like phenosafranine, gramicidine, etc., were likewise without effect.

D. Ultrasonic vibration

The quickly dried bakers' yeast could not be depleted of its phosphate content and its coenzymes by washing. Actually fermentation was very little changed if the cofactors were added as described in the methods.

For studying the effect of coenzymes the yeast had to be made more permeable, which we did by ultrasonic vibration of 60 to 80 mins. In this case part of the ATP-ase was destroyed, but there was still enough left to keep constant the speed of fermentation during 50 to 60 mins, while only a part of the added inorganic phosphate was esterified.

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Respiration was more damaged than fermentation, but was still appreciable and the PASTEUR effect was nearly unchanged. For these experiments the whole vibrated yeast must be used. The supernatant, after removal of the debris of the cells, shows only a very small respiration.

In Table IV the influence of cofactors is shown. With a usual amount added, the speed is increased about two to three fold. Cozymase alone has only a small effect but all factors together without cozymase give also only 2/3 of the maximum. In Table V, the MQ of the ultrasonic vibrated yeast is shown with all cofactors added. In the absence

TABLE IV

INFLUENCE OF COFACTORS ON ULTRASONICALLY TREATED DRIED YEAST (80 min VIBRATED)

No. of protocol	mg dry yeast	Time in min	Cozymase 10^4 M	$Q_{CO_2}^{N_2}$ with addition of cofactors			
				no factor added	all factors without cozymase	cozymase alone	all factors
107	12.5	40		26			
	12.5	40			54		
	12.5	40	2			32	83
	12.5	40	6				86.5
124	12.5	50		34			
	12.5	50			46		
	12.5	50	2				76
	12.5	50	6				79

TABLE V

SONIC VIBRATED DRIED BAKERS' YEAST; *p*-NITROPHENOL

No. of protocol	mg dried yeast	Time in min	Nitrophenol conc. 10^3	Q			MQ	
				N_2/CO_2	O_2/CO_2	O_2	in the control	with nitrophenol
100	20	50	—	67	56	13.5	0.85	
	20	50	2	54	53.3	9.6		0
101	25	30	—	91	76	16	0.95	
	25	30	2	80	81	11		0
101a*	25	30	—	115	92.5	31	0.75	
	25	30	2	103.5	101	16.3		0.15
108	12.5	60	—	93.5	67	17.6	1.5	
**	12.5	60		71	54	15.5	1.1	
111	12.5	40	—	87	69.5	12.0	1.45	
**	12.5	40	—	54.7	43.5	7.8	1.3	
***	12.5	40	—	23.1	18.0	7.8	0.65	

* with addition of boiled yeast juice.
** no cozymase added.
*** no cofactors added.

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of the cofactors the MQ becomes smaller. This fact may not have a great significance because the anaerobic fermentation is only 1/3 of the normal. With $2 \cdot 10^{-3}$ *M* nitrophenol, the PASTEUR effect is completely abolished. This corresponds to the influence on the non vibrated yeast.

E. Experiments with Spiegelman's yeast. Cultivation

The foregoing experiments were repeated with the yeast LK 2 G 12 of SPIEGELMAN, here called K, which was cultivated as described in the methods. Table VI shows the metabolic quotients with the living yeast K in comparison with living bakers' yeast. As it is apparent from Table VI the anaerobically cultivated yeast as well as that which was cultivated with an excess of sugar for 18 hours show a metabolic type similar to that of brewers' yeast with very low respiration, in terms of Q less than a tenth of the anaerobic fermentation. However, if grown in broth for 66 hours, at least one day longer than sugar is available, the respiration rises 5 times, and accordingly, fermentation in oxygen is now only a third of the fermentation in nitrogen.

TABLE VI

METABOLIC QUOTIENTS OF LIVING YEAST

No. of prep.	Yeast types	Cultivation	$Q^{N_2}_{CO_2}$	$Q^{O_2}_{CO_2}$	Q_{O_2}	MQ
81	Bakers'	—	205	55.5	83	1.8
83	K	anaerobic 15 hours	220	188	23.3	1.4
94	K	aerobic 18 hours	312	248	20.5	3.1
96	K	aerobic 66 hours	324	114	102	2.05
96a	K*	aerobic 66 hours	354	295	123	0.50

* with $6 \cdot 10^{-4}$ *M* *p*-nitrophenol.

It was first shown by one of us[5] that the brewers' yeast and also bakers' yeast of low respiration increases its Q_{O_2} value if kept many hours in dilute sugar solution, during which time the sugar is practically consumed. Shortly afterwards OTTO WARBURG[11] studying the conditions of cultivation of bakers' yeast found that bakers' yeast which was cultivated anaerobically had a low respiration but if cultivated aerobically and with exhaustion of the sugar shows a great rise in respiration. The same is true for this yeast K of SPIEGELMAN.

Dried yeast preparation made from the culture 83 and 94 of Table VI had a very low oxygen uptake, less than 5% of the aerobic fermentation; but dried preparations made from culture 96 and similarly cultivated yeast had a respiration which amounted to about 15% of the fermentation. Although this was still low, the PASTEUR effect could easily be determined. The metabolic values were, *e.g.*

$$Q^{N_2}_{CO_2} = 77,\ Q^{O_2}_{CO_2} = 44,\ Q_{O_2} = 12.2,\ MQ = 2.7.$$

By washing the dried K yeast, inorganic phosphate can be completely removed, but the cofactors only partly. Even unwashed yeast is strongly activated by the addition of cofactors and behaves similarly to the ultrasonic vibrated bakers' yeast.

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F. Activation

A new phenomenon was observed which was not apparent with the other yeast. If a solution of cozymase (made up from the preparation of 50% purity as well as from that of 83%) was brought for some minutes to pH 10.0 to 11 with Na_2CO_3 with development of a light yellow colour and immediately reneutralized, an activator was produced which raised the rate of anaerobic fermentation the more, the more cozymase was used, while without treatment the maximum of fermentation was reached with 150 γ DPN per ml and addition of excess cozymase had no effect. With this activation which must be ascribed to some decomposition product (not nicotinamide) or to the half reduced form of DPN, the MQ became excessively great. Probably in oxygen the activation was somewhat inhibited. It may be questioned, whether this high MQ is due to a true PASTEUR reaction according to our definition.

Some examples are given in Table VII. The carbonate treated cozymase is called B. The increase in the amount of untreated cozymase does not change the fermentation rate but the increase in the amount of cozymase B activates anaerobic fermentation until it is more than doubled (see protocol No. 121 and 128). Fermentation in oxygen and respiration rises less so that the MQ has a value of about 4.

TABLE VII

COFACTORS FOR DRIED YEAST K (12 mg DRIED YEAST PER SAMPLE)

No. of protocol	Time in min	Cofactors except cozymase	Cozymase added γ	Cozymase B	Q			MQ
					N_2/CO_2	O_2/CO_2	O_2	
106	60	—	—		28.8			
	60	+	—		39	27	10.8	1.31
	60	+	450		52	29.6	12.2	1.85
109	60	—	—		28	16.8	12.1	0.9
	60	+	—		33.6	22.2	11.2	1.0
	60	+	450		44	25.6	13.2	1.4
121	55	+	—		25			
	55	+	450		44	25.4	7.0	2.65
	55	+		150	40.3			
	55	+		450	62.3	29.4	9.1	3.6
128	45	+	—		28			
	45	+	250*		43			
	45	+	150		45.6	25.2	8.4	2.4
	45	+		250*	76.6	34.7	9.0	4.6
	45	+		750*	106	52.2	12.7	4.2

* Sigma preparation.

G. Effect of nitrophenol

Para-nitrophenol in a concentration of $1 \cdot 10^{-3}$ *M* nearly abolishes the PASTEUR effect in dried SPIEGELMAN's yeast K quite similarly to bakers' yeast. An example is given in Table VIII (to compare with 96a of Table VI, with living yeast). However,

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because of the strong esterification of phosphate during fermentation the inhibitory effect on the oxidative phosphorylation cannot be demonstrated.

TABLE VIII

INHIBITION OF PASTEUR EFFECT WITH YEAST K

No. of protocol	mg dry weight	Time in mins	Nitrophenol conc. 10^3	Q			MQ	
				N_2/CO_2	O_2/CO_2	O_2	control	with nitrophenol
103	12	50	—	57	32	9.6	2.0	
	12	50	1	43	38.2	10.6		0.44

DISCUSSION

The foregoing experiments demonstrate for the first time that dead cell preparations show a reversible PASTEUR effect. A deeper insight into the mechanism so far was not obtained, because in such cases, where the yeast is nearly completely killed and the phosphate can be washed out, fermentation prevails too much over respiration and fermentative phosphorylation too much over oxidative phosphorylation. According to all indications, however, the PASTEUR effect results from a phosphorylating step, as is especially apparent from the simultaneous inhibition of the PASTEUR effect and the aerobic phosphorylation by para-nitrophenol.

OTTO WARBURG has recently shown[12] that yeast zymohexase (aldolase) is under special conditions inhibited by oxygen: if the zymohexase reaction is inhibited by cysteine and reactivated by ferrous salt, oxygen interferes with the reactivation because it oxidizes the bivalent iron to the trivalent state. We determined, therefore, the aldolase activity in our yeast after sonic vibration. There was no difference in oxygen or nitrogen. $5 \cdot 10^{-4}$ dinitrophenol which lowers the MQ to 0.5 has no influence on the speed of the zymohexase reaction. Moreover, the Q_P* for aldolase at 30° in our sonic vibrated yeast was about 200 while the $Q_{CO_2}^{N_2}$ under the same conditions was less than a hundred. It is, therefore, probable that the aldolase does not regulate the speed of fermentation. The same conclusion was reached formerly in respect to glycolysis in muscle and malignant tumour[13].

SUMMARY

Under special conditions dried yeast preparations can be obtained which show a typical PASTEUR effect with a normal quotient (MQ),

$$\frac{\text{mol } CO_2 \text{ fermentation suppressed by oxidation}}{\text{mol } O_2 \text{ consumed}}$$

of 1 to 2.5. Quickly dried bakers' yeast without further treatment was used in the first place. The PASTEUR effect is strictly reversible. Moreover, it is nearly completely inhibited by *para*-nitrophenol ($1 \cdot 10^{-3}$ *M*) and less completely by 2,4-dinitrophenol and 3,5-dinitro-*o*-cresol. While almost no phosphate is esterified anaerobically, some phosphorylation occurs in oxygen, and this latter phosphorylation is then completely inhibited by *para*-nitrophenol.

* $Q_P = \mu l\ H_3PO_4$ ($1\ \mu l = 0.72\ \gamma$ P) of hexosediphosphate transformed into triosephosphate per mg dry weight per hr.

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Because such a dried bakers' yeast still contains 50% cells able to grow, the yeast was treated with ultrasonic vibration for 60 to 80 mins. Although this destroys some ATP-ase, the fermentation is still fairly constant for 60 to 80 min in the presence of low concentrations of phosphate. All observations can be repeated with the vibrated yeast with the same results. This yeast is now permeable to cofactors (cozymase, ATP, etc.) and the fermentation rate is tripled by addition of optimal concentrations of these factors.

Another yeast type, SPIEGELMAN's yeast K, gives the same results without ultrasonic treatment. It contains only 2% of living cells. It shows a specific activation by cozymase which has been treated for some minutes with carbonate (p_H 10.5). This activation is weaker in oxygen. This leads to a real or apparent increase of the MQ up to 4.

RÉSUMÉ

Sous certaines conditions l'on peut obtenir des préparations de levure séchée qui montrent un effet PASTEUR typique avec un quotient (MQ),

$$\frac{\text{mol de } CO_2 \text{ de fermentation réprimés par l'oxydation}}{\text{mol d'}O_2 \text{ consommés}}$$

normal de 1 à 2.5. Nous avons employé en premier lieu de la levure pressée, séchée rapidement sans traitement ultérieur. L'effet PASTEUR est strictement reversible. De plus, il est presque complètement inhibé par le *p*-nitrophénol ($1 \cdot 10^{-3}$ *M*) et moins complètement par le 2,4-dinitrophénol et le 3,5-dinitro-*o*-crésol. En anaérobiose l'on n'observe aucune estérification de phosphate, tandis qu'en présence d'oxygène la phosphorylation a lieu et, dans ces conditions, elle est complètement inhibée par le *p*-nitrophénol.

Une telle levure pressée, séchée, contient encore 50% de cellules capables de croissance; c'est pourquoi nous l'avons exposée à l'action des ultrasons pendant 60 à 80 minutes. Bienque ce traitement détruise une partie de l'ATP-ase, la fermentation reste encore à peu près inchangée pendant 60 à 80 minutes en présence de faibles concentrations de phosphate. Les résultats de toutes les expériences faites avec de la levure non traitée sont reproductibles avec de la levure préalablement soumise aux vibrations. Une telle levure est perméable aux "cofacteurs" (cozymase, ATP, etc.) et la vitesse de la fermentation est triplée par l'addition de ces facteurs à concentration optimale.

Un autre type de levure, la levure SPIEGELMAN K, donne les mêmes résultats sans traitement préalable aux ultrasons. Cette levure contient seulement 2% de cellules vivantes. Elle est activée de façon spécifique par une cozymase, ayant subi un traitement préalable de quelques minutes par le carbonate (p_H 10.5). Cette activation est plus faible en atmosphère d'oxygène, ce qui cause une augmentation réelle ou apparente du MQ jusqu'à la valeur de 4.

ZUSAMMENFASSUNG

Unter besonderen Bedingungen kann man getrocknete Hefepräparate erhalten, die einen typischen PASTEUR-Effekt und einen normalen Quotient (MQ)

$$\frac{\text{Mol Gärungs-}CO_2 \text{ verschwunden in } O_2}{\text{Mol } O_2 \text{ verbraucht}}$$

von 1 bis 2.5 zeigen. In erster Linie wurde rasch getrocknete und nicht weiter behandelte Presshefe verwendet. Der PASTEUR-Effekt ist streng reversibel. Ausserdem wird er fast vollständig durch *p*-Nitrophenol ($1 \cdot 10^{-3}$ *M*) und unvollständig durch 2,4-Dinitrophenol und 3,5-Dinitro-*o*-kresol unterdrückt. Während unter anaeroben Bedingungen beinahe kein Phosphat verestert wird, findet unter aeroben Bedingungen etwas Phosphorylierung statt, die dann durch *p*-Nitrophenol vollständig gehemmt wird.

Da solche Presshefe noch 50% lebender Zellen enthält, wurde sie während 60 bis 80 Minuten mit Ultraschallwellen behandelt. Obwohl diese einen Teil der ATP-ase zerstören, bleibt die Gärung bei geringer Phosphatkonzentration doch noch während 60 bis 80 Minuten nahezu konstant. Die obigen Ergebnisse sind mit der, mit Ultraschall vorbehandelten Hefe reproduzierbar. Die Hefe wird durch diese Vorbehandlung für die sogenannten "Cofaktoren" (cozymase, ATP, usw.) durchlässig, die, bei optimaler Konzentration, die Geschwindigkeit der Gärung verdreifachen.

Eine andere Hefeart, die SPIEGELMAN K-Hefe, zeigt ohne Ultraschallbehandlung dieselben Ergebnisse. Sie enthält nur 2% lebender Zellen. Sie wird spezifisch durch Cozymase aktiviert, welche vorher ein Paar Minuten mit Carbonat (p_H 10.5) behandelt wurde. Diese Aktivierung ist in Sauerstoffatmosphere schwächer. Hiedurch wird eine scheinbare oder wirkliche Zunahme des MQ bewirkt.

References p. 12.

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