CCXXXIII. NOTE ON THE ACTIVATION OF OXIDO-REDUCTIONS BY CO-ZYMASE.

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THE question of the existence of a co-reductase has frequently been discussed. The differences of opinion upon careful examination have proved often to originate from the fact that this problem has been understood in quite a different way by different authors. In a paper which recently appeared, Harden and Macfarlane [1931] have returned to this question and summarise their results as follows.

"The addition of lactate, succinate or methylglyoxal restored the power of reducing methylene blue to yeast preparations washed to a point at which no fermentation of hexosediphosphate occurred. From these results it is concluded that a co-enzyme is probably not required for the action of the reductase in yeast preparations."

We found previously [Euler, Nilsson and Runehjelm, 1927] in full agreement with Harden and Macfarlane, that the reduction of methylene blue, when performed in presence of lactate and succinate, takes place even in the absence of co-zymase.

On the other hand, co-zymase is required as an activator of the carbohydrate redoxase [Euler and Nilsson, 1926, 1, 2]. It was previously pointed out by Nilsson [1930], that a direct comparison between oxido-reductions in yeast and in muscle, in which different substrates take part as hydrogen donators is justified only in special cases. "Dazu kommt noch, dass die Specificität der bei verschiedenen Donatoren wirksamen Redoxasen nicht genügend erkannt wurde, so dass z. B. die Verbrennung von Milchsäure oder von Bernsteinsäure mit der Verbrennung von Kohlenhydratabbauprodukten in der Hefe und im Muskel direkt verglichen wurde, ohne dass man sich darüber klar machte, dass bei diesen verschiedenen Reaktionen vielleicht in sämtlichen Fällen ganz verschiedene Enzyme oder Enzymsysteme wirksam sind."

Harden and Macfarlane state, that on the addition of co-zymase and hexosediphosphate to washed yeast fermentation occurs which may give rise to easily oxidisable reaction products, which in their turn cause a reduction of the methylene blue.

. In such a case this reduction would be of a secondary character and it would consequently not correspond to the directly activated reaction in which the co-zymase acted as a co-reductase.

The possibility of explaining the chemical mechanism of the reduction of methylene blue in yeast in the manner recently suggested by Harden and Macfarlane has by no means been overlooked by us; it is noted in one of our earliest papers on this subject [Euler and Nilsson, 1926, 1].

However, our more recent results speak more and more in favour of the opinion that the reaction in which the co-enzyme directly takes part is of an oxido-reductive character. As this view has already been discussed in a previous publication we only wish to emphasise here that reductions, which are activated by the co-enzyme, take place even when fermentation or glycolysis is completely stopped by specific poisons. The product of the oxidation reaction which appears in this case has the composition of a monophosphoglyceric acid [Nilsson, 1930].

This conclusion is supported by the following facts.

Table 73 (p. 92) and Table 81 (p. 98) of Nilsson's paper show the reduction of acetaldehyde in presence and in absence of co-enzyme after addition of NaF.

Table 74 (p. 93) shows that NaF completely inhibits all fermentation. From Tables 74 and 81 it will be seen that the reduction of acetaldehyde does not occur without co-enzyme and that this reduction is not inhibited by NaF, when added in quantities which completely inhibit fermentation. A comparison with Table 72 (p. 92) shows that on the contrary the reduction of acetaldehyde is activated by such concentrations of NaF.

Since it became clear to us that the transformation of acetaldehyde in dried yeast consists in the reduction of the aldehyde, we used acetaldehyde instead of methylene blue in our oxido-reduction experiments, because it is possible to follow with this substance the course of the reduction from time to time.

We quote here, however, one of our earlier experiments with methylene blue, which shows that, in agreement with our reduction experiments with acetaldehyde, reduction of methylene blue by washed yeast + purified co-enzyme and hexosediphosphate occurs in presence of fluoride.

Apoenzyme + phosphate + hexosediphosphate + methylene blue		Reduction time
+ Purified co-enzyme	{ without NaF	1.5 minutes
	{ with NaF	1.75 „
Without co-enzyme		More than 3 hours

The difference between the reduction times 1.5 and 1.75 lies within the limits of the errors of observation.

Concerning our opinion on the reactions which occur in this case and in the case of a normal fermentation we refer to the publication of Nilsson quoted above [Nilsson, 1930] and to a summarising paper by Euler and Nilsson [1930].

In this connection the question whether or not co-enzyme acts as an activator of aldehydemutase is of special interest.

The fact that acetaldehyde, added to a suspension of dried yeast, disappears, was at first interpreted by us as a simple dismutation of the aldehyde and because co-zymase was found to be indispensable for the reaction the opinion was expressed that co-zymase has the character of a co-mutase [Euler and Myrbäck, 1927], which assumption was also in agreement with the result that co-zymase is identical with the co-enzyme of the reductase.

This question entered a new stage when it became apparent by the experiments of Nilsson that the acetaldehyde disappears in the presence of dried yeast, not by a simple dismutation but by a reduction very probably of the same type as the reduction of methylene blue.

This new fact caused a modification of our former theoretical views on this subject as is seen from Nilsson's paper [1930].

Thus the rôle of the co-zymase in oxido-reductions in the presence of yeast is cleared up to a certain degree; such is not the case with regard to the transformation of acetaldehyde in the liver, studied in detail by Parnas [1910]. By estimations of alcohol and acid Parnas has shown that it is here probably a question of a real aldehyde dismutation. But as all Parnas's experiments were carried out with mashed liver, or with raw liver juice, thus, with an enzymic material containing rather large amounts of carbohydrates, it is possible that a dismutation of mixed character also takes place, which may have influenced his results to some extent.

In this Institute the question has been again treated by Euler and Brunius [1928]. The most important results then obtained seem to be that purified and dialysed enzyme solutions, themselves inactive, were able to catalyse the aldehyde transformation after the addition of purified co-zymase.

Continuing this investigation we wished to compare the aldehyde transformation which is catalysed by the liver enzyme with the mixed dismutation as it takes place in suspensions of dried yeast.

These experiments also seem to support the opinion that in the liver a real acetaldehyde mutase occurs, which only acts in the presence of co-zymase. The fact that the transformation of acetaldehyde is activated by hexosediphosphate indicates, however, that an oxido-reduction may also occur analogous to that in fermentation by dried yeast, where hexosemonophosphate is proved to take part in a mixed dismutation with acetaldehyde [Nilsson, 1930].

Our results now obtained will be seen from the following tables.

1. *Action of phosphate in presence and in absence of hexosediphosphate.*

Enzyme solution: dialysed liver juice. Co-zymase: 140 Co/cc. $ACo = 40,000$.
Concentration of the acetaldehyde: 1.9 %.

Phosphate buffer: 0.67 M ; $p_H = 6.4$.

Concentration of the hexosediphosphate: solution of sodium candiolin, approximately 6 %.

Volume of the reaction mixture: 10 cc. In this volume 1 cc. acetaldehyde solution + 3 cc. enzyme solution + 2 cc. solution of co-zymase.

ACTIVATION OF OXIDO-REDUCTIONS BY CO-ZYMASE 2171

Mins.	Acetaldehyde transformed in 2 cc. of the reacting mixture (mg.).			
	No hexosediphosphate		1.5 cc. hexosediphosphate	
	No phosphate	2.5 cc. phosphate	No phosphate	2.5 cc. phosphate
30	0.89	0.83	0.83	1.42
60	1.44	1.48	1.61	2.27
90	1.94	2.17	2.08	2.76

2. Action of co-enzyme in presence and in absence of hexosediphosphate.

All reagents as in the table above.

Volume of the reaction mixture: 10 cc., containing 1 cc. solution of acetaldehyde + 3 cc. solution of dialysed liver juice + 2.5 cc. phosphate buffer.

Mins.	Acetaldehyde transformed in 2 cc. of the reacting mixture (mg.).			
	No hexosediphosphate		1.5 cc. hexosediphosphate	
	No co-enzyme	2 cc. co-enzyme	No co-enzyme	2 cc. co-enzyme
30	0.10	0.85	0.25	1.40
60	0.12	1.55	0.39	2.25
90	0.23	2.21	0.76	3.03

SUMMARY.

1. For aldehyde transformation, when catalysed by dialysed liver juice and purified co-enzyme, phosphate is not required. There is thus a difference from the mixed dismutation, as it occurs in solutions fermented by dried yeast.
2. The transformation of acetaldehyde in dialysed liver juice is increased by the addition of hexosediphosphate or of phosphate.
3. Both in the presence and in the absence of hexosediphosphate co-enzyme is indispensable for the transformation of acetaldehyde by liver juice.

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