Table 9. *Magnitude of arsenate-stable respiration in yeast in various nutritional states*

State of yeast	Q_{O_2} Control	Q_{O_2} Arsenate (mM)	Inhibition (%)
Impoverished	−4·3	−3·9	10
Normal	−7·4	−6·0	19
Enriched	−21	−7·3	65
Normal + glucose	−77	−11·9	85

Table 10. *Respiratory quotient of arsenate-stable respiration of yeast in presence of glucose*

The fermentation caused by the inhibition of respiration by arsenate was inhibited by iodoacetate. Each manometer vessel contained 1·0 ml. of 5% yeast suspension (about 14 mg. dry wt.), 0·5 ml. of 0·8% glucose, 0·5 ml. of 0·1 M tris, pH 6·5, 0·5 ml. of 6 mM sodium arsenate and 0·5 ml. of 6 mM iodoacetate. Duplicate pairs were set up with and without KOH to absorb the respiratory CO_2.

State of yeast	R.Q. in glucose + arsenate + iodoacetate	R.Q. of endogenous respiration
Impoverished	0·85	0·86
Normal	0·89	0·87
Normal	0·78	0·87

DISCUSSION

It is clear from the results presented that the substrate for the respiration when no external oxidizable material is present is, in ordinary commercial baker's yeast, not of carbohydrate nature. This agrees with earlier workers' measurements of R.Q. and is consistent with the fact that no fermentation of stored carbohydrate takes place. The effect of DNP is to make the stored carbohydrate available for breakdown, and this takes place by both oxidation and fermentation, the oxidation of the non-carbohydrate substrate being suppressed. The nature of the non-carbohydrate material remains unknown; its amount in the yeast cell is reduced by aeration, and increased by treatment of the cell with glucose; the R.Q. of its oxidation is 0·85.

SUMMARY

1. When baker's yeast respires without added substrate its respiratory quotient is 0·85.

2. No stored polysaccharide disappears during this respiration.

3. The respiration is associated with an esterification of inorganic phosphate ($Q^{O_2}_P = -40$).

4. In the presence of 2:4-dinitrophenol (DNP) aerobically a fermentation of stored carbohydrate is set up and the respiration is changed to an oxidation of carbohydrate.

5. The fermentation induced by DNP is not inhibited by 10^{-3} M iodoacetate.

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The Pasteur Effect in Normal Yeast and its Inhibition by Various Agents

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As Dickens (1951) has most recently pointed out, no theory yet put forward to explain the Pasteur effect can be considered to exclude numerous other possible theories. The present paper seeks to define the problem more closely as it occurs in yeast, and to dispose of at least one of the conflicting theories that have been proposed to explain the phenomenon.

The definition of the Pasteur effect is commonly stated in two parts: (1) the partial or complete suppression by oxygen of the formation of the products of anaerobic carbohydrate breakdown, and (2) the diminution by oxygen of the rate of carbohydrate breakdown. The first part of the definition may be given quantitative expression by means of

the Meyerhof quotient ($M.Q. = Q^{N_2}_{CO_2} - Q^{O_2}_{CO_2}/Q_{O_2}$); so far no expression giving a quantitative measure of the second part of the definition has been used, and for this purpose the Pasteur quotient is proposed, defined as $P.Q. = Q^{O_2}_{Glucose}/Q^{N_2}_{Glucose}$. In the baker's yeast used in the present experiments the numerical value of the P.Q. was usually 0·20–0·25, at low glucose concentrations, a figure agreeing with the many data found in the literature.

Observations of the Pasteur effect have usually been made by measuring the rates of respiration and of aerobic and anaerobic fermentation. The respiratory oxygen has not always been shown to represent oxidation of glucose, which makes evaluation of the P.Q. impossible. In the present case it will be shown that the oxygen consumption is a measure of the glucose oxidized. Further, not all the glucose used by the yeast is oxidized or fermented, and a different value of the P.Q. will be obtained according to whether the rate of glucose catabolism or of total glucose utilization is used.

All substances which inhibit respiration induce an aerobic fermentation and thereby increase the value of the P.Q. to 1·0. It is only when such an increase of the P.Q. is observed without any simultaneous inhibition of the respiration that the Pasteur effect is said to be inhibited. A large number of substances have been shown to inhibit the Pasteur effect in various mammalian tissues (see Dickens, 1951). The present paper gives, for one particular brand of baker's yeast: (1) an evaluation, in terms of the Pasteur quotient as defined above, of the effect of oxygen on the catabolism and total utilization of glucose; and (2) the effect of a wide range of inhibitors on this effect of oxygen. In a later paper an account will be given of the phosphorylation processes in the same yeast, and the effect of the same inhibitors on them (Stickland, 1956*b*).

MATERIALS AND METHODS

Yeast. Ordinary pressed baker's yeast (City Brand Pure Yeast, made by J. Mann and Co., Leeds) was used; other brands, used occasionally, showed some slight quantitative differences in behaviour. For some experiments the yeast was washed with water; the washings were water-clear and contained no protein (material precipitable by trichloroacetic acid) or P (either inorganic or combined) and washing of the yeast was in general dispensed with.

Manometric measurement of glucose catabolism. The standard method of measuring O_2 consumption and CO_2 production in Warburg manometers was used. For all manometric experiments 0·5 ml. of a 2% (fresh wt.) suspension of yeast in water, 0·5 ml. of 0·1 M aminotrishydroxymethylmethane, pH 6·5, and 0·5 ml. of 0·8% glucose were present in each vessel; total vol., 3·0 ml. For anaerobic measurements the vessels were filled with N_2 purified by passage over heated copper; it was confirmed that there was no change in the CO_2 content of the buffer during fermentation under these conditions. Fermentation in air was followed by measuring the change of pressure in a manometer with no KOH to absorb the respiratory CO_2, and making a correction for the respiration from a simultaneous measurement of this quantity, on the assumption that the R.Q. was unity. It was shown that the phenomena to be described could still be observed at O_2 tensions down to about 20 mm. Hg. All manometric measurements were made at 25°, and lasted 1 hr. after the equilibration of the manometers, unless anything to the contrary is stated.

Measurement of total glucose utilization. For these experiments the glucose (0·5 ml. of 0·8% solution) was placed in the side arm of the manometer vessel, and was tipped after equilibration was complete; manometric readings were taken, usually for 60 min.; then the vessels were quickly removed and 2·0 ml. of 10% trichloroacetic acid solution was added to their contents. After thorough rinsing of the side arm, the mixtures were centrifuged. The glucose content of a measured sample of the neutralized supernatant was determined by the method of Hanes (1929).

Measurement of polysaccharide synthesis. The yeast residues obtained in the previous procedure were washed with ethanol by centrifuging to remove glucose, and then hydrolysed in 2 N-HCl for 2 hr. at 100°. This was shown to be sufficient to produce a steady maximum reducing power in the solution. The glucose content of the neutralized solutions was determined by the method of Hanes (1929). No attempt was made to identify the substance or substances whose hydrolysis gave rise to these reducing sugars, but the course of the hydrolysis was parallel to that of glycogen, and for the purpose of this paper the material in question will be referred to as 'polysaccharide'. Its quantity will be given as the equivalent amount of glucose.

The average polysaccharide content of the baker's yeast used in these experiments was 340 mg./g. dry wt. of yeast (range 270–360 mg./g. dry wt.), which on incubation of the yeast in glucose solution for 1 hr. increased aerobically to about 470 mg./g. dry wt. and anaerobically to about 440 mg./g. dry wt. This reserve carbohydrate is not available for fermentation (Stier & Stannard, 1936; Spiegelman & Nozawa, 1945), but some respiration does take place in the absence of external substrates. This endogenous respiration is not an oxidation of polysaccharide.

Method of expressing results. As the Pasteur effect is said to be inhibited only when the P.Q. is increased without any inhibition of the respiration, it is essential to have a means of expressing numerically the extent of any increase in the P.Q., in order to compare it with the degree of inhibition of the respiration under the same conditions. The P.Q. in normal yeast is usually about 0·22, and this value can be increased to 1·00 by inhibiting the respiration (it can also in some circumstances exceed unity). Therefore the degree of elevation of the P.Q. can be simply expressed as a proportion of the difference between the uninhibited value and 1·00; e.g. if the control value were 0·22, and this were increased in the presence of an inhibitor to 0·80, the degree of elevation of the P.Q. may be written as $(0·80–0·22)/(1·00–0·22) = 74\%$. If this elevation of the P.Q. is greater than the inhibition of the respiration, some degree of inhibition of the Pasteur effect has been produced; if the elevation of the P.Q. is 100%, and the respiration unaffected, then the Pasteur effect has been completely inhibited.

RESULTS

Respiratory quotient

The validity of the measurements of aerobic fermentation depends on the fact that the R.Q. of yeast respiring in the presence of glucose is 1·00, so this point must be established first.

The R.Q. of yeast in the absence of glucose is about 0·85 (Meyerhof, 1925; Lundsgaard, 1930; Stickland, 1956*a*); in the presence of glucose the R.Q. cannot be measured directly, since CO_2 is one of the products of fermentation as well as of respiration. Meyerhof (1925) gives reasons for assuming a value of 1·00 in the presence of glucose, and Lundsgaard (1930) found an R.Q. of 0·99 in yeast respiring in glucose solution in the presence of a concentration of iodoacetate sufficient to suppress completely the fermentation. In the present experiments this observation of Lundsgaard has been repeated (see below). In six experiments with iodoacetate, at various concentrations from $2·5 \times 10^{-4}$ M to 10^{-3} M, in which the inhibition of the anaerobic fermentation ranged from 80 to 100 %, the average R.Q. was found to be 1·01, and in the corresponding control series without iodoacetate, 0·99. It was also found that the yeast used in these experiments showed only a very low rate of respiration in the absence of glucose, so that at least 85 % of the O_2 consumption must represent glucose oxidation, and on that ground alone the R.Q. could not be less than 0·98.

Pasteur effect

(*a*) *From measurements of glucose catabolism.* The averages of fifty experiments on thirty-four samples of yeast are presented in Table 1. This shows that under the conditions described fermentation is completely suppressed by O_2, and the rate of catabolism of glucose is at the same time reduced to about one-fifth (P.Q. = 0·22). The calculation assumes that all the respiration represents glucose oxidation; this is justified on the grounds given above.

(*b*) *From measurements of total glucose utilization.* The rate of disappearance of glucose, as shown in Table 2, is greater than can be accounted for by the rate of catabolism, as was observed by Meyerhof (1925), and the amount of glucose that cannot be accounted for is about the same in the presence and absence of O_2. The result of this is that the P.Q. is much higher when total glucose utilization is taken into account, the figure being 0·52; i.e. the glucose utilization is reduced only to one-half by O_2.

Polysaccharide synthesis

In the previous section it was shown that much of the glucose that disappears cannot be accounted for by either respiration or fermentation. The amount of glucose unaccounted for is roughly the same

Table 1. *The Pasteur quotient of baker's yeast from measurements of glucose catabolized*

The value of $Q^{O_2}_{CO_2}$ is corrected for the simultaneous respiration on the basis of R.Q. = 1·00.

$Q^{N_2}_{Glucose}$ is derived from $Q^{N_2}_{CO_2}$ on the assumption that exactly 2 moles of CO_2 arise from 1 mole of glucose.

$Q^{O_2}_{Glucose}$ is derived from $Q^{O_2}_{CO_2}$ and Q_{O_2} on the same basis with respect to the fermentation, and on the assumption that 6 moles of O_2 represent the oxidation of 1 mole of glucose. The figures in parentheses after the averages are the extreme values observed (in fifty experiments).

Q_{O_2}	−71	(−49 to −90)
$Q^{O_2}_{CO_2}$	1	(−5 to +6)
$Q^{O_2}_{Glucose}$	−11	(−8 to −14)
$Q^{N_2}_{CO_2}$	104	(76 to 148)
$Q^{N_2}_{Glucose}$	−52	(−38 to −74)
P.Q.	0·22	(0·16 to 0·29)

Table 2. *The Pasteur quotient of baker's yeast from measurements of total glucose utilization*

The various determinations were made as described in the Methods section. The averages are those of nineteen experiments. Concentrations of yeast, glucose and buffer as in text. $Q_{Glucose}$ is obtained by making 180 g. of glucose = 22·4 l.

	Glucose			
	In air (mg.)	In N_2 (mg.)	$Q^{O_2}_{Glucose}$	$Q^{N_2}_{Glucose}$
Catabolism (from manometric measurements as in Table 1)	0·24 (0·18–0·29)	1·02 (0·82–1·19)	−11	−45
Utilization (from direct estimation of glucose)	0·80 (0·65–0·98)	1·55 (1·31–1·84)	−36	−69
Glucose utilization not accounted for by catabolism	0·56 (0·39–0·75)	0·52 (0·40–0·69)	—	—
Polysaccharide formation	0·36 (0·30–0·52)	0·28 (0·18–0·41)	+16	+12
P.Q. (catabolism)	0·24 (0·18–0·30)			
P.Q. (total glucose)	0·52 (0·41–0·60)			

aerobically and anaerobically, but the proportion is far greater aerobically (70 %) than anaerobically (34 %). Table 2 shows that of this missing glucose a large part (64 % aerobically and 54 % anaerobically) can be accounted for by an increase in the polysaccharide content of the yeast; the fate of the rest in the aerobic experiments of Pickett & Clifton (1943) could not be determined, but there was no fat synthesis as had been suggested by Meyerhof (1925). The present results are in good quantitative agreement with those of Meyerhof (1925) and Pickett & Clifton (1941, 1943).

Linearity of the processes

Fig. 1 shows that respiration, fermentation, glucose disappearance and polysaccharide synthesis all proceed roughly linearly, under the conditions described, for 80 min., so that it is justifiable to use single determinations at 1 hr. to measure rates. In some experiments manometers were removed for estimations at 20, 40 and 60 min., but in most cases duplicates at 60 min. were taken.

Effect of glucose concentration

Meyerhof (1925), working with a concentration of glucose of 2·5 or 5 %, gave figures for respiration and fermentation which lead to a value of about 0·4 for the P.Q. in baker's yeast (0·6 for bottom yeast). From the use of 0·25 % of glucose in his experiments on the rate of disappearance of glucose determined chemically, he gives data which lead to a P.Q. averaging 0·6. The latter figure is similar to that found in the present experiments at a similar glucose concentration (namely, 0·52), but the former is considerably higher than that shown in Table 1 (namely, 0·22). The effect of glucose concentration on the P.Q. was therefore investigated, with the results shown in Fig. 2. At low glucose concentrations $Q^{O_2}_{CO_2}$ was zero, but began to have a measurable value at a glucose concentration of about 0·5–1 %, and then increased rapidly with the concentration. Q_{O_2} was constant over the whole range above 0·1 %, and $Q^{N_2}_{CO_2}$ increased steadily with the glucose concentration, though less rapidly above 0·5 %. The P.Q. over the middle range of concentrations (0·1–1 %) had its normal low value, in this case about 0·25; at very low concentrations the P.Q. rose because the anaerobic fermentation decreased with fall of glucose concentration more than did the respiration, and at higher concentrations the P.Q. increased because of the appearance of a very considerable aerobic fermentation.

A comparison of the rates of total glucose utilization at a low (0·2 %) and a high (5·0 %) glucose concentration is shown in Table 3. The usual methods were used, except that at the high glucose concentration, to obtain sufficient accuracy in the measurement of the amount of glucose used, the experiment had to be continued for 3 hr. The figures in Table 3 give the average changes per hr. over the 3 hr. period. The only points of difference to be noted are the aerobic fermentation at the higher concentration, already referred to, and the lower yield of polysaccharide at the higher concentration,

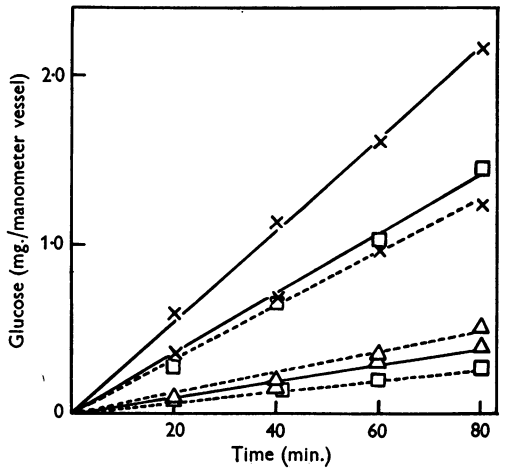


Fig. 1. Linear course of the glucose metabolism of yeast. Initial concentration of glucose was 4·0 mg./manometer vessel (7·4 mM). ×—×, Total glucose used in N_2; ×- - -×, total glucose used in air; □—□, glucose catabolized in N_2; □- - -□, glucose catabolized in air; △—△, polysaccharide synthesized in N_2; △- - -△, polysaccharide synthesized in air.

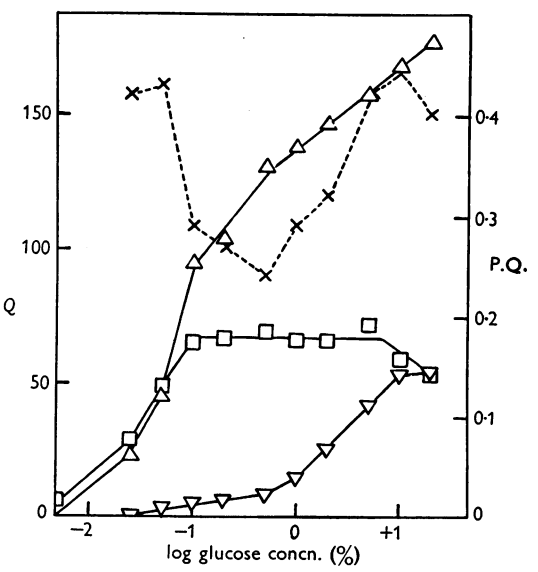


Fig. 2. Effect of glucose concentration on the rates of catabolism of glucose by yeast, and on the P.Q. □—□, Q_{O_2}; △—△, $Q^{N_2}_{CO_2}$; ▽—▽, $Q^{O_2}_{CO_2}$; ×- - -×, P.Q.

Table 3. *Glucose catabolism and total utilization at a high and a low glucose concentration*

	Glucose (mg./manometer vessel)			
	Aerobic		Anaerobic	
Glucose concn. (%)	0·2	5·0	0·2	5·0
Glucose catabolized				
Oxidized	0·58	0·57	0	0
Fermented	0·04	0·40	2·91	4·03
Total	0·62	0·97	2·91	4·03
P.Q.	0·21	0·24	—	—
Total glucose utilized	1·80	2·20	3·87	5·30
P.Q.	0·46	0·42	—	—
Glucose unaccounted for	1·18	1·23	0·96	1·27
Polysaccharide found	0·78	0·32	0·53	0·38

which is accounted for by the longer duration of the experiment. The absolute amount synthesized in the 3 hr. was greater than that at the lower concentration in 1 hr., and presumably represents a saturation of the yeast with polysaccharide.

Effect of inhibitors

Table 4 gives a general survey of the results with a number of poisons on glucose catabolism, calculated in such a way as to bring out any inhibition of the Pasteur effect. The ordinary respiratory inhibitors (sodium azide, KCN, lowered O_2 tension) increased the P.Q. to the same degree as they lowered the respiration, i.e. did not inhibit the Pasteur effect; narcotics (phenylurea, urethane, and many others), though they inhibited to varying degrees both respiration and fermentation, again did not inhibit the Pasteur effect; sodium arsenate and arsenite, although having anomalous effects on respiration and fermentation, did not inhibit the Pasteur effect. Sodium iodoacetate (Lundsgaard, 1930), by inhibiting the fermentation and leaving the respiration relatively unaffected, brought about a rise in the P.Q. which would by the definitions so far advanced constitute an inhibition of the Pasteur effect; it cannot be so regarded, and the definition must be emended to exclude such phenomena (see below). 2:4-Dinitrophenol (DNP) inhibited the Pasteur effect powerfully (Ehrenfest & Ronzoni, 1933). Propionitrile at concentrations of the order of 0·1 M also inhibited, as did ethyl carbylamine, a result already hinted at by Warburg (1926*a*).

From the list of substances in Table 4 were chosen representative inhibitors for the study of their effect on glucose utilization, polysaccharide synthesis and, as reported in a later paper, phosphorylation. Those used were: (*a*) sodium azide and KCN, non-inhibitors of the Pasteur effect; (*b*) DNP and propionitrile, inhibitors of the Pasteur effect; (*c*) urethane, a typical narcotic which inhibits respiration and fermentation, but not the Pasteur effect; (*d*) sodium arsenate, sodium arsenite and sodium iodoacetate, for reasons of general interest not specifically connected with the Pasteur effect.

Sodium azide. Table 4 has shown that with progressively increasing concentrations of sodium azide the respiration was increasingly inhibited, and the P.Q. simultaneously increased towards a limiting value of 1·00.

Table 5 gives data for the total rate of disappearance of glucose and the rate of polysaccharide synthesis at two concentrations of sodium azide. At 10^{-4} M, the total anaerobic glucose utilization was increased over the control roughly in the same proportion as the fermentation; aerobically, the respiration was largely inhibited, and aerobic fermentation appeared, total catabolism being increased, the glucose utilization being increased almost as much; the amount of glucose unaccounted for was not much diminished, and polysaccharide synthesis remained considerable. Thus this concentration of azide merely changed over the mode of breakdown of glucose in O_2 from oxidation to fermentation, brought the rates of catabolism and total utilization of glucose in O_2 up to the anaerobic level, and left polysaccharide synthesis substantially unaltered. At the higher concentration of azide (10^{-3} M) a similar situation was found, except that now the total glucose utilization scarcely exceeded the amount catabolized, and polysaccharide synthesis was almost entirely suppressed, both aerobically and anaerobically.

Potassium cyanide. In Table 4 it was seen that with increasing concentrations of KCN the respiration fell and the P.Q. rose to approximately unity, the anaerobic fermentation rate being unaffected (Warburg, 1925). It is to be noted that a considerable degree of inhibition of respiration appeared before very much aerobic fermentation arose (at 3×10^{-6} M). This was observed consistently, but might conceivably be due to a difference in the HCN concentrations between the vessels in which the CO_2 was absorbed and those in which it was not. The KCN–KOH mixtures of Robbie (1946) were

used for CO_2 absorption in the presence of HCN, and the internal evidence of the experiments themselves (constancy of the degree of inhibition of the respiration during the entire period of the experiment) suggested that the HCN concentrations were being maintained at the level required.

Table 6 shows that KCN up to 10^{-4} M had no effect on the glucose utilization or polysaccharide synthesis, beyond increasing the former quantity up to the anaerobic level.

2:4-*Dinitrophenol.* From the example given in Table 4 it may be seen that DNP increased both respiration and anaerobic fermentation, the P.Q. being raised at moderate concentrations chiefly by the increased respiration, not by a marked aerobic fermentation. Only at very high concentrations did aerobic fermentation become quantitatively important, and at these levels the anaerobic fermentation was already partially inhibited. However, the P.Q. could be raised to values much above 1·00.

The results for glucose utilization and polysaccharide synthesis in the presence of DNP (Table 7) are complicated by the fact that this agent (uniquely among the inhibitors used in the present work) changed the endogenous metabolism in such a way that fermentation of the polysaccharides of the yeast cell was set up (Rothstein & Berke, 1952) and the endogenous respiration was changed from a non-carbohydrate to a purely carbohydrate nature (Stickland, 1956*a*).

The data of Stickland (1956*a*) show that the quantity of intracellular polysaccharide broken down in an experiment such as that recorded in Table 7 would be in the controls nil, and in the presence of DNP at 3×10^{-3} M, aerobically 0·1 mg. and anaerobically 0·03 mg. (These quantities were not determined on the same specimen of yeast, but were constant in a number of other samples.) The endogenous metabolism is thus appreciable, but not large enough to disturb the following conclusions.

Table 4. *Effect of inhibitors on the catabolism of glucose by baker's yeast, and on the Pasteur quotient derived from measurements of catabolism*

Inhibitor	Concn. (M)	Q_{O_2}	$Q^{O_2}_{CO_2}$	$Q^{N_2}_{CO_2}$	$Q^{O_2}_{Glucose}$	$Q^{N_2}_{Glucose}$	P.Q.	Elevation of P.Q. (%)	Inhibition of respiration (%)	Inhibition of Pasteur effect
Sodium azide	0	−62	2	92	−11	−46	0·24	.	.	.
	5×10^{-5}	−45	27	125	−21	−63	0·33	12	27	–
	10^{-4}	−29	88	149	−49	−75	0·65	54	53	–
	0	−68	6	118	−14	−59	0·24	.	.	.
	10^{-4}	−20	135	163	−71	−82	0·86	82	71	–
	2×10^{-4}	−9	158	160	−80	−80	1·00	100	87	–
	0	−66	−1	79	−11	−40	0·27	.	.	.
	10^{-3}	−5	98	96	−50	−48	1·04	106	92	–
KCN	0	−70	0	120	−12	−60	0·20	.	.	.
	10^{-6}	−62	16	(120)*	−18	(−60)*	0·30	13	12	–
	3×10^{-6}	−27	19	(120)*	−14	(−60)*	0·23	4	61	–
	10^{-5}	−10	58	(120)*	−31	(−60)*	0·52	40	86	–
	3×10^{-5}	0	93	(120)*	−47	(−60)*	0·78	73	100	–
	10^{-4}	0	125	(120)*	−63	(−60)*	1·05	106	100	–
	3×10^{-4}	0	132	(120)*	−66	(−60)*	1·10	112	100	–
Decreased O_2 tension (air pressure, mm. Hg)	760	−75	0	103	−13	−52	0·25	.	.	.
	80	−75	0	103	−13	−52	0·25	0	0	–
	25	−47	40	103	−28	−52	0·54	39	37	–
	8	−22	83	103	−45	−52	0·86	81	71	–
Phenylurea	0	−55	4	86	−11	−43	0·26	.	.	.
	10^{-2}	−37	18	72	−15	−36	0·42	22	33	–
	3×10^{-2}	−3	46	43	−23	−22	1·04	105	95	–
Urethane	0	−64	0	90	−11	−45	0·24	.	.	.
	3×10^{-1}	−42	5	40	−12	−20	0·60	47	34	–
	1	−2	14	15	−7	−8	0·88	84	97	–
	3	0	5	6	−3	−3	1·0	100	100	–
Sodium arsenate	0	−58	0	76	−10	−38	0·26	.	.	.
	3×10^{-6}	−39	5	63	−9	−32	0·28	3	33	–
	10^{-5}	−25	6	36	−7	−18	0·39	18	57	–
	0	−62	−1	76	−10	−38	0·26	.	.	.
	$2·5 \times 10^{-5}$	−15	14	28	−10	−14	0·71	61	76	–
	5×10^{-5}	−13	10	24	−7	−12	0·58	43	79	–
	0	−75	0	97	−13	−49	0·27	.	.	.
	10^{-4}	−15	15	27	−10	−14	0·71	60	80	–
	3×10^{-4}	−12	15	28	−10	−14	0·71	60	84	–
	10^{-3}	−11	19	27	−11	−14	0·79	71	85	–

Table 4 (*cont.*)

Inhibitor	Concn. (M)	Q_{O_2}	$Q^{O_2}_{CO_2}$	$Q^{N_2}_{CO_2}$	$Q^{O_2}_{Glucose}$	$Q^{N_2}_{Glucose}$	P.Q.	Elevation of P.Q. (%)	Inhibition of respiration (%)	Inhibition of Pasteur effect
Sodium arsenite	0	−71	−1	94	−13	−47	0·28	.	.	.
	10^{-4}	−29	13	53	−11	−27	0·41	18	61	−
	10^{-3}	−26	12	49	−10	−25	0·40	17	65	−
	10^{-2}	−14	0	5	−2	−3	0·7	60	81	−
Sodium iodoacetate	0	−74	0	97	−12	−49	0·24	.	.	.
	10^{-4}	−84	0	65	−14	−33	0·42	24	−14	?
	$2·5 \times 10^{-4}$	−68	0	12	−11	−6	2	200	8	?
	5×10^{-4}	−53	0	5	−9	−3	3	300	28	?
	10^{-3}	−34	0	1	−6	−1	6	600	54	?
2:4-Dinitrophenol	0	−63	−3	92	−11	−46	0·24	.	.	.
	3×10^{-4}	−81	4	100	−18	−50	0·36	16	−29	+
	10^{-3}	−104	37	117	−55	−59	0·93	91	−65	+ + +
	3×10^{-3}	−63	66	66	−50	−33	1·51	170	0	+ + +
Propionitrile	0	−64	5	108	−13	−54	0·24	.	.	.
	$7·5 \times 10^{-2}$	−61	80	108	−50	−54	0·93	91	5	+ +
	0	−76	0	106	−13	−53	0·25	.	.	.
	$1·25 \times 10^{-1}$	−79	59	90	−45	−45	1·00	100	−4	+ + +
	0	−54	−2	93	−9	−46	0·20	.	.	.
	$2·7 \times 10^{-1}$	−54	83	103	−51	−52	0·98	98	0	+ + +
Ethyl carbylamine	0	−57	2	88	−10	−44	0·23	.	.	.
	10^{-2}	−47	67	84	−42	−42	1·00	100	18	+ +

The following substances were tested, at a range of concentrations causing partial inhibition of the respiration, and were found not to inhibit the Pasteur effect: *sec.*-amyl alcohol, *n*-butylurethane, *n*-butyronitrile, *n*- and *iso*-capronitrile, cresyl blue, methylene blue, 5-nonanone, 1-pentanol, 2-pentanone, phenylurethane, phenosafranine, *n*- and *iso*-valeronitrile.

Calculations from the data of Warburg (1926*b*) and Melnick (1941) on the effect of carbon monoxide on yeast metabolism show that this substance does not inhibit the Pasteur effect in yeast.

Calculations from the data of Quastel & Wheatley (1932) and Runnström & Sperber (1938) show that cysteine, though inhibiting yeast respiration, does not inhibit the Pasteur effect.

p-Nitrophenol (10^{-3} M) and 4:6-dinitro-*o*-cresol (3×10^{-4} M) behaved similarly to DNP, and acetonitrile (M) behaved similarly to propionitrile.

* It was confirmed in other experiments (see, e.g., Table 6) that KCN has no effect on $Q^{N_2}_{CO_2}$.

Table 5. *Effect of sodium azide on glucose utilization and polysaccharide synthesis*

Experimental procedure as described in text. Glucose catabolized is calculated from manometric measurements of respiration and fermentation. Total glucose utilized is from direct chemical estimation. Initial glucose was 4·0 mg./manometer vessel.

	Glucose (mg./manometer vessel)					
	Aerobic			Anaerobic		
	Control	$+NaN_3$ (10^{-4} M)	$+NaN_3$ (10^{-3} M)	Control	$+NaN_3$ (10^{-4} M)	$+NaN_3$ (10^{-3} M)
(*a*) Glucose catabolized						
Oxidized	0·24	0·10	0·01	0	0	0
Fermented	0·01	1·09	1·19	0·82	1·09	1·04
Total	0·25	1·19	1·20	0·82	1·09	1·04
P.Q.	0·31	1·09	1·15	—	—	—
(*b*) Total glucose utilized	0·78	1·59	1·23	1·44	1·68	1·25
P.Q.	0·54	0·95	0·98	—	—	—
(*c*) Glucose unaccounted for	0·53	0·40	0·03	0·62	0·59	0·21
(*d*) Glucose found as polysaccharide	0·35	0·22	0	0·24	0·20	0·03

Anaerobically DNP had no large inhibitory effect on the amount of glucose catabolized, but did decrease the total amount of glucose used, the difference appearing in a diminution of the amount of glucose not accounted for, and consequently in a great lowering of polysaccharide synthesis. Aerobically there was a marked increase in the glucose catabolism even to a higher level than the anaerobic (i.e. P.Q. 1·00), but the total glucose utilized was again not greatly affected, so that the chief change was the disappearance of the 'glucose unaccounted for' fraction, and, of course, of the polysaccharide synthesis. At the higher concentrations an actual disappearance of polysaccharide, even in the presence of glucose, can be observed.

Table 6. *Effect of potassium cyanide on glucose utilization and polysaccharide synthesis*

Details are as given in Table 5.

	Glucose (mg./manometer vessel)									
	Aerobic					Anaerobic				
	Control	+KCN (3×10^{-6} M)	+KCN (10^{-5} M)	+KCN (3×10^{-5} M)	+KCN (10^{-4} M)	Control	+KCN (3×10^{-6} M)	+KCN (10^{-5} M)	+KCN (3×10^{-5} M)	+KCN (10^{-4} M)
(*a*) Glucose catabolized										
Oxidized	0·22	0·16	0·04	0·00	0·00	0	0	0	0	0
Fermented	0·01	0·06	0·32	0·74	1·09	1·10	1·01	1·02	0·99	1·07
Total	0·23	0·22	0·36	0·74	1·09	1·10	1·01	1·02	0·99	1·07
P.Q.	0·24	0·22	0·35	0·75	1·02	—	—	—	—	—
(*b*) Total glucose utilized	0·65	0·86	1·00	1·34	1·57	1·59	1·65	1·56	1·72	1·62
P.Q.	0·41	0·52	0·64	0·78	0·97	—	—	—	—	—
(*c*) Glucose unaccounted for	0·39	0·61	0·64	0·60	0·48	0·49	0·64	0·54	0·73	0·55
(*d*) Glucose found as polysaccharide	0·31	0·35	0·33	0·28	0·32	0·31	0·32	0·32	0·31	0·32

Table 7. *Effect of DNP on glucose utilization and polysaccharide synthesis*

Details are as given in Table 5.

	Glucose (mg./manometer vessel)							
	Aerobic				Anaerobic			
	Control	+DNP (10^{-3} M)	+DNP (3×10^{-3} M)	+DNP (10^{-2} M)	Control	+DNP (10^{-3} M)	+DNP (3×10^{-3} M)	+DNP (10^{-2} M)
(*a*) Glucose catabolized								
Oxidized	0·24	0·27	0·26	0·28	0	0	0	0
Fermented	0·01	0·36	0·58	0·64	0·82	1·04	1·08	0·70
Total	0·25	0·63	0·84	0·92	0·82	1·04	1·08	0·70
P.Q.	0·31	0·60	0·78	1·31	—	—	—	—
(*b*) Total glucose utilized	0·78	1·06	0·95	0·78	1·44	1·63	1·34	0·90
P.Q.	0·54	0·65	0·71	0·87	—	—	—	—
(*c*) Glucose unaccounted for	0·53	0·43	0·11	−0·14	0·62	0·59	0·26	0·20
(*d*) Glucose found as polysaccharide	0·35	0·13	−0·03	−0·07	0·24	0·18	0·03	−0·05

Propionitrile. Propionitrile, the other typical inhibitor of the Pasteur effect, differs in its action in several ways from DNP. In Table 4 it can be seen that the value of the P.Q. was never raised significantly above 1·00 (this is true of many other experiments not reported in detail). In Table 8 the effect of propionitrile on glucose utilization and polysaccharide synthesis is shown. Propionitrile (in this example at 0·25 M though more usually 0·15 M was enough to produce the same effect) increased the amount of glucose catabolized aerobically nearly fourfold, almost to the anaerobic level, but increased the total amount of glucose used only by about 30 %. This resulted in a smaller increase in the P.Q., when this was expressed in terms of the total glucose utilized, and also in a smaller amount of glucose unaccounted for, although the rate of polysaccharide synthesis remained almost unaffected by propionitrile.

In a single experiment with ethyl carbylamine (10^{-2} M) the same results were observed, the P.Q. being raised from 0·22 to 0·96, although the rate of polysaccharide synthesis, both aerobic and anaerobic, was unaffected.

Urethane. Urethane may be taken as typical of that majority of substances listed in Table 4, which are known to be narcotics, and which inhibit respiration and fermentation to approximately the same degree, and which increase the P.Q. only in proportion to the inhibition of the respiration. They do not increase the value of $Q^{O_2}_{Glucose}$, or do so only slightly, but cause an increase in P.Q. by decreasing the value of $Q^{N_2}_{Glucose}$.

Table 9 shows that, at 0·3 M, a concentration sufficient to inhibit respiration and fermentation partially, the whole metabolism, including polysaccharide synthesis, proceeded normally but at a diminished rate; at 0·9 M, at which concentration respiration and fermentation were almost abolished, little glucose was unaccounted for and no polysaccharide was formed.

Sodium arsenate. The peculiar features of the action of sodium arsenate (Table 4) were that it inhibited both respiration and fermentation by some 70 % at low concentrations ($2·5 \times 10^{-5}$ M), and that this inhibition was scarcely increased by increasing the concentration up to 10^{-3} M, i.e. there was a residuum of both respiration and fermentation which was arsenate-stable.

Table 8. *Effect of propionitrile on glucose utilization and polysaccharide synthesis*

Details are as given in Table 5. PN = propionitrile.

	Glucose (mg./manometer vessel)			
	Aerobic		Anaerobic	
	Control	+PN (0·25 M)	Control	+PN (0·25 M)
(*a*) Glucose catabolized				
Oxidized	0·29	0·30	0	0
Fermented	0·00	0·66	1·19	1·01
Total	0·29	0·96	1·19	1·01
P.Q.	0·24	0·95	—	—
(*b*) Total glucose utilized	0·98	1·27	1·84	1·60
P.Q.	0·53	0·79	—	—
(*c*) Glucose unaccounted for	0·69	0·31	0·65	0·59
(*d*) Glucose found as polysaccharide	0·38	0·30	0·41	0·30

Table 9. *Effect of urethane on glucose utilization and polysaccharide synthesis*

Details are as given in Table 5.

	Glucose (mg./manometer vessel)					
	Aerobic			Anaerobic		
	Control	+Urethane (0·3 M)	+Urethane (0·9 M)	Control	+Urethane (0·3 M)	+Urethane (0·9 M)
(*a*) Glucose catabolized						
Oxidized	0·24	0·16	0·04	0	0	0
Fermented	0·00	0·06	0·20	1·01	0·45	0·08
Total	0·24	0·22	0·24	1·01	0·45	0·08
P.Q.	0·24	0·49	3	—	—	—
(*b*) Total glucose utilized	0·97	0·74	0·19	1·70	1·03	0·17
P.Q.	0·57	0·72	1·1	—	—	—
(*c*) Glucose unaccounted for	0·73	0·52	0	0·69	0·58	0·09
(*d*) Glucose found as polysaccharide	0·52	0·28	0	0·30	0·16	0

Table 10. *Effect of sodium arsenate on glucose utilization and polysaccharide synthesis*

Details are as given in Table 5.

	Glucose (mg./manometer vessel)					
	Aerobic			Anaerobic		
	Control	$+AsO_4^{3-}$ (3×10^{-5} M)	$+AsO_4^{3-}$ (3×10^{-4} M)	Control	$+AsO_4^{3-}$ (3×10^{-5} M)	$+AsO_4^{3-}$ (3×10^{-4} M)
(*a*) Glucose catabolized						
Oxidized	0·29	0·09	0·06	0	0	0
Fermented	0·00	0·23	0·22	1·19	0·60	0·53
Total	0·29	0·32	0·28	1·19	0·60	0·53
P.Q.	0·24	0·60	0·47	—	—	—
(*b*) Total glucose utilized	0·98	0·45	0·43	1·84	0·80	0·73
P.Q.	0·53	0·62	0·54	—	—	—
(*c*) Glucose unaccounted for	0·69	0·13	0·15	0·65	0·20	0·20
(*d*) Glucose found as polysaccharide	0·38	0·05	0·01	0·41	0·04	0·03

Table 11. *Effect of sodium arsenite on glucose utilization and polysaccharide synthesis*

Details are as given in Table 5. The reducing action of the arsenite on the ferricyanide used in the glucose estimations was allowed for by carrying out the necessary control determinations.

	Glucose (mg./manometer vessel)					
	Aerobic			Anaerobic		
	Control	$+AsO_3^{3-}$ (10^{-4} M)	$+AsO_3^{3-}$ (10^{-3} M)	Control	$+AsO_3^{3-}$ (10^{-4} M)	$+AsO_3^{3-}$ (10^{-3} M)
(*a*) Glucose catabolized						
Oxidized	0·28	0·11	0·10	0	0	0
Fermented	0·00	0·14	0·13	1·05	0·59	0·55
Total	0·28	0·25	0·23	1·05	0·59	0·55
P.Q.	0·27	0·42	0·42	—	—	—
(*b*) Total glucose utilized	0·79	0·87	0·64	1·67	1·23	1·05
P.Q.	0·47	0·71	0·57	—	—	—
(*c*) Glucose unaccounted for	0·51	0·62	0·41	0·62	0·64	0·50
(*d*) Glucose found as polysaccharide	0·31	0·22	0·19	0·22	0·24	0·15

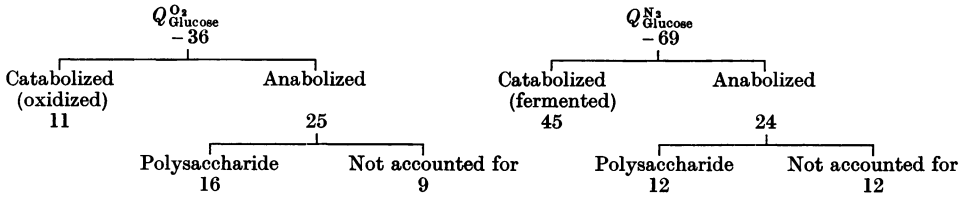
Table 12. *Effect of sodium iodoacetate on glucose utilization and polysaccharide synthesis*

Details are as given in Table 5. IAc = iodoacetate.

	Glucose (mg./manometer vessel)					
	Aerobic			Anaerobic		
	Control	+IAc ($2{\cdot}5 \times 10^{-4}$ M)	+IAc (5×10^{-4} M)	Control	+IAc ($2{\cdot}5 \times 10^{-4}$ M)	+IAc (5×10^{-4} M)
(*a*) Glucose catabolized						
Oxidized	0·24	0·22	0·20	0	0	0
Fermented	0·00	0·04	0·03	1·07	0·07	0·03
Total	0·24	0·26	0·23	1·07	0·07	0·03
P.Q.	0·22	4	8	—	—	—
(*b*) Total glucose utilized	0·76	0·68	0·33	1·50	0·21	0·07
P.Q.	0·50	3	5	—	—	—
(*c*) Glucose unaccounted for	0·52	0·42	0·10	0·43	0·14	0·04
(*d*) Glucose found as polysaccharide	0·34	0·26	0·05	0·24	0	0

Table 13. *Fate of the glucose utilized by yeast under aerobic and anaerobic conditions*

All the figures are in terms of $Q_{Glucose}$.



In Table 10 it can be seen that aerobically, though catabolism was unaffected by arsenate (oxidation being partly replaced by fermentation), total glucose utilization was inhibited, with the result that much less glucose was unaccounted for, and polysaccharide synthesis was almost suppressed. This was true of two arsenate concentrations, differing tenfold, and similar results were obtained in other experiments at 10^{-5} and 10^{-4} M.

Sodium arsenite. The effects of arsenite on the rate of catabolism of glucose, and on the value of the P.Q. derived from them, were very similar to those of arsenate (Table 4). This inhibitor differed, however, in causing less inhibition of the total utilization of glucose, and leaving the polysaccharide synthesis at a much higher level, both aerobically and anaerobically (Table 11).

Sodium iodoacetate. Sodium iodoacetate is the only substance known which at a carefully chosen concentration (usually $2{\cdot}5 \times 10^{-4}$ M, but with some specimens of yeast slightly more or less) causes no inhibition of respiration and almost complete inhibition of the fermentation (Lundsgaard, 1930).

Its effect on glucose utilization (Table 12) was, aerobically, to decrease the rate, so that the gap between glucose used and glucose catabolized was narrowed; of the glucose left unaccounted for the same proportion, though a greatly diminished amount, was synthesized to polysaccharide. Anaerobically, catabolism and polysaccharide synthesis were equally eliminated by iodoacetate.

DISCUSSION

The extent of the Pasteur effect in yeast has previously been considered only from the point of view of the amount of glucose catabolized (see Dickens, 1951), and values of the P.Q. varying from 0·2 to 0·4, according to the concentration of glucose employed, may be calculated from the data of Meyerhof (1925), Lundsgaard (1930) and many other authors for catabolism by baker's yeast. The same two authors also give figures showing the total amount of glucose used by the yeast, from which may be calculated values of the P.Q. from 0·5 to 0·6, as found in the present paper.

The distribution of the glucose used, between catabolism and synthesis into cell material, in air and in nitrogen, is shown in Table 13. It is plain that almost the same rate of synthesis, chiefly into polysaccharide, is found aerobically and anaerobically, and the effect of oxygen is restricted to limiting the amount of glucose broken down.

Mechanism of the Pasteur effect

It is not enough therefore to account for the Pasteur effect by a reversible partial inactivation by oxygen of hexokinase (cf. Colowick & Price, 1945), as the effect of oxygen is not only to diminish the rate of formation of glucose 6-phosphate, but also, more markedly, to change the subsequent reactions it undergoes.

The only hypothesis so far propounded which could account for these facts is that of Engelhardt & Sakov (1943) which suggests a complete reversible inhibition by oxygen of some stage between glucose 6-phosphate and triose phosphate, so that the glucose 6-phosphate would be forced into the path of the Warburg–Dickens oxidation route. The rates would be automatically fixed by the concentration of the enzymes catalysing the Warburg–Dickens reactions, the rate of oxidation directly, and the rate of attack on glucose by the amount of adenosine triphosphate rendered available by the oxidation.

It is not easy on such lines to account for the effect of increasing glucose concentration (Fig. 2). If oxygen were acting by inhibiting completely the fermentative route of glucose breakdown, then glucose must at higher concentrations counteract this inhibition. No mechanism for such an effect suggests itself.

Endogenous metabolism

The endogenous metabolism of the baker's yeast used in these experiments was negligibly small (Stickland, 1956*a*), the average of a number of specimens being $Q^{N_2}_{CO_2} = 0$, $Q_{O_2} = -5$. The specimens used for these measurements were only in part the same specimens as those used in the present paper, but this yeast showed a great uniformity of behaviour, and the average of one set of specimens may be legitimately compared with the average of

another set. The evidence of inhibition by potassium cyanide suggests that glucose suppresses the endogenous respiration, but other evidence suggests that it does not (Stickland, 1956*a*). For the purposes of the arguments about the Pasteur effect presented here, the endogenous metabolism may be safely ignored.

Definition of inhibition of the Pasteur effect

Although the term Pasteur effect is completely defined by any one of the normally current definitions, the expression inhibition of the Pasteur effect, when one is thinking in terms of the action of oxygen in diminishing the rate of attack on glucose, is not so easy to define. In the present paper it has so far been described only as an increase in the Pasteur quotient not accompanied by an inhibition of the respiration. However, it is clear now that equality of $Q^{O_2}_{Glucose}$ and $Q^{N_2}_{Glucose}$, together with an undiminished Q_{O_2}, are not enough to constitute an inhibition of the Pasteur effect; it must be stipulated that the $Q^{O_2}_{Glucose}$ shall be increased towards the anaerobic level, not the $Q^{N_2}_{Glucose}$ brought down to the aerobic level. Hence the definition must include a term excluding cases in which the fermentation is powerfully inhibited, as found with sodium iodoacetate.

Inhibition of the Pasteur effect in yeast

The Pasteur effect in mammalian tissues has been shown to be inhibited by a very wide range of substances (see Dickens, 1951). In yeast the greater number of these have no action, and the only two classes of substances having such an effect are (1) nitrophenols, and (2) some nitriles and ethyl *iso*-nitrile.

The nitrophenols (exemplified by DNP) increase the P.Q. to values well above unity (up to 1·8), i.e. more glucose is broken down aerobically than anaerobically. On the other hand, the total rate of utilization of glucose aerobically never exceeds that observed anaerobically. This difference is due to the fact that aerobically the whole of the glucose that disappears can be accounted for by fermentation and respiration, and no synthetic reactions remain (cf. Clifton, 1937), whilst anaerobically a considerable part of the glucose used cannot be so accounted for (from one-third to one-half as much as in the control in several experiments), though even anaerobically polysaccharide synthesis is almost completely abolished by 3×10^{-3} M DNP. The chief effect of DNP is therefore not to increase the amount of glucose attacked, but to direct it from synthetic pathways into catabolism (cf. Pickett & Clifton, 1941). It is clear then that enough adenosine triphosphate must be made available, even in the presence of DNP, to maintain the rate of attack on glucose at the same level as in the absence of DNP; any 'uncoupling' of phosphorylation must apply only to that part of the adenosine triphosphate required for synthetic processes.

The nitriles (exemplified by propionitrile) behave quite differently. The P.Q. in a large number of experiments has never been observed to exceed unity, and the increase in catabolism of glucose is paralleled by an increase in total glucose utilization, the polysaccharide synthesis being unaffected.

Of the other respiratory inhibitors tested, potassium cyanide is the simplest in action, as it causes oxidation of glucose merely to be replaced by fermentation and leaves the synthetic processes, including polysaccharide synthesis, untouched; sodium azide shows the same behaviour at 10^{-4} M, but at 10^{-3} M the synthetic reactions also are inhibited.

Iodoacetate has the reverse effect, eliminating the anaerobic processes and leaving the aerobic almost unaffected (at $2{\cdot}5 \times 10^{-4}$ M in the example given, Table 12), including polysaccharide synthesis.

Glucose itself may be said at very high concentrations to inhibit the Pasteur effect partially. This effect, as with propionitrile, is brought about by causing the appearance of some aerobic fermentation, and does not involve any interference with synthetic reactions.

SUMMARY

1. For the quantitative expression of that aspect of the Pasteur effect which consists of a lowering by oxygen of the rate of glucose utilization, the term Pasteur quotient (P.Q.) is proposed:

$$\text{P.Q.} = Q^{O_2}_{Glucose}/Q^{N_2}_{Glucose}.$$

2. The value of P.Q. in baker's yeast at medium glucose concentrations is 0·2–0·25, when expressed in terms of glucose catabolized, and 0·5–0·6 in terms of total glucose utilized.

3. At high glucose concentrations the P.Q. rises from 0·2 to 0·4, through the appearance of aerobic fermentation.

4. The rates of glucose breakdown and polysaccharide synthesis, aerobically and anaerobically, are reported.

5. Of the many substances which inhibit the Pasteur effect in some mammalian cells, only two classes also inhibit it in yeast: (i) nitrophenols, and (ii) lower aliphatic nitriles and *iso*nitriles.

6. The action of these two groups of substances differ in the following respects: (*a*) 2:4-Dinitrophenol (DNP) increases the amount of glucose catabolized aerobically above that catabolized anaerobically, i.e. P.Q. > 1·0; propionitrile never increases the P.Q. above 1·0. (*b*) DNP entirely

inhibits polysaccharide synthesis aerobically, propionitrile does not.

7. The bearing of these results on the mechanism of the Pasteur effect is discussed.

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Phosphorylations and Dephosphorylations in Yeast and their Inhibition by Various Agents

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Lynen (1941) and Lynen & Koenigsberger (1951) have related the Pasteur effect to phosphorylation processes, attributing the lowered rate of attack on glucose in oxygen to the lowered level of inorganic phosphate which results from the greater aerobic rate of phosphorylation, and which limits the rate of oxidation of triose phosphate.

In the preceding paper (Stickland, 1956) the action of selected inhibitors on the carbohydrate metabolism of yeast, and especially on the Pasteur effect, was studied. The present paper studies more exactly the processes of phosphorylation and dephosphorylation as they appear in one brand of baker's yeast, and the effect of the same inhibitors on these processes.

MATERIALS AND METHODS

Yeast. The same brand of commercial pressed baker's yeast was used as in the previous paper (Stickland, 1956).

Measurement of the rate of phosphorylation. Baker's yeast as bought contains about 4 mg./g. dry wt. of P extractable by trichloroacetic acid solution, of which about one-half is present as inorganic orthophosphate. When glucose is added, the total extractable P remains constant, and the inorganic P level falls (Macfarlane, 1936). This fall is rapid, and more rapid under aerobic than under anaerobic conditions (Lynen, 1941). With the yeast used in the present experiments, the fall in inorganic P aerobically had reached its maximum in about 2 min., and was not linear even for 10 sec. To cope with this rapidity of reaction, and with the fact that aeration by bubbling O_2 through the solutions was excluded because volatile inhibitors were to be used, the following methods were employed.

(*a*) Aerobic rate. Into each of a series of 4 in. $\times$ 0·5 in. Pyrex test tubes were measured 2·0 ml. of a 15% suspension of yeast (about 80 mg. dry wt.) and 0·5 ml. of 0·1 M aminotrishydroxymethylmethane, pH 6·5. These mixtures were allowed to stand at room temperature for about 30 min. A volume (0·5 ml.) of 0·8% glucose solution was added rapidly, and the tube immediately closed by a thumb and shaken violently for the necessary time. Shaking was stopped 1 sec. before the period was finished, and at the proper time 2·0 ml. of 10% trichloroacetic acid solution was run in from a burette. Enough O_2 would be present in solution to keep the respiration going for several seconds. The time of the experiment was taken as that between the commencement of the shaking and the first entry of the trichloroacetic acid, and this interval could be measured with an error of probably 1 or 2 sec. For the control uninhibited rates, duplicate tubes were usually taken at 0, 5, 10, 15 and 20 sec.; the intervals were lengthened when the phosphorylation was inhibited.

Inhibitors, when present, were added, dissolved at the appropriate concentration in 0·5 ml. of tris. The concentration given is that present in the final mixture, after the addition of the glucose solution. The yeast was therefore exposed to a concentration of inhibitor 1·2 times that stated for periods not less than 15 min. and not greater than 60 min. before the addition of the glucose.