Table 2. Fractional Adsorption of UDPG-Dehydrogenase with Calcium Phosphate Gel

Adsorption of enzyme from solution in 0·01 *M* phosphate *p*H 6·8	
Total volume of gel added (ml.)	Total units* of enzyme in solution
0	12·4
9·0	36·3
18·0	49·5
30·0†	60·2
3·0	50·5
6·0	46·5
21·0	31·5
51·0	0

* Measured by the rate of reduction of diphosphopyridine nucleotide (7×10^{-4} *M*) in presence of glycine buffer (0·15 *M*, *p*H 8·7) and UDPG ($1{\cdot}5 \times 10^{-4}$ *M*). Micro-cells with total volumes of 0·7 ml. were used in a Beckman *DU* spectrophotometer at 30° C. The unit of enzyme activity is defined as a change in optical density at 340 mμ of 0·100 per min. at 30° C.

† At this point all the added gel was discarded ; 23 per cent of the total protein had disappeared from solution.

in a typical experiment, 23 per cent of the total protein had been adsorbed to the gel.

It can be seen that the fractional procedure brings about removal of an interfering system as previously mentioned, together with a substantial amount of protein. During the latter part of the step, the enzyme is adsorbed to the gel. The gel is washed with 0·01 *M* phosphate *p*H 6·8 and the enzyme eluted with 0·1 *M* phosphate buffer *p*H 7·4. Quantitative recovery of the enzymes (peak values) have been obtained in these experiments.

The results indicate that in procedures for the purification of other enzymes or proteins which involve the use of adsorption-elution techniques, the fractional methods described here may be of considerable value. Such procedures are relatively easy to apply provided that rapid assay methods are available.

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The Pasteur Effect in a Respiration-deficient Mutant of Baker's Yeast

The respiration-deficient mutant of baker's yeast, *Saccharomyces cerevisiae R XII A*, induced by high-temperature cultivation, was used for examining the point of action of oxygen in the Pasteur effect. The mutant was identical with the *petite colonie* mutant described by Ephrussi[1] and Raut[2]. The argument was that, if oxygen acted directly on some enzyme involved in glucose degradation (for example, glyceraldehyde phosphate dehydrogenase), it would decrease the rate of glucose catabolism even in the mutant lacking essential components of the cytochrome system.

It was found that in the presence of glucose there was no demonstrable uptake of oxygen by the suspension and that the Q_{O_2} (=100–130) was not affected by the introduction of oxygen into the suspension. The uptake of glucose, as measured by

Table 1. Inorganic Phosphate and Labile Phosphate Levels in the Respiration-Deficient Mutant (μgm. Phosphorus/mgm. Dry Weight ± *S.E.*)

Incubation conditions	Inorganic phosphate	Labile phosphate
No substrate, anaerobically	4·24 ± 0·24	5·84 ± 0·31
No substrate, 10 min. aeration	3·63 ± 0·26	6·39 ± 0·67
5 per cent glucose, anaerobically	2·37 ± 0·23	7·15 ± 0·71
5 per cent glucose, 10 min. aeration	2·40 ± 0·21	7·26 ± 0·75

quantitative analysis as well as by distribution of radioactivity following application of universally labelled ^{14}C-glucose, revealed no differences between aerobic and anaerobic conditions. As another criterion of the Pasteur effect, the change of inorganic phosphate-level was examined. Here again no change due to oxygen could be observed to take place in the steady-state level in the presence of glucose (Table 1).

If these results can be generalized they may serve as a weighty support for the theory that oxygen brings about the Pasteur effect not by affecting directly any enzyme involved in the anaerobic breakdown of glucose but rather by making it possible for other catabolic pathways to function (much in line with Lynen's views[3,4]).

It was observed, however, that under conditions when no substrate was present in the medium, oxygen was effective in producing changes in the amounts or, at any rate, in the distribution of some metabolically active components of the mutant cell. Some of these changes (for example, in the penetration of free glucose into the cell) are dealt with elsewhere[5]. It was found here that oxygen decreased the level of inorganic phosphate in the absence of glucose, with simultaneous production of labile phosphate bonds (Table 1), the process being completely blocked by 10^{-4} *M* potassium cyanide but only partly inhibited (at most by 20 per cent of the steady-state level change) by 5×10^{-4} *M* 2,4-dinitrophenol. The generation of high-energy phosphate bonds was accompanied by only a minute oxygen consumption ($Q_{O_2} = 0{\cdot}25$–$0{\cdot}32$; $Q_{CO_2} = 1{\cdot}09$–$1{\cdot}48$) so that the apparent phosphorus : oxygen ratio reached values far exceeding those commonly reported for whole cells and even for purified enzyme systems. Thus endogenous phosphorylation in the mutant (and very likely in ordinary baker's yeast, ref. 6) appears to be qualitatively different from phosphorylation proceeding with glucose, and produces high-energy bonds at the expense of other energy sources (physicochemical state of proteins, nucleic acids ?) than those involved in oxidative phosphorylation known heretofore.

Detailed results will be published in *Folia microbiologica*.

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