Summary

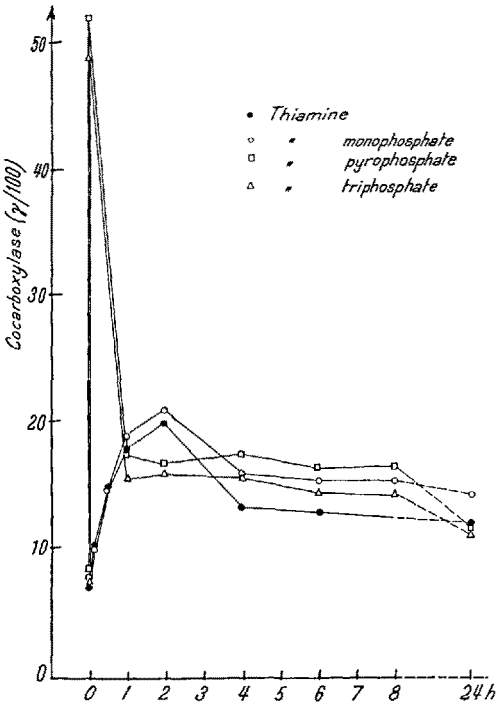
This study deals with the effect of minute amounts of oxygen upon the fermentation of glucose by resting cells of the tame wine yeast Fendant grown for 17 and 66 h, respectively, in a synthetic substrate containing glucose, vitamin-free casein hydrolysate, citrate buffer and mineral salts. The results prove conclusively the dependence of the metabolic activity of the yeast cells from young cultures on the duration of flushing with oxygen-free nitrogen or argon before the addition of glucose, the rate of carbon dioxide production in the approximately linear phase of fermentation decreasing considerably with increasing flushing times. This inactivation of the cells is removed, as a whole or partly, at oxygen tensions greater than 0·01–0·05% by volume. Resting cells from old Fendant cultures show the effects mentioned only to a very small extent. It is assumed that in resting cells from young Fendant yeast cultures one or more components of the zymase system are inactivated on flushing with nitrogen or argon. The activity is restored, quantitatively or partly, by minute amounts of oxygen introduced prior to the addition of glucose.

The Level of Blood Cocarboxylase after Administration of Thiamine and its Phosphoric Esters

Various aspects of thiamine and diphosphothiamine (DPT) metabolism have been investigated by several authors[1]. Since MARKEES and MEYER[2] pointed out the therapeutical importance of DPT, the fate of this coenzyme, when administered parenterally, has been taken into account. The level of blood cocarboxylase was studied after thiamine[3] and DPT[4] injection. The excretion of thiamine by the kidneys after administration of thiamine and DPT was studied by TATSUO ABE[5]. According to this author, the administration of both thiamine and DPT is followed by the excretion of free thiamine, and it has been found that when DPT is injected, the elimination of thiamine is extended over a longer period of time.

The purpose of this work was to investigate the modifications of the content of blood cocarboxylase when thiamine or one of its phosphoric esters (mono-, pyro-, tri-) was administered.

The experiments were performed on 6 male dogs kept on a standard diet. The amount of blood cocarboxylase in each animal was determined by the method of WESTENBRINK[6] before intravenous injection of free thiamine (3 mg/kg) and at different times after this administration over a period of 24 h. At one week intervals, the same type of experiment was repeated by injecting intravenously monophosphothiamine (4 mg/kg), diphosphothiamine (5 mg/kg) and triphosphothiamine (6 mg/kg) respectively. The results of these experiments are given in the Figure where the points of the curves represent the average of the results obtained for all 6 animals.



The content of blood cocarboxylase in dogs after intravenous injection of thiamine, monophoshothiamine, diphosphothiamine, and triphosphothiamine.

From the Figure it can be seen that the administration of thiamine and monophosphothiamine brings about an increase in blood cocarboxylase, its maximum being 2 h after injection. Furthermore, monophosphothiamine appears to be somewhat more active than free thiamine. The administration of diphosphothiamine and triphosphothiamine is immediately followed by a sharp increase in blood cocarboxylase activity. However, between the first and the third hour, the level of blood cocarboxylase is lower than when thiamine and monophosphothiamine are injected.

These results indicate that the administration of all three thiamine phosphoric esters is more effective than that of thiamine itself in maintaining the level of blood cocarboxylase higher than in normal conditions for at least 24 h. The Figure shows clearly that triphosphothiamine has the same behaviour as DPT. This fact provides strong evidence that *in vivo* triphosphothiamine is not immediately split into monophosphothiamine and pyrophosphate, as was observed *in vitro*[1].

The thiamine phosphoric esters used in this work were prepared according to VISCONTINI *et al.*[2]. We are indebted to Prof. VISCONTINI for his suggestions in the preparation of these substances.

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Institute of Biological Chemistry, University of Rome, June 15, 1953.

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[1] M. VISCONTINI, G. BONETTI, C. EBNÖTHER, and P. KARRER, Helv. chim. Acta *34*, 1388 (1951).

[2] M. VISCONTINI, G. BONETTI, and P. KARRER, Helv. chim. Acta *32*, 1478 (1949).

Résumé

On a étudié sur le chien les courbes de l'activité cocarboxylasique du sang après injection de thiamine et des esthers mono di- et triphosphoriques de cette vitamine.

Antigenicity and Enzyme Activity of *Salmonella typhosa*

The relationship of antigenic make-up to the virulence of the typhoid bacilli has been the subject of extensive studies[1]. No attempt however appears to have been made to study the enzymatic activity of the various types of strains (Vi, O and H) in relation to their antigenicity and virulence. In the course of investigations along this line certain marked differences were observed in the oxidative metabolism of glutamic acid and tyrosine by the various strains possessing different antigenic characteristics. The present communication describes these results.

The following strains were used:

(1) BHATNAGAR's strain ViI[2] having predominantly the Vi antigen and no H antigen at all (O inagglutinable, low virulence).
(2) WATSON's V strain possessing all the three Vi, O, and H antigens (O inagglutinable, highly virulent).
(3) H 901 having H and O antigen (O agglutinable, low virulence).
(4) O 901 possessing O antigen only (highly sensitive to O-agglutinins and of low virulence).

All these cultures were maintained on the beef heart infusion agar medium, pH 7·6.

The metabolic studies were carried out by the conventional WARBURG's technique. 1 ml of M/15 phosphate buffer of pH 7·0 along with 1 ml of bacterial suspension was placed in the main compartment of the flask. In the centre cup was kept 0·2 ml of 10% KOH and a 2 cm² filter paper. 1·0 ml M/100 L-glutamic acid or L-tyrosine (B.D.H.) was taken in the side arm. The bacterial suspension was made from a 24 h growth at 37°C washed twice with 0·85% saline and finally adjusted to 40% transmission in a Lumetron photoelectric colorimeter Type 400 A using red filter (650 mμ). After equilibration (38·5°C), the substrate was tipped in the main compartment and the oxygen consumption was measured for a period of 2 h. The results at 60 and 120 min are presented in the Table.

Metabolism of glutamic acid and tyrosine by different antigenic strains of *S. typhosa*

Substrates	Time in minutes	Oxygen consumption (μl)			
		ViI	WATSON's V	H–901	O–901
L-Glutamic-acid . . .	60	141·2	89·8	73·7	65·3
	120	628·1	234·0	109·6	110·8
L-Tyrosine .	60	63·0	35·1	12·0	10·8
	120	269·5	67·3	18·0	18·0
Endogenous .	60	13·8	21·8	19·4	28·6
	120	23·4	30·0	25·2	34·9

[1] A. FELIX and R. M. PITT, Brit. J. Exptl. Path. *15*, 346 (1934); Lancet. *1*, 186 (1934); J. Hyg. *35*, 428 (1935).
[2] S. S. BHATNAGAR, C. G. J. SPEECHLY, and M. SINGH, J. Hyg. *38*, 663 (1938).

It is evident from this Table that the strains tested metabolised the two substrates in a markedly different manner. The maximum oxygen consumption in case of glutamic acid was shown by the strain most rich in Vi antigen (ViI) amounting to approximately six times that of the O-agglutinable strains (H–901, O–901) devoid of Vi antigen. The next in order was the WATSON's V, which gave only 30% respiration as compared to that of the ViI. On the other hand, H–901 and O–901 gave almost the same metabolism with respect to glutamic acid, the oxygen consumption in 2 h being approximately 110 μl.

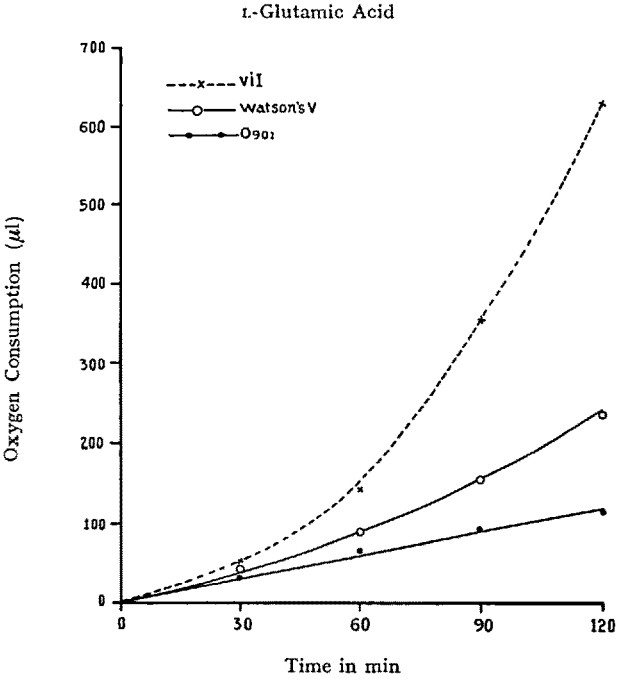

Fig. 1.—Metabolism of l-glutamic acid by different antigenic strains of *S. typhosa*.

The results with tyrosine (Table) follow essentially the same pattern, with the ViI strain showing approximately 42% of the corresponding activity for glutamic acid in 2 h. The differences between the various antigenic strains are well marked in this case also. Metabolic activity of the WATSON's strain and that of the other 2 strains corresponds to about 25 and 7% respectively of the ViI strain.

The time reaction curves for the ViI, WATSON's V and O–901 with glutamic acid are presented in Figure 1. All the three strains seem to show about the same metabolic activity up to first half an hour period after which the curve for ViI rises sharply. In the case of tyrosine also, the differences between the various strains are well marked as can be seen from Figure 2, where the oxygen consumption (μl) has been plotted against time of reaction.

It will be seen from the results presented that ViI strain has the maximum metabolic activity towards both glutamic acid and tyrosine, whereas WATSON's V strain, which antigenically occupies an intermediate position between the completely O-inagglutinable strain (ViI) and the O-agglutinable strains, metabolises these two substrates to a lesser degree. H–901 and O–901 strains show more or less the same activity. It would appear that 'Vi' antigen is in some way responsible for the differences observed in metabolism.