CXIII. THE EFFECT OF CYANIDE ON THE SCHARDINGER ENZYME.

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SZENT-GYÖRGYI [1926] claims to have brought forward evidence that in the aerobic oxidation of aldehydes under the influence of the aldehyde oxidase of milk (Schardinger's enzyme) an activation of the molecular oxygen by iron in accordance with Warburg's theory is necessary. His evidence consists in the observation that cyanide in a concentration of $M/200$ produced a 75 % inhibition of the uptake of oxygen by the enzyme-aldehyde system, and he concludes that the cyanide acts by inactivating the iron, as it does in the cases of iron catalysis studied by Warburg and others. The question is of importance in connection with Warburg's theory of tissue oxidations, and in view of the facts that Dixon and Thurlow [1925] have shown that the xanthine oxidase system is independent of such activation by iron, and have further shown [1924, 2] that the xanthine oxidase is identical with, or at any rate very closely similar to, the Schardinger enzyme, it was clear that a more detailed study of the action of cyanides on the latter enzyme should be made.

The experiments were carried out in a Barcroft apparatus at 40° . The enzyme solution used was a 20 % solution in phosphate buffer of the caseinogen preparation of Dixon and Thurlow [1924, 1]. This preparation is very similar to that used by Szent-Györgyi, differing only in the method of precipitation. The acetaldehyde was freshly prepared from pure crystalline aldehyde-ammonia by distillation with dilute sulphuric acid. It was made up in solution in water, and at Dr Szent-Györgyi's suggestion the solution was made alkaline to phenolphthalein with NaOH, allowed to stand a few moments, and re-neutralised immediately before use in order to destroy any traces of peracids which might have been formed and which might interfere with the action of the cyanide. The same amount of aldehyde solution was placed in both flasks of the apparatus in order to compensate the vapour pressure. The cyanide solution was always a freshly made up and neutralised solution of pure crystalline potassium cyanide.

Several series of experiments were carried out, and similar results were obtained in all. A typical set of curves is given in Fig. 1.

It will be seen that $M/100$ cyanide produces some inhibition of the oxygen uptake, but much less than that observed by Szent-Györgyi with $M/200$,

possibly owing to slight differences in the experimental conditions. With $M/1000$ cyanide, however, no inhibition is produced, which shows that the system is *not* cyanide-sensitive in Warburg's sense. Even with $M/300$ cyanide no effect can be observed, as Fig. 2 shows, and the inhibition only begins to be noticeable with about $M/200$.

Fig. 1.

Fig. 2.

Fig. 1. Each apparatus contained: 1 cc. 20 % caseinogen enzyme preparation; 1 cc. phosphate buffer (p_H 7.6); 1 cc. 0.5 % acetaldehyde; and the following concentrations of KCN: A, 0; B, $M/1000$; C, $M/100$. The control flasks contained the same solutions, but with buffer instead of the enzyme solution.

Fig. 2. As in the previous experiment, but a different enzyme preparation was used, and the concentrations of KCN were: A, 0; B, $M/300$.

We may compare the behaviour of this system with that of a typical cyanide-sensitive system—the succinoxidase of muscle, which was studied by Fleisch [1924]. Fleisch showed that in this case a concentration of cyanide of less than $M/1000$ was amply sufficient to produce a practically complete inhibition of the oxygen uptake in the presence of succinate. The enzyme was not, however, destroyed by the cyanide, as the methylene blue reduction was unaffected.

In order to determine whether the inhibiting action of $M/100$ cyanide on the Schardinger enzyme was due to actual destruction of the enzyme, the solutions used in the experiment given in Fig. 1 were transferred at the conclusion of the experiment from the flasks of the Barcroft apparatus to Thunberg vacuum-tubes. 1 cc. of 1/5000 methylene blue was added to each tube and the tubes were then evacuated and placed in the thermostat at 32° . There was no need to add fresh aldehyde, as only about 10 % of that originally added had been oxidised during the course of the experiment. The results obtained were as follows:

Contents of tube			Reduction-time min.
Solution from apparatus with no KCN...	...	...	98
" " $M/1000$ KCN	...	...	90
" " $M/100$ KCN	...	...	2000
1 cc. original solution + 1.1 cc. buffer + 0.9 cc. aldehyde			9

These figures show quite definitely that the inhibiting effect of the higher concentration of cyanide is due to an actual destruction of the enzyme itself. In all three solutions there had been considerable destruction during the oxygen uptake, which was doubtless due to the peroxide formed during the reaction, as already observed during the oxidation of hypoxanthine [Dixon, 1925; see also Thurlow, 1925]. The much greater destructive effect of the $M/100$ cyanide is however evident.

A precisely similar inhibitory effect of cyanide in concentrations greater than about $M/150$ was observed by Dixon and Thurlow [1925] using hypoxanthine as substrate, and shown by them to be due to the same cause.

Some unpublished experiments made by the writer and Miss Thurlow in 1924 may be given here, as they show clearly the destructive effect of cyanide on the milk oxidase, with either aldehyde or hypoxanthine as substrate. The experiments were carried out in Thunberg tubes in the usual way. The enzyme solution used was one of the caseinogen enzyme preparation in phosphate buffer at p_H 7.6.

Contents of tubes	Reduction-time min.
5 cc. enzyme + 0.5 cc. methylene blue + 0.1 cc. (0.2 mg.) hypoxanthine	5
5 cc. enzyme + 0.5 cc. methylene blue + 0.1 cc. (0.2 mg.) hypoxanthine + 0.1 cc. $M/10$ KCN	4.5
5 cc. enzyme + 0.5 cc. methylene blue + 0.1 cc. (0.2 mg.) hypoxanthine + 0.5 cc. $M/10$ KCN	7
5 cc. enzyme + 0.5 cc. methylene blue + 0.1 cc. 10 % aldehyde	7
5 cc. enzyme + 0.5 cc. methylene blue + 0.1 cc. 10 % aldehyde + 0.1 cc. $M/10$ KCN	8.5
5 cc. enzyme + 0.5 cc. methylene blue + 0.1 cc. 10 % aldehyde + 0.5 cc. $M/10$ KCN	17

These figures show that while $M/500$ cyanide produces no appreciable effect, $M/100$ cyanide has a definite destructive action on the enzyme; which is in good agreement with the experiments given above. The effect appears to be more marked with aldehyde than with hypoxanthine, but this does not necessarily mean that we are dealing with two distinct enzymes, as it is possible that there may also be an action of the cyanide on the aldehyde at the enzyme surface.

The destruction may become very marked if the oxidase is allowed to stand for a short time with the cyanide before adding the aldehyde and methylene blue, as the following experiment shows. 1 cc. of 20 % caseinogen preparation + 1 cc. $M/30$ KCN were allowed to stand 5 minutes at room temperature, and then 1 cc. 1 % aldehyde + 1 cc. buffer and 1 cc. methylene blue added. The reduction-time was 30 minutes, whereas 1 cc. of the same enzyme solution + 1 cc. buffer + 1 cc. aldehyde + 1 cc. methylene blue reduced in $4\frac{1}{2}$ minutes.

The experiments described in this paper show clearly that the inhibition produced by the higher concentrations of cyanide cannot be regarded as evidence for oxygen-activation. Since it occurs with methylene blue as the hydrogen acceptor under precisely the same conditions as with oxygen it must be referred rather to an actual destruction of the enzyme.

SUMMARY.

Inhibition of the aerobic oxidation of aldehyde by the Schardinger enzyme is produced only by concentrations of cyanide greater than $M/200$. It is due to an actual destruction of the enzyme itself, and does not constitute evidence of oxygen-activation.

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