CLXXXI. ALCOHOL DEHYDROGENASE OF ANIMAL TISSUES

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It was first found by Batelli & Stern [1910] that extracts from various animal tissues are able to oxidize alcohol. More recent investigations and attempts to isolate the enzyme responsible for propyl alcohol oxidation were carried out by Reichel & Köhle [1935]. Alcohol oxidation in tissue slices from normal and fasting animals was studied by Leloir & Munoz [1938]. Bernheim [1938] investigated the influence of alloxan on the alcohol oxidation in tissue extracts.

The present work was undertaken in connexion with the study of the liver aldehyde mutase [Dixon & Lutwak-Mann, 1937]. This enzyme catalyses the dismutation of $CH_3.CHO$ to C_2H_5OH and $CH_3.COOH$ and it was of interest to investigate the alcohol dehydrogenase in the liver by methods similar to those employed for the mutase study.

Materials

Horse liver was used throughout as the source of the animal alcohol dehydrogenase. Livers from other animals (dog, pig, sheep) were also found to contain the enzyme in large quantities. A number of preparations were made in which acetone and ammonium sulphate were used for precipitation.

1. *Acetone preparation.* Minced horse liver is mixed with an equal volume of water and after standing a few hours at room temperature the large particles are removed by centrifuging. The aqueous extract is heated to 52° for 15 min. to remove inactive protein, then cooled and precipitated with 3 vol. acetone. The precipitate is centrifuged and resuspended by grinding with a small amount of water. The insoluble part is discarded and the extract is then precipitated with 2 vol. acetone. (Sometimes a 3 : 1 mixture of acetone and alcohol is used.) This procedure is repeated twice. The final acetone precipitate is dried *in vacuo* over $CaCl_2$. This preparation usually contained in addition to the alcohol dehydrogenase an active aldehyde mutase, but gave negative results for the aldehyde oxidase.

2. *Ammonium sulphate preparations.* (*a*) The aqueous liver extract is precipitated with 3 vol. saturated ammonium sulphate, the precipitate is filtered off and partially dried in air, then completely dried *in vacuo* over H_2SO_4 and KOH. (*b*) The liver extract is heated to 52° for a few minutes, the resulting precipitate discarded and the filtrate precipitated using 30 g. solid ammonium sulphate for every 100 ml. fluid. (*c*) The preparation obtained in (*a*) is taken up in a small quantity of water and dialysed against running tap water for 24 hr. The precipitate formed on dialysis is filtered off and the fluid precipitated with acetone in the same way as described in the acetone preparation.

A number of experiments were also carried out using acetic bacteria as the source of a system capable of alcohol oxidation. Cultures of *Acetobacter suboxydans* grown on glycerol-yeast water medium were spun off, washed twice with water and finally suspended in water. This suspension was used for experiments and was found to retain its activity for several days at 0°.

Coenzymes. Cozymase (70% pure) was prepared from baker's yeast by a modification and combination of Myrbäck's methods as described by Green & Brosteaux [1936]. Coenzyme II from red blood cells and flavoprotein from Lebedew juice were prepared by the methods of Warburg & Christian [1931; 1932]. Cocarboxylase and adenosinetriphosphate were obtained as described by Lohmann [1931] and Lohmann & Schuster [1937].

Methods

The enzymic oxidation of alcohol was tested mainly by two methods. (1) Anaerobically, in presence of methylene blue as hydrogen acceptor following the usual Thunberg technique. Tubes with hollow stoppers were used from which the substrate, methylene blue and other reactants were emptied into the tube containing the enzyme solution after the latter had been exhausted and filled twice with O_2-free N_2, and the reduction time of methylene blue at 37° was measured. (2) Aerobically, O_2 uptake was measured using Barcroft differential manometers with side bulbs [Keilin & Hartree, 1935]. The enzyme solution in *M*/30 phosphate buffer was placed in the right-hand flask only and the phosphate buffer in the other flask. The inner tubes contained KOH-soaked filter papers. Alcohol and other substances were emptied into the flasks from both side bulbs after 15 min. equilibration at 37°.

Properties of the alcohol dehydrogenase system

Coenzymes. The yeast alcohol dehydrogenase has been shown to require for its activity the presence of cozymase (diphosphopyridine nucleotide, coenzyme I) and of flavoprotein (yellow enzyme). In order to determine whether these two substances play a similar role in the oxidation of alcohol by preparations from animal tissues it was essential to have a coenzyme-free enzyme preparation.

Ordinary horse liver acetone preparations were unsuitable for this purpose since they always contain large amounts of cozymase which cannot be removed even by prolonged dialysis [Reichel & Köhle, 1935; Dixon & Lutwak-Mann, 1937]. The last-named authors used dog liver acetone preparations which contain practically no cozymase owing to its quick decomposition by this particular liver tissue. A very convenient and simple method has now been found to obtain horse liver preparations completely free from cozymase. If the liver extract be first treated with ammonium sulphate (as under (*a*), p. 1364) and either used directly or precipitated with acetone after removal of ammonium sulphate (as under (*c*), p. 1364) it then appears to be cozymase-free. On testing these preparations for aldehyde mutase, unless cozymase was added they showed no trace of activity. It can also be seen from Table I, Exps. 1–6, that the alcohol dehydrogenase prepared in this way catalyses the reaction only on addition of cozymase. Coenzyme II (triphosphopyridine nucleotide), cocarboxylase, adenosinetriphosphate, glutathione, known to act as coenzymes in various enzymic

Table I. *Effect of cozymase and flavoprotein on various liver alcohol dehydrogenase preparations*

Thunberg tubes with 20 mg. enzyme preparation, 1 mg. methylene blue, 2 ml. phosphate buffer *M*/30, *p*H = 7·6.

Ammonium sulphate preparation	ml. alcohol 96%	mg. cozymase	mg. flavoprotein	Reduction time min.
Exp. 1	0·1	—	—	∞
2	0·1	0·02	—	(Several hr.)
3	0·1	0·10	—	35
4	0·1	0·40	—	7
5	0·1	1·00	—	6
6	0·1	2·00	—	6
7	0·1	0·50	2·0	7
8	—	0·50	2·0	∞
Acetone preparation				
9	0·1	—	—	60
10	0·1	0·30	—	20
11	0·1	0·60	—	20
12	0·1	0·60	2·0	18

systems, and also nicotinamide, were found to be completely inactive. Recently Quibell [1938] has also found cozymase to be the specific coenzyme of the animal alcohol dehydrogenase.

That cozymase is also involved in the oxidation of alcohol by the acetic bacteria was shown in the following way. Fresh untreated suspensions of *Acetobacter suboxydans* very vigorously oxidize alcohol, both aerobically and anaerobically, and it is difficult to produce an acceleration of the reaction by addition of cozymase. When, however, the suspension was saturated with ammonium sulphate and after a few hours exhaustively dialysed, a marked increase in the velocity of the reaction was found on addition of cozymase.

Table II. *Effect of cozymase on oxygen uptake in presence of alcohol by acetic bacteria*

In Barcroft manometers, bacterial suspension in phosphate buffer $M/30$, pH = 6·5, 2 mg. alcohol, 0·3 ml. 20% KOH in inner tube, total volume 3·3 ml.

	O_2 uptake μl./20 min.
Fresh suspension	320
Do. +0·4 mg. cozymase	350
Treated with ammonium sulphate and dialysed	90
Do. +0·4 mg. cozymase	200

With regard to flavoprotein it can be seen from Table I, Exps. 7, 12, that it has only very little effect on the reaction. Dewan & Green [1938] were also unable to see any marked influence of flavoprotein in animal enzyme systems.

Coenzyme-factor. Stability of the alcohol dehydrogenase

The belief that the alcohol dehydrogenase from animal tissues is a very labile and unstable enzyme [Batelli & Stern, 1910; Reichel & Köhle, 1935] is disproved by the following observations. Two types of liver alcohol dehydrogenase preparations, (1) the usual acetone preparation, and (2) an ammonium sulphate preparation, made by method (*a*) above, were tested by the methylene blue technique every 2 weeks for a period of 4 months. The crude ammonium sulphate preparation seemed to be the more active one and its activity remained practically unchanged during the long period of observation. The acetone preparation gave lower initial values and seemed to deteriorat egradually on standing. This suggested the possibility of a special agent necessary for alcohol dehydrogenase activity, present apparently in the cruder ammonium sulphate preparation but partly destroyed in the acetone-treated preparation, in which the agent also seemed to undergo progressive decomposition on storing.

At this stage a note was published by Dewan & Green [1937] on the necessity of a special oxidation catalyst which they called the "coenzyme factor" in all enzyme systems where cozymase was involved. I had the opportunity of testing the influence of the coenzyme factor prepared from heart muscle and kindly given to me by Drs Dewan & Green. The experiment has been described in their paper [1938, p. 633]; it shows a very pronounced acceleration of the reaction on the addition of the factor preparation from heart muscle to an acetone liver preparation of alcohol dehydrogenase.

In order to prove that the differences described above in the behaviour of the crude ammonium sulphate and the acetone preparations were due solely to their unequal contents of the coenzyme factor, the presence of the factor in the liver had to be shown. Liver mince was washed with tap water for 24 hr. until

Table III. *Stability of the liver alcohol preparations on storing and effect of the coenzyme factor*

Thunberg tubes with 40 mg. enzyme preparation, 0·5 mg. cozymase, 1 mg. methylene blue, 0·1 ml. 96% alcohol, 2 ml. phosphate buffer *p*H = 7·6. 1 ml. coenzyme factor preparation (sheep liver).

Age of alcohol dehydrogenase preparation		Reduction time, min. Ammonium sulphate	Acetone
Fresh preparation	Without factor	4 min.	58 min.
	With factor	4 min. 50 sec.	4 min. 30 sec.
2 weeks old	Without factor	4 min.	60 min.
	With factor	4 min. 50 sec.	4 min. 30 sec.
2 months old	Without factor	5 min. 30 sec.	3 hr.
	With factor	4 min.	4 min.
4 months old	Without factor	6 min.	Several hr.
	With factor	4 min.	6 min.

almost colourless, ground in water with sand, the coarse particles were removed by centrifuging or straining through muslin and the resulting uniform suspension was used. Alone it gave negative results when tested for alcohol dehydrogenase, aldehyde mutase and aldehyde dehydrogenase, but added to the acetone preparation of alcohol dehydrogenase it produced a very marked acceleration of the methylene blue reduction (Table III). Washed liver suspensions as used in these experiments retain their activity for several days when kept at 0°.

The coenzyme factor has been shown to be acetone-sensitive and thermolabile [Dewan & Green, 1937] but resistant to ammonium sulphate treatment [Euler & Hellström, 1938]. This explains the poor activity of the acetone preparations from the beginning and also their apparent continual deterioration on standing. From Table III it can be seen that when supplemented by the coenzyme factor there is no change in the activity of the acetone preparation during the whole period (4 months) of the experiment. In the ammonium sulphate preparation no acceleration was seen on addition of the factor. It is however enough to heat the liver extract for a short time to 52° before the ammonium sulphate precipitation (as described under (*b*) above), to obtain a preparation giving results similar to those of the acetone preparations.

Effects similar to those with the washed liver suspensions were also obtained with preparations of coenzyme factor made from skeletal muscle, spleen and brain tissue. Dewan & Green [1938] and Euler & Haasse [1938] state that the factor is present in a great variety of animal tissues.

In addition to the above results which seem to disprove completely the alleged instability of the alcohol dehydrogenase, I was never able to confirm statements regarding the sensitivity of the enzyme towards O_2 or on standing in solution. There was no significant loss of activity on aeration at 37° or on prolonged dialysis or in the solution after standing for 3–4 days.

Optimum p*H*. Reichel & Köhle [1935] state that the *p*H optimum of their preparations of liver alcohol dehydrogenase is 6·7–7·3. Using the methylene blue technique the optimum was found to be at *p*H 7·6, with a rapid fall below *p*H 7. The shift towards the alkaline side may be caused by the addition in these experiments of the coenzyme factor which is very acid-sensitive and comparatively resistant to alkali.

When however the bacterial alcohol oxidase was tested at various reactions from *p*H 4·5 to 8·3, the optimum was found at *p*H 5.

Estimation of the reaction product formed anaerobically

Data for anaerobic experiments on the alcohol dehydrogenase are given in Tables I and III. It remained however to estimate the product of the reaction with and without coenzyme factor.

In order to obtain enough material for a chemical determination samples 10 times larger than those normally required for ordinary Thunberg experiments were put up. Large (70 ml.) Thunberg tubes were found most convenient; their hollow stoppers were filled with *M* phosphoric acid to inactivate the enzyme at the end of the reaction, which was indicated by the reduction of the methylene blue. After efficient cooling in ice-water the fluid was quantitatively transferred to the distillation flask of the Friedemann *et al.* [1927] apparatus. The distillation into $NaHSO_3$ was continued for 15 min. and the estimation of acetaldehyde was carried out iodimetrically.

The actual composition of the various samples and controls together with the results obtained are given in Table IV.

Table IV. *Chemical estimations of acetaldehyde*

In Thunberg tubes (70 ml.) 5 ml. dialysed enzyme solution (=0·3 g. acetone preparation), 4 ml. phosphate buffer *M*/20, *p*H 7·6, 10 mg. methylene blue (MB), 5 mg. cozymase, 0·3 ml. 96% alcohol, 4 ml. factor preparation. In hollow stoppers 10 ml. *M* phosphoric acid.

In control experiments acid was added to the enzyme and factor before the other reagents.

1 ml. *M*/100 I_2=0·22 mg. acetaldehyde.

1 mol. MB (355) corresponds to 1 mol. acetaldehyde (44).

	Reduction time min.	ml. *M*/100 I_2
Controls		
1. Enzyme + factor + cozymase + alcohol + MB	—	1·95
2. Factor (sheep liver, heart muscle)	—	0·10
3. Enzyme + factor (liver) + cozymase + MB + 1 mg. acetaldehyde	—	6·80
Experiments		
1. Enzyme + cozymase + alcohol + MB	80	4·45
2. Enzyme + cozymase + alcohol + heart muscle factor + MB	12	6·00
3. Enzyme + cozymase + alcohol + skeletal muscle factor + MB	16	6·60
4. Enzyme + cozymase + alcohol + liver factor + MB	8	2·20
5. Enzyme + cozymase + alcohol + liver factor + MB	10	2·35
6. Enzyme + cozymase + 1 mg. aldehyde + liver factor + MB, incubated for 10 min. at 37°	—	6·60

It can be seen from Table IV that acetaldehyde is produced in samples without additional coenzyme factor and also when a washed heart muscle suspension is added as the source of the factor. Acetaldehyde dinitrophenylhydrazone was also obtained in similar experiments by precipitation with 2:4-dinitrophenylhydrazine after removal of the proteins with trichloroacetic acid and adsorption of methylene blue on kaolin.

In all cases however where washed liver suspensions provided the coenzyme factor, acetaldehyde formation could not be detected. A control experiment (Table IV, Exp. 6) where, instead of alcohol, a known amount of acetaldehyde was incubated for 8 min. with the experimental mixture and recovered quantitatively, excluded the possibility of acetaldehyde disappearance due to dismutation or oxidation. No satisfactory explanation of this phenomenon could be found and it is not easy to see what could be the reaction product of alcohol dehydrogenation in this case.

Aerobic experiments on the alcohol dehydrogenase

Several preliminary experiments were first carried out on 1 : 1 aqueous liver extracts in which O_2 uptake was measured on addition of alcohol and cozymase. A very rapid and marked increase in O_2 uptake by the extracts was observed with alcohol and similarly, but to a smaller extent, with glycerol. It should however be pointed out that there was always a large "blank" O_2 uptake in the extracts which did not disappear even on very long autolysis and dialysis. The observed increase in O_2 uptake on addition of alcohol very likely represented some complicated reaction between alcohol (or its oxidation product) and the substrates already present in the extract.

When however the O_2 uptakes of the acetone or ammonium sulphate preparations of liver alcohol dehydrogenase were tested manometrically on addition of alcohol and cozymase, it was found that practically no O_2 was taken up unless a carrier was provided. Methylene blue was found to be a suitable carrier, pyocyanine acted equally well (Table V, Exps. 2–4), quinone in small concentrations was useless, in larger concentrations it inhibited the enzyme. Even in the presence of methylene blue the reaction still remained very slow. Some acceleration was evident when *M*/6 semicarbazide was added as acetaldehyde fixative (Table V, Exp. 7). Addition of suspensions of coenzyme factor from liver or skeletal muscle had no effect on the O_2 uptake; when however a washed heart muscle suspension was used a definite acceleration of the reaction was obtained (Table V, Exps. 8, 10). The explanation probably lies in the fact that when tested for O_2 uptake in presence of *p*-phenylenediamine, the heart muscle suspension showed a considerable activity due to cytochrome oxidase even without addition of cytochrome *c*, whereas the liver and skeletal muscle suspensions yielded negative results.

Table V. *O_2 uptake of the liver alcohol dehydrogenase preparation*

In Barcroft manometers. 30 mg. enzyme preparation, 5 mg. alcohol, 0·5 mg. cozymase, 0·1 and 1 mg. methylene blue (MB), 1 mg. pyocyanine hydrochloride, 2 mg. flavoprotein, 0·5 ml. *M*/2 semicarbazide, 1 ml. washed heart muscle or skeletal muscle preparation. Phosphate buffer *M*/30, *p*H 7·4, total volume 3·3 ml.

Enzyme + cozymase + alcohol with the addition of:	O_2 uptake μl./80 min.
1. —	20
2. 0·1 mg. MB	50
3. 1·0 mg. MB	120
4. 1·0 mg. pyocyanine	110
5. 2·0 mg. flavoprotein	30
6. 2·0 mg. flavoprotein + 1·0 mg. MB	90
7. *M*/2 semicarbazide	170
8. Washed heart muscle suspension	240
9. Washed skeletal muscle suspension	40
10. Washed heart muscle suspension	200

Chemical estimations of the reaction product in aerobic experiments were made, but gave unsatisfactory results owing mainly to the very prolonged reaction time (3–4 hr.) before theoretical values were reached. The presence of volatile acid (acetic acid) in these experiments especially with some of the more crude preparations explained the small yields of acetaldehyde. It pointed to the fact that acetaldehyde partly underwent either oxidation or dismutation to acetic acid.

Various alcohols. The activity of the liver alcohol dehydrogenase was tested in presence of other alcohols, mainly by the Thunberg technique. Propyl

alcohol gave values similar to ethyl alcohol; with methyl and amyl alcohols positive results were obtained. With saligenin, glycerol and α-glycerophosphate negative results were obtained even on addition of the coenzyme factor.

Table VI. *Various alcohols*

Thunberg tubes with 20 mg. enzyme preparation, 30 mg. substrate, 0·5 mg. cozymase, 1 mg. methylene blue, 1 ml. coenzyme factor, 2 ml. phosphate buffer *p*H 7·6.

	Reduction time
1. Ethyl alcohol	1 min. 35 sec.
2. Propyl alcohol	2 min.
3. Amyl alcohol	5 min. 35 sec.
4. Methyl alcohol	11 min.
5. Saligenin	∞
6. Glycerol	∞
7. α-Glycerophosphate	∞

The comparatively slow oxidation of methyl alcohol (Table VI, Exp. 4) suggested the possibility of a poisoning of the enzyme by H.CHO as the reaction product. But it was found that H.CHO in small concentrations has little effect on the liver alcohol dehydrogenase and incidentally it was discovered that it undergoes in the liver preparations a typical dismutation to CH_3OH and H.COOH. Large quantities of H.CHO (and also of CH_3.CHO) produce a definite inhibition of the alcohol dehydrogenase which is partly reversible after removal of the H.CHO by dialysis.

The presence of methyl alcohol has no inhibitory effect on the oxidation of C_2H_5OH in liver enzyme preparations. In this respect the bacterial alcohol oxidase shows an interesting behaviour. It hardly oxidizes CH_3OH at all, but in the presence of CH_3OH the oxidation of C_2H_5OH is almost completely abolished. That this is due to a specific influence of the CH_3OH on the alcohol enzyme and not to a general poisoning effect on the bacteria was shown by the fact that other enzymes (aldehyde and/or glucose oxidation) of these acetic bacteria were found to be entirely unaffected by CH_3OH.

Inhibitors

Dixon [1937] has found that *M*/1000 iodoacetate completely inhibits the yeast alcohol dehydrogenase. However, negative results or only slight inhibition was obtained with *M*/100 iodoacetate on the animal alcohol dehydrogenase (Table VII; Table VIII, Exps. 3–6). It seemed desirable to examine more closely this difference in the behaviour of the yeast and liver enzymes.

It was thought that the negative results with the liver enzyme preparation might be due to the high content of combined and free SH-groups (glutathione) of the proteins and consequently to a complete decomposition of the iodoacetate during the incubation with the liver enzyme solution. To test this possibility the following procedure was chosen. The yeast alcohol dehydrogenase was prepared by the method used by Dixon [1937] and found to be completely inhibited by *M*/100 iodoacetate. Then varying amounts of the liver enzyme preparation, some of them after exhaustive dialysis and also in some cases after heat-inactivation, were incubated with iodoacetate (final concentration *M*/20) for 5–10 min. at 37°. Then they were mixed with the yeast enzyme and the mixture was again incubated for some time at 37°. The activity was then tested by means of the methylene blue technique in the usual way. It was found that the inhibitory effect of iodoacetate upon the yeast enzyme was almost completely abolished in samples where comparatively large quantities of the

Table VII. *Effect of iodoacetate on the liver alcohol dehydrogenase*

30 mg. enzyme preparation, 0·5 mg. cozymase, 0·1 mg. alcohol 96%, 1 mg. methylene blue, 1 ml. factor preparation, iodoacetate (IAA) to give $M/50$ or $M/100$ final concentration. The enzyme solution was incubated with iodoacetate for 15 min. at 37° before the actual experiment.

	Reduction time
A. Ammonium sulphate preparation	
1. No IAA	6 min.
2. +IAA $M/100$	7 min.
3. +IAA $M/50$	7 min. 50 sec.
B. Acetone preparation	
Not dialysed:	
1. No IAA (without "factor")	28 min.
2. +IAA $M/100$ (without "factor")	29 min.
Dialysed:	
3. No IAA (without "factor")	30 min.
4. +IAA $M/100$ (without "factor")	36 min.
5. No IAA (with "factor")	3 min. 10 sec.
6. +IAA $M/100$ (with "factor")	5 min. 50 sec.

liver preparation, boiled or unboiled, were incubated with iodoacetate (Table VIII, B, Exps. 1, 3). If, however, small amounts of the liver enzyme were used, or after a very prolonged dialysis, the decomposition of iodoacetate by the SH-groups of the liver enzyme was comparatively small and there was enough iodoacetate left to exert its usual poisoning effect upon the yeast alcohol dehydrogenase (Table VIII, B, Exps. 2, 4). The liver enzyme nevertheless remained uninhibited (Table VIII, A, Exps. 4, 6).

Table VIII. *Effect of iodoacetate in mixtures of yeast—and liver—alcohol dehydrogenase preparations*

Thunberg tubes. 3% enzyme solutions in phosphate buffer $M/50$, *p*H 7·4; 0·5 mg. cozymase, 1 ml. $M/2000$ MB.

	ml. yeast enzyme	ml. liver enzyme	Final concentration of IAA	Reduction time
A. 1.	1·00	—	—	3 min. 35 sec.
2.	1·00	—	$M/100$	∞
3.	—	0·50, not dialysed	—	18 min.
4.	—	0·50, not dialysed	$M/100$	20 min.
5.	—	0·50, dialysed	—	25 min.
6.	—	0·50, dialysed	$M/100$	33 min.
7.	1·00	0·50, not dialysed	—	3 min. 50 sec.
8.	1·00	0·10, not dialysed	—	3 min.

B. Samples of liver enzyme + IAA (final concentration $M/50$) were incubated for 10 min. at 37°. 1 ml. of yeast enzyme solution was added and the mixture again incubated for 10 min. at 37° before testing with MB.

Yeast enzyme added to:	Reduction time
1. 0·50 ml. liver enzyme (not dialysed) + IAA	5 min. 45 sec.
2. 0·10 ml. liver enzyme (not dialysed) + IAA	60 min.
3. 0·50 ml. liver enzyme (boiled, not dialysed) + IAA	7 min. 30 sec.
4. 0·50 ml. liver enzyme (dialysed) + IAA	50 min.

Yet another experiment was done to prove that the liver alcohol dehydrogenase is resistant to iodoacetate. In a liver preparation which contained both the alcohol dehydrogenase and the aldehyde mutase, $M/80$ iodoacetate inhibited the mutase almost completely and had only a very small effect on the dehydrogenase.

The bacterial alcohol oxidase tested aerobically gave similarly negative results after prolonged incubation with iodoacetate. In anaerobic experiments a slight but reproducible inhibition was recorded.

A few other substances known to act as inhibitors in various enzyme systems were tested and their actions both on the liver alcohol dehydrogenase and aldehyde mutase compared. Urethane, oxalate, maleate and pyrophosphate produce a fairly large inhibition of the alcohol dehydrogenase (30–50 %) but none of the mutase. Both enzymes are cyanide stable. Quinine, morphine and nicotine in low concentration act as inhibitors of the mutase but have no effect on the alcohol dehydrogenase.

In experiments on the aldehyde mutase which will be shortly described in detail, it was found that ammonium sulphate inhibits and also partly destroys irreversibly the aldehyde mutase but leaves the alcohol dehydrogenase unaffected.

Methylene blue in concentrations suitable as a carrier for the alcohol dehydrogenase slows down considerably the mutase activity.

Animal tissues other than liver

When tested by the methods described in this paper, positive results were obtained with alcohol dehydrogenase preparations from intestine and kidney, but brain and muscle gave negative results.

Discussion

The mechanism of the oxidation of alcohol in yeast has been much studied and recently Negelein & Wulff [1937] in Warburg's laboratory have isolated in crystalline form the alcohol dehydrogenase from yeast. However, the alcohol dehydrogenase of animal tissues has received much less attention in spite of its occurrence in various animal tissues. From the experimental evidence given in this paper several conclusions can be drawn regarding the properties and the behaviour of the animal alcohol dehydrogenase under various conditions.

It has been shown that the enzyme is able to oxidize alcohol both anaerobically and aerobically but it requires for its activity the presence of some additional substances. The coenzyme necessary for this reaction is cozymase, and this property seems to be general for all enzyme systems concerned with alcohol oxidation (yeast, animal tissues, bacteria and also probably plants).

Acetone-treated enzyme preparations require for their completion under anaerobic conditions the addition of the coenzyme factor, so that this factor must be regarded as an essential part of the enzyme system. The omission of the coenzyme factor led in older observations to the unjustified view that the enzyme is particularly unstable.

In tissue slices [Leloir & Munoz, 1938] or in liver extracts alcohol undergoes rapid oxidation, but the acetone- or ammonium sulphate-treated enzyme preparations react with O_2 slowly and only if a carrier such as methylene blue is provided. The fact that an acceleration of O_2 uptake can be achieved through heart muscle cytochrome oxidase suggests the probability of a similar process actually taking place when alcohol is oxidized in the intact liver tissue. It also explains why it has been frequently assumed that the alcohol oxidase system from animal tissues is a CN-sensitive system. We found that under anaerobic conditions cyanide either has no effect upon the enzyme or slightly accelerates the reaction, probably by fixing the aldehyde. Thus the cyanide inhibition observed is due solely to the poisoning of the cyanide-sensitive cytochrome oxidase system.

It is difficult to regard flavoprotein as a part of the animal alcohol dehydrogenase system. It has only a very small effect on the course of the reaction, when added to preparations repeatedly treated with acetone.

When alcohol is oxidized anaerobically by the enzyme preparation either alone or with the addition of a washed muscle suspension as coenzyme factor, acetaldehyde is found as the reaction product. It is difficult to account for the fact that no acetaldehyde is found when washed liver suspensions are used as the source of the coenzyme factor. Such washed liver suspensions alone show no aldehyde dehydrogenase or mutase activity, and when acetaldehyde is added and incubated with the experimental mixture composed of the liver enzyme preparation, liver suspension (coenzyme factor), cozymase and methylene blue, it is recovered quantitatively. The possibility remains of the alcohol dehydrogenation being directed into a hitherto unknown route in presence of the washed liver suspension, or of its being linked with some secondary reaction. But to prove this assumption purification and closer analysis of the liver suspension is necessary.

In experiments in which the course of the reaction is very slow, e.g. in aerobic conditions or anaerobically without additional coenzyme factor, the yield of acetaldehyde is smaller than would be expected from the amount of alcohol added and some acetic acid is produced. This can be accounted for by the presence in the preparations of aldehyde mutase and in some of the more crude preparations also of aldehyde oxidase. It explains why in the intact liver tissue acetic acid is found almost exclusively as the product of alcohol oxidation.

In contrast to the yeast alcohol dehydrogenase, the animal (and also the bacterial) alcohol dehydrogenase seems to be only little affected by iodoacetate, even if large concentrations of the iodoacetate are allowed to act upon the enzyme for some time. This finding, together with the fact that flavoprotein does not seem to be a constituent of the animal enzyme, suggests the possibility of some essential difference between the structures of the yeast and the animal alcohol dehydrogenases.

Dixon & Lutwak-Mann [1937] have shown that by suitable methods of preparation and purification it is possible to obtain the liver aldehyde mutase free from alcohol dehydrogenase. The present results, where the actions of various inhibitors were tested both on the alcohol dehydrogenase and on the mutase, furnish further evidence that these two enzymes are distinct systems.

Summary

Various methods of preparation of the liver alcohol dehydrogenase and its properties are described.

By using suitable preparations it can be shown that cozymase and cozymase factor are essential components of the system.

According to the treatment, preparations can be obtained which contain no cozymase but have enough coenzyme factor (precipitation with ammonium sulphate) and vice versa (precipitation with acetone). The alcohol dehydrogenase in the preparations remains unaltered over a period of months.

Under certain experimental conditions acetaldehyde is obtained quantitatively as the reaction product of alcohol oxidation. The probability of other courses of alcohol oxidation is discussed.

The alcohol dehydrogenase in the preparations cannot react directly with O_2. The presence of carriers (methylene blue, pyocyanine) is necessary. The reaction with O_2 is markedly accelerated by addition of cytochrome oxidase preparations.

Ethyl, propyl, methyl and amyl alcohols are oxidized by the liver alcohol dehydrogenase preparations. With glycerol, α-glycerophosphate and saligenin negative results are obtained.

Iodoacetate has little effect upon the animal alcohol dehydrogenase. The enzyme is not sensitive to cyanide. Evidence is provided for the non-identity of the liver alcohol dehydrogenase and aldehyde mutase.

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