XCVI. THE EFFECT OF NARCOTICS ON SOME DEHYDROGENASES.

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ACCORDING to the well-known work of Warburg [1921] the inhibiting effect of narcotics on cell respiration, on fermentation by yeast and on oxidation of amino-acids on a charcoal surface is due to the preferential adsorption of these substances on the particular surfaces, and the reaction velocity is inhibited owing to the displacement of the reacting substances in question by the narcotics. That the narcotic action increases with the increase in the molecular weight in a homologous series, coupled with the fact that the surface activity of these substances increases also in the same order, is a strong support for the theory of Warburg. Nothing is known, however, about the particular constituents of the cell on which this displacement of adsorption takes place, though a good deal of emphasis has been laid on the rôle of lipoids in the phenomenon of narcosis [Loewe, 1913]. Henderson [1930] in his recent review of the theories of narcosis finds considerable difficulties in deciding on the substrate on which narcotics act in the known reversible manner. This question of the effect of narcotics on some particular constituents of the cells is naturally interesting and requires elucidation before the narcosis of cell respiration can be properly understood.

In a study of the cytochrome-indophenol oxidase system of cells, Keilin [1929] has put forward the view that cyanide inhibits the oxidation of cytochrome by the indophenol oxidase system. Narcotics, however, have little or no effect on the activity of the oxidase-cytochrome system. That is, they do not affect the oxidation of reduced cytochrome, but they inhibit the activity of dehydrogenases and hence the reduction of oxidised cytochrome. The inhibiting effect of narcotics on cell respiration is thus due to their inhibiting action on dehydrogenases in the cells.

The present paper contains a study of the effect of some narcotics on three dehydrogenase systems, namely xanthine-hypoxanthine dehydrogenase (xanthine oxidase), aldehyde dehydrogenase (Schardinger enzyme) and succinic dehydrogenase. Incidentally some experiments have been made on the question of the identity of xanthine oxidase with Schardinger enzyme.

EXPERIMENTAL.

The xanthine oxidase and Schardinger enzyme were prepared in the same sample in the form of the caseinogen preparation of Dixon and Thurlow

Fig. 1.

Fig. 1. Effect of ethylurethane on xanthine oxidase-hypoxanthine system.

A. Control.

B. Urethane 5 %.

Fig. 2.

Fig. 2. Effect of phenylurethane on xanthine oxidase system.

○—○—○ Denotes control.

×—×—× Denotes urethane-saturated.

Fig. 3. Effect of narcotics on Schardinger enzyme.

A. Phenylurea 0.02 M.

B. Control and valeronitrile 0.11 M.

C. Ethylurethane 5 %.

The Barcroft cups in all these cases (Figs. 1, 2 and 3) were made up as follows: 0.5 cc. of 20 % caseinogen solution and 1.2 cc. phosphate buffer p_H 7.6 were placed in both the cups. In the control, 0.3 cc. of 0.2 % hypoxanthine + 1 cc. of water was added in the right hand cup, whilst the left hand cup received 1.3 cc. water. The hypoxanthine was contained in a small Keilin tube which was tipped into the cup after temperature equilibration in the usual way and this was taken as the zero time. In the case of Schardinger enzyme, 0.5 cc. of 0.25 % piperonal was added in place of hypoxanthine, the quantity of water being reduced to 1.1 cc. When narcotics were added, the amount of water was reduced proportionately, the total volume being kept constant at 3 cc. in both the cups. The narcotic was added in both the cups, and in the case of phenylurethane an excess was present.

[1924, 1] from highly active milk. The succinic dehydrogenase was prepared from washed sheep's heart muscle according to the method of Keilin [1929].

The experiments were made both by the methylene blue technique (anaerobically) and by the oxygen-uptake method in a Barcroft micro-respirometer. The main results are summarised below. All the experiments were made at 37°.

(1) With xanthine oxidase using hypoxanthine as substrate, ethylurethane 5 %, phenylurethane, saturated solution, diethylurea 5 % and phenylurea 0.3 % had very little effect on the velocity of oxygen-uptake (Figs. 1 and 2).

(2) With Schardinger enzyme, using piperonal as substrate, ethylurethane 5 %, phenylurea 0.02 *M*, and valeronitrile 0.11 *M* had very little effect on the initial velocity of oxygen-uptake (Fig. 3).

(3) In the anaerobic reduction of methylene blue in presence of xanthine oxidase and Schardinger enzyme, similar results were obtained. The amount of methylene blue used was 1 cc. of 1:5000 solution in 5 cc. total volume.

(4) Narcotics had a strong inhibiting action on the succinic dehydrogenase system, the results of the oxygen-uptake and methylene blue reduction methods agreeing with each other. This action was found to be (in the case of ethylurethane which was the only narcotic examined) completely reversible. As the data are interesting, these results are given in some detail.

Effect of narcotics on succinic dehydrogenase.

The narcotics used in this investigation were ethylurethane, phenylurethane, diethylurea, phenylurea, propionitrile, valeronitrile and vanillin. The dehydrogenase was a washed muscle preparation containing approximately 100 mg. per 1 cc. of the suspension. The Barcroft cups were made up as follows: 0.5 cc. muscle preparation, 1 cc. phosphate buffer p_H 7.6, 1 cc. narcotic of known concentration. The right-hand cup contained in addition 0.5 cc. *M*/10 sodium succinate, whilst the same amount of water was added to the left-hand cup. In the control experiments, of course, no narcotic was added. The total volume

Fig. 4. Effect of diethylurea on the succinic dehydrogenase system.
A. Control. B. Diethylurea 0.66 *M*.

Fig. 5. Effect of phenylurea and vanillin on the succinic dehydrogenase system.
A. Control. B. Phenylurea 0.02 *M*. C. Vanillin 0.011 *M*.

in every cup was 3 cc. and the temperature of the bath 37°. In Figs. 4 to 7 the results are graphically shown. It will be observed that with all these narcotics the activity of the oxygen-absorbing system is greatly reduced.

Fig. 6.

Fig. 6. Effect of nitriles on the succinic dehydrogenase system.
A. Control. B. Valeronitrile 0.11 *M*. C. Propionitrile 0.66 *M*.

Fig. 7.

Fig. 7. Effect of urethanes on the succinic dehydrogenase system.
A. Control. B. Phenylurethane 0.003 *M*. C. Ethylurethane 0.66 *M*.

The majority of the narcotic concentrations were chosen in such a way (by preliminary tests) that the inhibition fell to about 50 % of the control. In other cases this value has been extrapolated. The following table summarises the results.

Table I.

Narcotic	Concentration of narcotic which inhibits about 50 % (<i>M</i>)		
	Oxygen-uptake in presence of succinic dehydrogenase	Respiration of nucleated erythrocytes— Warburg	Anaerobic reduction of methylene blue by succinic dehydrogenase
Ethylurethane	0.65	0.33	0.6
Phenylurethane	0.003	0.003	0.002
Diethylurea	0.35	0.52	0.2
Phenylurea	0.028	0.018	0.028
Propionitrile	0.48	0.36	—
Valeronitrile	0.08	0.06	—
Vanillin	0.011	0.02	0.022

The results obtained in this work are compared with those of Warburg on respiration by avian blood corpuscles. The last column contains the results obtained by the methylene blue technique. Since it will be interesting to compare the results of these methylene blue reduction experiments with the results obtained in oxygen absorption experiments, some of them are given here. It will be observed that the effects of narcotics in the case of the succinic dehydrogenase system are really on the hydrogen-activating mechanism of the system. Figs. 8–12 illustrate the main results obtained. Each Thunberg tube contained 1 cc. phosphate buffer of p_H 7.6, 0.5 cc. methylene blue 1:5000, 0.5 cc. muscle preparation, and 0.5 cc. sodium succinate *M*/10 unless

Fig. 8.

Fig. 9.

Fig. 8. Effect of varying enzyme concentration on the succinic dehydrogenase system.

Fig. 9. Effect of varying substrate concentration on the succinic dehydrogenase system.

Fig. 10. Effect of varying methylene blue concentration on the succinic dehydrogenase system.

The curve shows the activity per unit quantity of methylene blue. The original concentration of methylene blue is 1 in 5000.

Fig. 11.

Fig. 11. Effect of urea on the succinic dehydrogenase system.

A. Activity without narcotic.

Fig. 12.

Fig. 12. Effect of urethanes on the succinic dehydrogenase system.

A. Activity without narcotic.

B. Ethylurethane 17.8 %.

C. Phenylurethane 0.05 %.

otherwise stated, the total volume being 5 cc., made up either by addition of water or narcotic. The activity is the reciprocal of the time of reduction of methylene blue in minutes multiplied by 100. The data include the effect of varying enzyme concentration, the effect of different methylene blue concentrations, the effect of different substrate concentrations and the effect of different narcotics of different concentrations. The data given in the last column of Table I have been obtained from the experimental curves. It will be observed that the velocity of the reduction of methylene blue (activity) is directly proportional to the enzyme concentration, that the rate of methylene blue reduction at constant enzyme concentration depends on the concentration of methylene blue below a certain concentration, and that the rate of reduction of methylene blue is nearly constant within the range of substrate concentration studied in this paper [compare Ahlgren, 1926]. It will also be seen from Table I that in every case the higher homologue has a greater inhibiting (narcotic) action, a fact also observed by Batelli and Stern [1913, 1] but criticised by Vernon [1914], and the magnitudes of the individual values are also often not far from the values obtained by Warburg in his experiments on respiration by blood cells.

It will thus be observed from the above results that the muscle system containing succinic dehydrogenase and indophenol oxidase gives results in presence of narcotics entirely in accordance with the theory of Keilin. It is therefore interesting to enquire why narcotics fail to show any effect on xanthine oxidase or Schardinger enzyme. Curiously enough, both these systems are also not sensitive to cyanide, whereas oxidation of succinate in presence of the muscle system is sensitive to cyanide. This is no doubt due to the fact that cyanide inhibits the indophenol oxidase system which is absent from the xanthine or Schardinger enzyme. It is to be noted at this place that nitriles (propionitrile, valeronitrile) had no effect on the reduction of methylene blue by succinic dehydrogenase, though they inhibited the oxygen absorption. This is to be explained by the fact that the .CN group does not affect the hydrogen-activating system. Regarding the difference in the behaviour of these two types of dehydrogenases in presence of narcotics nothing definite can be said at present, but some related facts may be stated here. Batelli and Stern [1913, 2] showed that there is some connection between the ability of narcotics to coagulate nucleoproteins and to inhibit the action of succinic dehydrogenase. The muscle preparation used in this work is a coarse suspension which easily settles down in presence of narcotics. The caseinogen preparation of xanthine oxidase or Schardinger enzyme is, however, more soluble and gives a slightly opalescent solution which is not precipitated in presence of narcotics of the concentration used in this investigation. It was found, however, in several instances that by increasing the concentration of the narcotic to a high value (for instance, ethylurethane 14 %), some diminution in the velocity of oxygen-uptake in presence of xanthine oxidase could be observed. This effect may be due to a real coagula-

tion effect. As however such high concentrations are not to be considered as narcotic concentrations, these results were not followed up by any further work. Mention may also be made here of the interesting attempt of Bernard [1885], who tried to classify narcotisable and non-narcotisable ferments on the basis of their being associated either with living protoplasmic mass or with non-living and non-protoplasmic mass.

THE IDENTITY OF XANTHINE OXIDASE WITH SCHARDINGER ENZYME.

Warburg in his experiments used vanillin as a narcotic, and it has been found by me in this paper to have a narcotic action on succinic dehydrogenase. Since vanillin is an aldehyde, it could not be used as a narcotic as such with the caseinogen preparation of Dixon and Thurlow, as this preparation catalyses the oxidation of both hypoxanthine and aldehydes. Some preliminary experiments on the oxidation of hypoxanthine in presence of the caseinogen preparation, however, showed that the presence of vanillin affects the rate of hypoxanthine oxidation. It has previously been observed by Dixon and Thurlow [1924, 2] that uric acid inhibits the oxidation of aldehydes in presence of Schardinger enzyme and they advance this as a strong point in favour of the view that xanthine oxidase and Schardinger enzyme are identical. This view is also strongly supported by the high degree of specificity of the enzymes found by Coombs [1927]. The result obtained by me points to the fact that in presence of vanillin the two substrates are competing with each other, producing a competitive inhibition. The competition is so great that in experiments in which a saturated solution of vanillin was placed in both the cups of a Barcroft apparatus and hypoxanthine only in the right cup, no movement of the liquid in the manometer took place in half an hour. This showed that the oxygen-uptake in the two cups was balanced and the presence of a saturated solution of vanillin had completely excluded the hypoxanthine from the enzyme surface. Since Wieland and Macrae [1930] consider that they have observed a summation effect with two substrates (hypoxanthine and aldehyde), it was thought worth while to obtain some comparative data on this point. The experiments have shown that with hypoxanthine on one hand and vanillin (*m*-methoxy-*p*-hydroxybenzaldehyde) and piperonal (3:4-methylenedioxybenzaldehyde) on the other hand, no additive effect was observed. In every case the combined velocity of oxygen-uptake was midway between the velocities of oxygen-uptake of the separate systems. Figs. 13 and 14 show the results. The concentration of vanillin used was 0.5 % and of piperonal 0.25 %, other conditions being the same as described before. It will be seen that as the aldehyde concentration increases the total oxygen-uptake falls, though, had there been two different enzymes, the oxygen-uptake would have been greater than when hypoxanthine was present alone. The decrease in the oxygen-uptake is due to the fact that the aldehyde, which has a much lower rate of oxygen-uptake, displaces a portion of the hypoxanthine and hence lowers the rate of oxygen-uptake of the whole system. This result

points very strongly to the conclusion that the two dehydrogenases are identical.

Fig. 13.

Fig. 13. Effect of vanillin on hypoxanthine oxidation.

A. 0.3 cc. hypoxanthine : no vanillin.	E. 1.0 cc. vanillin : no hypoxanthine.
B. 0.3 cc. hypoxanthine + 0.2 cc. vanillin.	F. 0.5 cc. vanillin : no hypoxanthine.
C. 0.3 cc. hypoxanthine + 0.5 cc. vanillin.	G. 0.2 cc. vanillin : no hypoxanthine.
D. 0.3 cc. hypoxanthine + 1.0 cc. vanillin.	

Fig. 14.

Fig. 14. Effect of piperonal on hypoxanthine oxidation.

A. 0.3 cc. hypoxanthine : no piperonal.	D. 0.5 cc. piperonal only.
B. 0.3 cc. hypoxanthine + 0.2 cc. piperonal	E. 0.2 cc. piperonal only.
C. 0.3 cc. hypoxanthine + 0.5 cc. piperonal.	

In these experiments (Figs. 13 and 14) the concentration of vanillin was 0.5 % and of piperonal 0.25 %, both prepared in phosphate buffer of p_H 7.6. The Barcroft cups were made up as follows. The right-hand cup usually received a constant amount of 0.3 cc. hypoxanthine 0.2 % solution (unless otherwise stated) and varying amount of either vanillin or piperonal. 0.5 cc. of the 20 % caseinogen solution was kept in the small Keilin tubes and was tipped into both the cups after temperature equilibration, and this was taken as the zero time. The total volume was adjusted at the beginning to 3 cc. by the addition of phosphate buffer in both the cups. Control experiments were also made without any hypoxanthine.

SUMMARY.

1. Narcotics have no effect on xanthine oxidase or Schardinger enzyme (caseinogen preparation) in ordinary concentrations, either as regards oxygen absorption or anaerobic reduction of methylene blue.

2. Narcotics have a great inhibiting action on the succinic dehydrogenase system, the effect increasing with higher homologues of the same series. The degrees of inhibition of oxygen-uptake and methylene blue reduction agree with each other in the majority of cases. Nitriles have, however, no effect on methylene blue reduction, though they inhibit the oxygen-uptake strongly.

3. A competitive inhibition of hypoxanthine oxidation is observed in presence of vanillin and piperonal. This is considered to be a strong point in favour of the view that xanthine oxidase and Schardinger enzyme are identical.

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