RIBOFLAVIN ENHANCEMENT OF RADIOACTIVE PHOSPHATE EXCHANGE BY YEASTS

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It has recently been suggested by von Hevesy¹ that processes occurring at the cell surfaces or just inside cell boundaries play an important part in phosphorus metabolism in yeast. We have obtained evidence that we interpret in a similar vein, and suggest the existence of a 'P-complexing factor' at the cell surface.

We have found it possible to increase the radioactive phosphate (P^{32}) exchanged by yeast in the presence of glucose by the addition of trace amounts of riboflavin to the suspending medium. The increases observed range from 20 to 200 per cent over the phosphate exchanged by controls in glucose alone. This effect has been found in two widely different species of yeasts.

The organisms used were *Candida albicans*, a pathogenic non-sporulating yeast, and *Saccharomyces cerevisiae* (Carlsberg No. 237). Cells were grown in wort or in a medium (GGY) consisting of: glucose, 2.0 gm.; glycine, 1.0 gm.; yeast extract ('Difco'), 0.1 gm.; and distilled water, 100 ml., and incubated for 24 hours at 20° C. with constant agitation. The yeast crop was harvested by centrifugation, washed twice with glass-distilled water, and suspended in glass-distilled water to a known density. The P^{32} was usually introduced at this time. Aliquots of the suspension were placed in flasks (with a fritted glass aeration tube leading to the bottom of the flask) in the following medium: yeast suspension, $M/5,000$ potassium dihydrogen phosphate, $M/15$ glucose, water or riboflavin (50 $\mu\text{gm./c.c.}$). The flasks were aerated with washed and filtered compressed air, or with 95 per cent nitrogen with 5 per cent carbon dioxide (passed through a heated copper coil). After the experimental period, the yeast cells were removed by centrifugation, washed twice with glass-distilled water and the yeast cream placed in aluminium dishes adapted for Geiger-Müller counting, and measured automatically². The results obtained in the two experiments reported here are typical of those we have obtained in many other experiments.

Addition of riboflavin (1 $\mu\text{gm./c.c.}$ final concentration) to suspensions of *Saccharomyces cerevisiae* or *Candida albicans* metabolizing glucose aerobically or anaerobically prompted a marked increase in P^{32} exchange. As shown in Table 1, this addition of riboflavin more than doubled the P^{32} exchanged aerobically by *S. cerevisiae* with either glucose or sucrose as source of carbohydrate. In the same experiment, 2,4 dinitrophenol in a concentration of $10^{-3} M$ inhibited P^{32} exchange approximately 40 per cent; this is in agreement with the inhibitory action of this substance on phosphate uptake by *S. cerevisiae* reported by Hotchkiss³.

If a P-complexing factor is formed at the cell surface, it is possible that, once formed, it might act

TABLE 1. *S. cerevisiae*, GROWN AEROBICALLY IN GGY MEDIUM FOR 24 HR. AT 25° C.
Not starved; exposed aerobically to P^{32} at 25° C., for 5 hr. with treatments as listed (40 mgm. yeast dry wt. per vessel)

Vessel	Treatment	Counts/min./mgm. dry wt. (standard units)
1	glucose + glycine	13.6
2	sucrose + glycine	12.5
3	glucose + glycine + 2,4 dinitrophenol	7.9
4	sucrose + glycine + 2,4 dinitrophenol	7.5
5	glucose + glycine + riboflavin	30.7
6	sucrose + glycine + riboflavin	32.8

in the absence of the energy-yielding processes apparently necessary for its formation⁴. An experiment was conducted along these lines wherein *C. albicans* was incubated aerobically at 20° C. in the presence of riboflavin and glucose, then equilibrated at + 4° C. and exposed to P^{32} . Table 2 shows that the starved suspension of the yeast exchanged twice as much P^{32} as the unstarved suspension (both suspensions were prepared from the same culture of yeast). It is also seen that riboflavin prompted a nearly four-fold increase in the exchange of P^{32} by the starved cells, but was without effect on the unstarved yeast. The occurrence of phosphate exchange at a very slow rate by yeast at + 4° C. has been reported by Lindahl *et al.*⁵.

TABLE 2. *C. albicans*, GROWN AEROBICALLY IN 'TULAUF' WORT AT 20° C. FOR 26 HR. WITH AGITATION.
Half the suspension not starved (stored at 4° C. for 18 hr.), the other half starved by agitating aerobically in water for 18 hours at 25° C. Both suspensions treated as below for 24 hr. at 25° C. aerobically with constant agitation (50 mgm. yeast dry wt. per vessel), then placed at - 4° C. for 45 min. P^{32} was then added and flasks held at + 4° C. in presence of P^{32} for 5 hr. Cells separated by centrifugation and washed in the cold

Vessel	Treatment	Counts/min. (standard units)	Counts/min./mgm. dry wt.
1	Cells starved		
2	glucose + riboflavin	100	2.2
	glucose	33.2	0.6
3	Not starved		
4	glucose + riboflavin	15.4	0.3
	glucose	19.5	0.3

The increase in rate of uptake of P^{32} in the residual-phosphorus fraction (presumably metaphosphate¹¹) of yeast brought about by cyanide⁶ might be considered in the light of the present data on riboflavin in view of the action shown by Pett⁷ for the increase in riboflavin synthesis by yeast treated with cyanide. Among the several compounds examined, yeast nucleic acid, uracil, and fructose 1,6 diphosphate proved without detectable effect on phosphorus exchange by *C. albicans* metabolizing glucose aerobically.

Yeasts are well known to be relatively rich in riboflavin, so it is rather surprising to find that added riboflavin exerts such marked effects as we have noted. An average value for the riboflavin content of baker's yeast may be taken from Pett⁷ as 20-30 $\mu\text{gm./gm.}$ dry wt., of which 80-90 per cent exists as bound riboflavin. In our experiments usually 30-50 mgm. dry weight of yeast was placed in each flask containing 50 ml.; thus a total of approximately 1 $\mu\text{gm.}$ of riboflavin was introduced into each flask with the yeast. If all this riboflavin were to have been released from the yeast (not possible under our conditions) a concentration of 0.02 $\mu\text{gm.}$ riboflavin per ml. would have resulted. The quantities of riboflavin introduced into each flask as impurities in the sugars and glycine (reagent quality,

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but not especially purified) are undoubtedly minute. While some species of the genus *Candida* are known⁸ to produce sufficient riboflavin on controlled low-iron media to colour the medium, we have never succeeded, using the strains of *Candida albicans* employed here, in obtaining more than very small yields of riboflavin with similar, purified, known-iron media.

While it is well known that the presence of external phosphate is not essential for the oxidation and assimilation of glucose by yeast suspensions⁹, it is also known that phosphate exchange^{4,6} and polymerization (as measured by the increase in basophilicity¹¹) are dependent on the presence of external metabolizable substrate.

In view of the correlation which has been pointed out³ between the action of 2,4 dinitrophenol on the assimilation of carbon sources by micro-organisms and its action on the uptake or exchange of inorganic phosphate by micro-organisms, it is of interest to compare the action of riboflavin with that of other agents on these two processes. Such a comparison has been made in Table 3 (references noted). It appears there is a decided 1:1 correlation between the processes of phosphate uptake and assimilation of carbon by micro-organisms. At the concentrations stated, the agents listed in Table 3 are without effect on the rate of aerobic or anaerobic breakdown of carbon sources. The effect noted¹⁰ for riboflavin on assimilation was observed with the two organisms reported on in this paper. From concomitant investigations¹⁰ on the variation in basophilicity of yeasts (see Wiame¹¹), it is believed that the 1:1 correlation between assimilation and phosphate uptake results from the, possibly coupled, simultaneous polymerization by yeasts of inorganic phosphate (to polyphosphates) and of carbon sources (to carbohydrate polymers).

TABLE 3

Substance	Concentration	Assimilation of carbohydrate	Uptake or exchange of inorganic phosphate
2,4 Dinitrophenol	10^{-4} M	inhibited ³	inhibited ³
Sodium azide	10^{-4} M	inhibited ³	inhibited ^{3,12}
Gramicidin*	40 μ gm./ml.	inhibited ³	inhibited ³
Glucose	10^{-2} M	a substrate	enhanced ^{4,6}
Riboflavin	2.5×10^{-4} M	enhanced ¹⁰	enhanced

* Acting on *Staphylococci*, other agents acting on yeast.

The observation of Malm¹² that phosphate exchange by yeast is sensitive to the pH of the medium can be taken as evidence that a surface reaction, easily affected by external pH, is involved in phosphate exchange (see Myrback and Vasseur¹⁴ for an extended discussion of the considerations underlying this line of reasoning). It is unlikely that phosphate enters the yeast cell in an ionized form, and it seems reasonable that phosphate is brought through the cell barrier by uniting with some 'complexing substance' existing at the cell surface. The compound of this substance with phosphate may then dissociate, or in other manner lose phosphate to the cell (possibly to coenzyme I; see Lindahl *et al.*⁵), and free 'complexing substance' to act again. From our observations we believe that such a role as postulated for the 'complexing substance' may be filled by riboflavin. Combined roles of phosphorus and hydrogen transference for trace-active substances may assume importance in considerations of *in vivo* processes.

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FACTORS INVOLVED IN THE DEACTIVATION OF PENICILLIN SOLUTIONS BY RUBBER TUBING

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COWAN¹ in 1945 demonstrated that aqueous solutions of penicillin could lose up to 50 per cent of their antibiotic activity by simple passage through the rubber tube of a continuous-drip apparatus. These results were confirmed by Huelsbusch, Foter and Gibby², who showed that certain samples of both natural and synthetic rubber could inactivate completely the penicillin in solution in twenty-four hours; and also by Hughes³, who showed that no improvement was obtained by repeated washing of the tubes before use.

At the request of the Medical Research Council through the Ministry of Supply Advisory Service on Rubber, we have been investigating this problem in the hope that we should be able to throw some light on the mechanism of inactivation and on possible methods of improving the quality of rubber supplies. The main technological details will be published elsewhere, and the purpose of this communication is to give a summary of the results relevant to medical practice.

The Effect of Compounding Ingredients and of Vulcanization. Unfortunately, none of the early workers was in a position to supply information on the compounding ingredients other than rubber which were present in the tubes which they tested. These ingredients may well represent quite a large proportion of the total weight, and as some of them are highly active substances it was thought possible that the trouble might be due to one or more of these rather than to the rubber itself. On testing singly a number of such substances, including natural rubber, sulphur, zinc oxide, stearic acid, paraffin wax and a number of accelerators of vulcanization—mercaptobenzthiazole, dibenzthiazyl disulphide, tetramethylthiuram disulphide, zinc diethyldithiocarbamate and