XLI. ETHYL ALCOHOL METABOLISM IN ANIMAL TISSUES

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BATELLI & STERN [1910] studied the oxidation of ethyl alcohol using "brei" of different organs and animals. An oxidation to acetaldehyde and acetic acid was observed, the most active organ being horse liver. Horse kidney also showed some activity. The enzymes responsible for this two-step oxidation are: for the first step (alcohol $\rightleftharpoons$ aldehyde) an alcohol dehydrogenase, and for the second (aldehyde $\rightarrow$ acid) either the Schardinger enzyme or a mutase.

Alcohol dehydrogenase of animal origin has been studied by Reichel & Köhle [1935] but much more is known about that of yeast which has even been obtained in crystalline form [Negelein & Wulff, 1937]. It requires coenzyme I (diphosphopyridine-nucleotide) and can therefore use the yellow enzyme as a carrier. The mutase [Parnas, 1910] which might catalyse the second step is, according to Dixon & Lutwak-Mann [1937], different from the Schardinger enzyme and also needs coenzyme I.

Many experiments have been done on the whole animal [see Le Breton, 1936] and also by liver perfusion [Lundsgaard, 1937], but the tissue slice technique has not been much used in this problem.

Methods

The method of Nicloux *et al.* [1934] was used for estimating alcohol. We have found it quite specific since organic acids and acetone did not interfere under the conditions of our experiments. O_2 uptake was measured with Warburg manometers in a medium of phosphate-Ringer [Krebs, 1933].

In experiments with liver there is a considerable acid formation; 3 ml. of Ringer were therefore used and dry weights of tissue between 10 and 15 mg.

In each experiment the thermobarometers contained alcohol solution; these were treated in exactly the same manner as the other manometers and were used to determine the initial amount of alcohol.

After 2 hr. at 37·5° the manometers were cooled in ice-water for 10 min. before opening, the slices were removed and the contents quantitatively transferred with saturated picric acid for alcohol estimation. In some cases a correction was applied to allow for the alcohol removed with the slices, and with certain organs (testis) the whole contents of the Warburg vessel were distilled. During the experimental period a loss of 3–5 % alcohol occurred, but duplicates (thermobarometers) agreed within 3 %.

Large-scale experiments. Flasks similar to those described by Krebs [1933] were filled with 22 ml. of bicarbonate-Ringer and $O_2+5\%$ CO_2 (*p*H 7·4). The initial composition of the medium was ascertained by withdrawing 10 ml. of the liquid after the gassing. The flasks were then not opened until the end of the experiment. In some cases oxygen uptake was measured in phosphate-Ringer in a parallel experiment.

Acetic acid in 2–5 ml. of liquid was titrated after distillation with an air current in a way similar to that described in Pregl & Roth [1935]. Values were compared with those given by distilling a standard solution.

Bicarbonate was estimated manometrically at 25° on 1 ml. solution plus 1 ml. distilled water, after gassing with 5% CO_2 in N_2, by addition of 0·05 ml. 40% H_2SO_4. On treating 2 ml. of water in the same way 27–30 μl. of gas are evolved (probably because of a decrease in CO_2 solubility), but as changes were always measured the correction cancels out.

Lactic acid was estimated as described by Friedemann & Kendall [1929] and West [1931]. Samples were evaporated to half-volume in a water bath to remove alcohol. The same apparatus (West's) was used for distilling the acetaldehyde. Pyruvic acid was estimated with carboxylase as described by Westerkamp [1933].

Units. $Q = \mu$l. of gas or of the substance in question calculated as gas at N.T.P., divided by tissue dry weight in mg. and time in hr. The suffix DA is used for distillable acid (calculated as monovalent) and A for acid (decrease in CO_2-combining power of the liquid). Taking 1 mol. ethanol as equivalent to 1 mol. gas 1γ ethanol = 0·487 μl.

Experimental results

Results obtained using different tissues appear in Table I. Liver is the only organ that causes a large alcohol disappearance (Tables II and IV), $-Q_{\text{ethanol}}$ in O_2 being between 6 and 9 for rat and about 3 for pigeon liver. Pigeon muscle

Table I. *Aerobic disappearance of ethanol in the presence of tissue slices*

2–3 ml. of phosphate Ringer, *p*H 7·4. 2 hr. at 37·5°. Gas: O_2.

Organ	Substrate	Q_{ethanol}	$-Q_{O_2}$
Rat kidney	Nil	0	20·8
	Ethanol, 0·0016 *M*	−0·3	22·6
Rat kidney	Nil	0	19·9
	Ethanol, 0·0015 *M*	0	23·1
Rat kidney	Nil	0	18·7
	Ethanol, 0·0019 *M*	−0·6	21·4
Rat kidney*	Nil	0	16·4
	Ethanol, 0·0019 *M*	−3·5, −3·9	20·4, 20·3
Rat testis	Nil	0	5·0
	Ethanol, 0·0016 *M*	0	4·8
	Glucose, 0·01 *M*	0	10·5
	Glucose + ethanol, 0·0016 *M*	−0·4	9·7
Rat spleen	Nil	0	10·7
	Ethanol, 0·0016 *M*	−0·7	10·3
Rat diaphragm	Nil	+0·2	5·3
	Ethanol, 0·0016 *M*	+0·2	4·7
Rat intestine	Nil	0	5·1
	Ethanol, 0·0016 *M*	0	4·5
Pigeon muscle "brei"	Nil	+0·04	—
	Ethanol, 0·0006 *M*	−0·15, −0·08	—
Pigeon liver	Nil	0, 0	9·4, 9·8
	Ethanol, 0·0006 *M*	−2·8, −3·0	8·9, 8·9

* This animal had received 10 ml. of ethanol per kg. daily for 1 month.

(brei) and rat kidney cause a small but definite disappearance. The action of kidney was specially marked in a rat which had previously received alcohol orally for a month. With other tissues the disappearance, if it exists, falls within the experimental error.

Oxygen uptake is slightly decreased by alcohol in every organ (Table I) except in kidney where it is increased.

Fasting decreases alcohol oxidation in liver (Table II), as was observed for the whole animal by Le Breton [1936]. Livers of rats fasted 50–70 hr. gave

Table II. *Influence of fasting on aerobic disappearance of ethanol*

Livers from rats fasted 50–70 hr. Values in brackets are those given by the controls belonging to the same litter and fed 2 hr. before.

Substrate	$Q_{ethanol}$	$-Q_{O_2}$
Nil	0 (0)	11·8 (14·7)
Ethanol, 0·0006 *M*	− 5·0 (− 8·7)	10·6 (12·4)
Ethanol, 0·0006 *M*	− 5·4 (− 8·7)	10·5 (13·0)
Ethanol + pyruvate, 0·0135 *M*	− 17·5 (− 13·7)	19·3 (21·8)
Ethanol + fumarate, 0·0135 *M*	− 10·0 (− 11·1)	14·6 (14·8)
Nil	0 (0)	10·5 (12·8)
Ethanol, 0·0006 *M*	− 5·2 (− 7·2)	9·99 (13·0)
Ethanol, 0·0006 *M*	− 5·4 (− 7·1)	9·97 (13·1)
Ethanol + *dl*-malate, 0·0135 *M*	− 6·9 (− 9·0)	12·6 (14·9)
Ethanol + acetate, 0·0135 *M*	− 5·3 (− 7·5)	11·7 (15·2)
Nil	0 (0)	11·6 (12·6)
Ethanol, 0·0006 *M*	− 5·6 (− 8·1)	10·2 (12·3)
Ethanol, 0·0006 *M*	− 5·6 (− 7·7)	10·0 (12·1)
Ethanol + pyruvate, 0·0135 *M*	− 20·0 (− 12·3)	24·0 (18·7)
Ethanol + glucose, 0·0135 *M*	− 7·3 (− 7·9)	12·6 (12·6)

$-Q_{ethanol}$ of 5–5·6, whereas the controls from the same litter fed 2 hr. previously gave values of 7–8.

Livers of rats which had received alcohol (10 ml. per kg. per day for 1 month) gave values of about 8, i.e. at the upper limit of normal variation.

A change in concentration of alcohol from $1·5 \times 10^{-4}$ to $2·2 \times 10^{-3}$ *M* does not appreciably change the $Q_{ethanol}$.

Anaerobically the $-Q_{ethanol}$ decreases to about 1.

Table III. *Anaerobic disappearance of ethanol in liver*

2·5 ml. bicarbonate-Ringer, *p*H 7·4. Gas: $N_2 + 5\%$ CO_2.

Substrate	$Q_{ethanol}$	$Q^{N_2}_{CO}$
Nil	0	1·1
Ethanol, 0·0008 *M*	− 1·2	1·1
Pyruvate, 0·02 *M*	0	9·2
Pyruvate + ethanol	− 3·0	8·1
Lactate, 0·02 *M*	0	1·2
Lactate + ethanol	− 1·9	1·0
Fumarate, 0·02 *M*	0	2·7
Fumarate + ethanol	− 1·2	1·7
Succinate, 0·02 *M*	0	—
Succinate + ethanol	− 1·1	0·5
Ethanol, 0·0008 *M*	− 1·3	2·0
Ethanol + fumarate, 0·0135 *M*	− 1·4	2·6
Ethanol + pyruvate	− 3·6	6·8
Ethanol, 0·0033 *M*	− 1·8	1·5
Oxaloacetate, 0·01 *M*	0	—
Oxaloacetate + ethanol	− 3·2	—
Fumarate, 0·01 *M* + ethanol	− 1·5	1·6

In none of our experiments did we find a well-defined alcohol formation. Sometimes in the controls we obtained values of 5–10 μl. alcohol but we do not consider these results as trustworthy. Neither aerobically nor anaerobically,

Table IV. *Effects of different substrates on the aerobic disappearance of ethanol in liver slices*

Substrate	$Q_{ethanol}$	$-Q_{O_2}$
Nil	0	11·2
Ethanol, 0·0008 *M*	−7·4, −7·8	10·6, 10·9
Lactate, 0·01 *M* + ethanol	−9·5, −9·6	11·7, 12·2
Pyruvate, 0·01 *M* + ethanol	−12·4	15·1
Succinate, 0·01 *M* + ethanol	−8·0	15·3
Fumarate, 0·01 *M* + ethanol	−8·9	11·1
dl-Malate, 0·01 *M* + ethanol	−8·8	11·5
Acetate, 0·01 *M* + ethanol	−8·1	11·7
Nil	0	11·3
Ethanol, 0·0019 *M*	−5·5	10·0
Ornithine, 0·01 *M*	0	11·8
Ornithine + ethanol	−5·8	9·8
Nil	0	13·2
Ethanol, 0·0015 *M*	−6·6	11·2
Lactate, 0·01 *M*	0	14·5
Lactate + ethanol	−9·5	12·4
Nil	0	10·0
Ethanol, 0·0018 *M*	−6·6	10·0
Acetate, 0·01 *M*	0	13·6
Acetate + ethanol	−5·4	11·8
Ammonium chloride, 0·01 *M*	0	10·3
Ammonium chloride + ethanol	−5·4	9·9
Glycine, 0·01 *M*	0	—
Glycine + ethanol	−6·6	10·5
Nil	0	11·7
Ethanol, 0·0016 *M*	−7·0	11·0
Succinate, 0·01 *M*	0	19·0
Succinate + ethanol	−8·4	16·4
Nil	0	10·9
Ethanol, 0·0005 *M*	−6·7, −7·0	10·4, 10·0
Lactate, 0·0033 *M* + ethanol	−9·4	11·3
Lactate, 0·0067 *M* + ethanol	−9·3, −9·3	11·4, 11·7
Fumarate, 0·0033 *M* + ethanol	−8·3	11·0
Fumarate, 0·0067 *M* + ethanol	−8·5	11·3
Nil	0	12·3
Ethanol, 0·0018 *M*	−7·6	10·7
Fumarate, 0·01 *M*	0	11·7
Fumarate + ethanol, 0·0018 *M*	−9·8	11·8
dl-Malate, 0·01 *M*	0	12·3
dl-Malate + ethanol	−9·6	11·8
dl-Alanine, 0·01 *M*	0	13·7
dl-Alanine + ethanol	−9·4	11·1
Glycerol, 0·01 *M*	0	10·9
Glycerol + ethanol	−5·6	10·1
Nil	0	13·6
Ethanol, 0·0018 *M*	−6·1	10·6
Butyrate, 0·01 *M*	0	18·2
Butyrate + ethanol	−5·7	14·0
dl-β-Hydroxybutyrate, 0·01 *M*	0	12·6
dl-β-Hydroxybutyrate + ethanol	−5·8	10·7
Pyruvate, 0·01 *M*	0	14·8
Pyruvate + ethanol	−11·8	14·2
Nil	0	12·8
Ethanol, 0·0018 *M*	−7·4	11·2
Citrate, 0·01 *M*	0	12·9
Citrate + ethanol	−9·4	14·2
Nil	0	12·3
Ethanol, 0·0033 *M*	−8·5, −8·7	11·9, 12·4
Oxaloacetate, 0·01 *M*	0	14·5
Oxaloacetate + ethanol	−13·6	16·3
Nil	0	11·8
Ethanol, 0·0033 *M*	−8·1, −8·1	11·2, 11·0
Lactate, 0·0016 *M* + ethanol	−9·3, −9·7	10·9, 11·5

with or without substrates was this blank increased. Only with pigeon muscle "brei" were values as high as 30 μl. obtained, but it is probable that some other substance interferes in the estimation as the distillation was slower than that of ethanol.

By incubating the intestine of a fed rat in Ringer for 2 hr. at 37° 0·5 mg. of alcohol was obtained. This was not identified but it is well known that alcohol can be formed by bacterial action.

Effect of different substrates. Of the different substrates tested aerobically (Tables II and IV) pyruvic acid was found to be the most active in increasing the $-Q_{\text{ethanol}}$ (from 7–8 to 12–20).

Increases were also observed with succinic, fumaric, malic, oxaloacetic and lactic acids, alanine and sometimes with citric acid (2 exps. out of 4). Acetic, butyric, β-hydroxybutyric and ascorbic acids, glycine, ornithine, glucose, ammonium chloride and insulin showed no action, while glycerol slightly depressed alcohol oxidation.

In the fasted rat the effect of pyruvic acid seemed to be even greater, $-Q_{\text{ethanol}}$ rising from 5 to 17 or 20. In one case glucose caused an increase from 5 to 7.

Influence of inhibitors. Table V shows the results obtained using various inhibitors. Cyanide greatly decreases alcohol oxidation, but does not stop it

Table V. *Influence of inhibitors*

Rat liver slices. Phosphate-Ringer, *p*H 7·4. Gas: O_2.

Substrate	Q_{ethanol}	$-Q_{O_2}$
Nil	0	11·3
Ethanol, 0·0015 *M*	−5·5	10·0
KCN, 0·001 *M*	0	1·2
KCN + ethanol	−0·6	1·4
KCN, 0·005 *M*	0	0·5
KCN + ethanol	−1·8	0·9
Nil (Ringer, no Ca)	0	8·8
Ethanol, 0·0015 *M*	−6·2	11·1
Fluoride, 0·01 *M*	0	8·2
Fluoride + ethanol	−5·2	7·8
Oxalate, 0·01 *M*	0	9·4
Oxalate + ethanol	−4·0	12·6
Arsenate, 0·01 *M* + ethanol	−5·2	8·1
Nil	0	10·0
Ethanol, 0·0016 *M*	−6·6	10·0
Malonate, 0·04 *M*	0	8·7
Malonate + ethanol	−5·3	8·0
Nil	0	13·6
Ethanol, 0·0015 *M*	−6·1	10·6
Phlorhidzin, 0·01 *M*	0	5·9
Phlorhidzin + ethanol	−3·9	7·3
Nil	0	12·8
Ethanol, 0·0006 *M*	−7·4	11·2
Iodoacetate, 0·007 *M*	0	3·3
Iodoacetate + ethanol	−2·7	4·4
Iodoacetate, 0·013 *M*	0	4·0
Iodoacetate + ethanol	−2·2	5·3
Nil	0	13·2
Ethanol, 0·0017 *M*	−6·6	11·2
Dinitrophenol, $1{\cdot}1 \times 10^{-5}$ *M*	0	14·2
Dinitrophenol + ethanol	−7·6	12·3
Dinitrophenol, $1{\cdot}1 \times 10^{-4}$ *M*	0	7·0
Dinitrophenol + ethanol	−4·2	9·3
Arsenate, 0·01 *M*	0	8·7
Arsenate + ethanol	−5·9	9·6

completely. A small increase in O_2 uptake is produced by alcohol when cyanide is present, this probably being due to cyanide-insensitive carriers (yellow enzyme), but it is not quantitatively important.

Iodoacetate which inhibits alcohol dehydrogenase [Dixon, 1937] and mutase [Dixon & Lutwak-Mann, 1937] brings down the $-Q_{\text{ethanol}}$ to 2 and in this case an increase in Q_{O_2} is also produced by alcohol. Fluoride, arsenate, phloridzin, malonate and oxalate also decrease alcohol oxidation; 2:4-dinitrophenol increases it at $1{\cdot}1 \times 10^{-5}$ M concentration and decreases it at higher concentrations.

Since incubation with cyanide has been shown to inhibit specifically the Schardinger enzyme [Dixon & Keilin, 1936; Leloir & Dixon, 1937] it was thought that it might prove interesting to try it on this system. Liver slices were incubated 30 min. with KCN $M/500$, washed 5 times in Ringer and then used for a large-scale experiment. We obtained: $-Q_{O_2} = 9{\cdot}2$, $Q_A = 7{\cdot}3$, $Q_{DA} = 6{\cdot}4$, while for the control without alcohol we obtained respectively: 9·3, 1·4, 1·6: i.e. no inhibition. Nevertheless it is uncertain if the Schardinger enzyme could be completely inhibited under these conditions, in view of the fact that in the presence of adequate substrates [Dixon & Keilin, 1936] the inhibition is not produced.

The oxidation product. The results of large-scale experiments are given in Table VI. Bicarbonate estimations indicate a great increase in acid formation,

Table VI. *Liver slices in bicarbonate Ringer*

Δ indicates the difference in composition of 1 ml. of the medium before and after 2 hr. at 37·5°. Gas: $O_2 + 5\%$ CO_2. Final volume: 12 ml.

Animal	Dry wt. of slices mg.	Substrate	Δ ethanol μl.	Q_{ethanol}	Δ bicarbonate μl.	Q_A	Δ distillable acid μl.	Q_{DA}	Q_{O_2}*	Q_{ethanol}*
Rat	199	Ethanol, 0·0019 M	−320	−9·7	−230	+7·0	+170	+5·2	−11·7	−9·1
	163	None	0	0	− 22	+0·8	0	0	−12·1	0
Rat	200	Ethanol, 0·0018 M	−294	−8·8	−219	+6·5	+173	+5·2	− 9·6	−8·4
	201	None	0	0	− 23	+0·7	0	0	−10·1	—
Pigeon	202	Ethanol, 0·0018 M	−127	−3·8	− 38	+1·13	+ 30	+0·9	—	—
	222	None	0	0	− 20	+0·5	+ 15	+0·4	—	—

* Measured in parallel experiment.

the Q_A value rising from 0·7–0·8 to 6·5–6·9. All or nearly all of this acid is distillable, the amount of it (Q_{DA} 5·2 and 5·2) corresponding roughly to the Q_A. Values obtained in other experiments were 10% higher or lower owing to the difficulty of obtaining very reliable results in the distillation and titration of small amounts of acetic acid.

In one of the experiments the acid was redistilled, neutralized, concentrated and the lanthanum test for acetic acid [Krüger & Tschirch, 1929; 1930] carried out. The reaction was intensely positive.

Table VI also shows an experiment on pigeon liver; $-Q_{\text{ethanol}}$ is lower (3·8). Acid formation is low but definite.

Little if any acetaldehyde seems to accumulate when rat liver slices oxidize alcohol. After incubating liver slices (*c.* 200 mg. dry weight) 1 hr. in the presence of $M/100$ alcohol and distilling the whole contents of the flask (with 0·1 N HCl) in West's apparatus, 1·6 ml. of $N/200$ iodine were required in the Clausen titration, while 1·5 ml. were used for the control.

Using liver "brei" instead of slices (*c.* 600 mg. dry weight) and the same concentration of alcohol, 10·6 ml. of iodine were required against 1·2 ml. for the control without alcohol. The bisulphite-binding substance was identified as acetaldehyde in the following way. HCl was added to the bisulphite solution to make the conc. 2*N*, excess SO_2 was blown out with N_2, and saturated 2:4-dinitrophenylhydrazine in 2*N* HCl was added. After 1 hr. at 37° crystallization began. After several recrystallizations from alcohol a hydrazone M.P. 166° (uncorr.) was obtained. Mixed M.P. with acetaldehyde hydrazone was 166°, M.P. = 168° (corr.), Campbell [1936]. In one case a hydrazone melting at 147° [Brady, 1931] was obtained.

The action of pyruvic acid. Data in Table III show the influence of pyruvic acid on the anaerobic disappearance of alcohol. An increase in $-Q_{\text{ethanol}}$ from 1 to 3 was produced, whereas fumaric acid had no such effect.

The anaerobic disappearance of pyruvic acid in the presence of alcohol and aldehyde was studied (Table VII). Both substances were active but the alcohol

Table VII. *Anaerobic disappearance of pyruvic acid*

Rat liver slices, 1 hr. at 37°. Gas: $N_2 + 5\%$ CO_2.

Substrate	$Q_{CO_2}^{N_2}$	$-Q_{\text{pyruvate}}$
Nil	6·6	0
Pyruvate, 0·005 *M*	12·9, 11·2	6·9, 7·0
Pyruvate + acetaldehyde, 0·01 *M*	17·7, 17·1	11·2, 10·9
Pyruvate + ethanol, 0·01 *M*	11·7, 10·8	13·5, 12·6

more so than the acetaldehyde. Anaerobic lactate formation from pyruvate was also increased by alcohol (Table VIII) from about 5 to 11. The fact that in this experiment Q_{lactate} is higher than $Q_{CO_2}^{N_2}$ is presumably due to a faster formation of lactate in the first 20 min., before the CO_2 measurement was begun.

Table VIII. *Anaerobic lactic acid formation*

Substrate	Q_{lactate}	$Q_{CO_2}^{N_2}$
Nil	3·8, 3·2	2·7, 2·8
Ethanol, 0·0017 *M*	2·4, 2·5	0·5, 0·7
Pyruvate, 0·013 *M*	5·6	4·2
Pyruvate, 0·013 *M* + ethanol	11·2	7·1
Pyruvate, 0·02 *M*	4·1	4·3
Pyruvate, 0·02 *M* + ethanol	11·7, 10·8	7·0, 6·4

The amount of lactate formed was somewhat in excess of the pyruvate disappearance (Table IX). Aerobically, lactate concentrations as low as 0·0016 *M* were still found active in causing increased disappearance of alcohol.

Table IX. *Anaerobic lactic acid formation from pyruvate*

Rat liver slices in bicarbonate-Ringer. Δ indicates the change in composition of 1 ml. of the medium after 1 hr. at 37·5°. Final volume: 14 ml. Gas: $N_2 + 5\%$ CO_2.

	Δ ethanol μl.	Q_{ethanol}	Δ lactate μl.	Q_{lactate}	Δ pyruvate μl.	Q_{pyruvate}
Pyruvate, 0·008 *M* Ethanol, 0·0014 *M* 134 mg. dry weight	−60	−5·8	212	20·5	−155	−7·5
Pyruvate, 0·008 *M* 126 mg. dry weight	0	0	134	13·9	− 93	−4·8

Discussion

Fasting has been found to decrease the speed of alcohol disappearance from the blood of whole animals [cf. Le Breton, 1936] and we have found that it also decreases alcohol oxidation by liver slices. The effect does not appear to be due to a loss of activity of the enzyme system since the addition of appropriate substrates increases the $-Q_{ethanol}$ to values even higher than those of normal liver. Probably there is a decrease in the amount of some of the substances (pyruvic, C_4 dicarboxylic acids) which have been shown to increase alcohol oxidation.

Chronic alcoholism increases alcohol disappearance in the whole animal but no appreciable difference was found in liver slices of rats so treated. Duplicate measurements of a kidney did however show a marked increase over that of the controls. Alcohol is considered to inhibit dehydrogenases and this might be the cause of its small depressing effect on O_2 uptake of liver slices, yet the same concentrations increase the respiration of kidney and of liver "brei". Another difference between "brei" and slices is that only in the former does acetaldehyde accumulate. Practically all the acid formed from alcohol is found to be acetic acid and about 65 % of the alcohol which disappears is recovered as acid. The rest of the alcohol (about 30 %) cannot be accounted for, the aldehyde presumably undergoing further reactions which have not as yet been analysed. If it is assumed that 60 % of the alcohol is transformed into acetic acid and the rest into acetaldehyde, about three-quarters of the normal oxygen uptake must be utilized for the oxidation of alcohol.

It is most probable that the accelerators (pyruvate was especially studied) act as hydrogen acceptors, since we have found that (*a*) pyruvic acid increases alcohol disappearance aerobically or anaerobically, (*b*) alcohol increases pyruvic acid disappearance and lactic acid formation anaerobically. This increase in lactic acid excludes the possibility of chemical combination between pyruvate and alcohol or aldehyde (unless the substance formed is estimated as lactic acid). Moreover, since the effect may appear anaerobically a coupled oxidation of the type described by Keilin & Hartree [1936] probably does not occur. The fact that lactate also increases alcohol oxidation shows that pyruvate acts not only as an acceptor but that, undergoing successive oxidations and reductions, it may also function as a carrier. Lactate concentrations as low as 0·0013–0·003 *M* still have an effect *in vitro* (blood concentration is 0·001–0·002 *M*).

The effects of succinic, fumaric, malic and oxaloacetic acids may be interpreted in a similar way. Since fumaric acid does not act as a hydrogen acceptor anaerobically it appears probable that the acceptor in this series is oxaloacetic acid. Therefore the probable interpretation is that the coupled reaction can only occur between two systems requiring coenzyme [Dewan & Green, 1937].

Our experiments support Szent-Györgyi's theory that C_4 dicarboxylic acids can act as carriers in animal oxidations and they also show that other reversible systems (pyruvic $\rightleftharpoons$ lactic) may act in the same way, at least in the case of alcohol oxidation in liver.

Summary

1. Ethanol oxidation was studied in rat and pigeon tissue slices.

2. Aerobically in rat liver the rate of oxidation is high; $-Q_{ethanol}$ 7–9. It is lowered by fasting to 5. In pigeon liver it is about 3.

3. In other organs except kidney and pigeon muscle oxidation is very small or absent.

4. Oxygen uptake is slightly lowered by ethanol in slices of every organ except kidney, where it is increased.

5. More than half of the alcohol that disappears in liver is converted into acetic acid, practically no acetaldehyde being accumulated.

6. Pyruvic, lactic, succinic, fumaric, malic and oxaloacetic acids and alanine increase aerobic alcohol disappearance.

7. Anaerobically $-Q_{\text{ethanol}}$ is about 1, increasing to 3 with pyruvic and oxaloacetic acids. Fumaric acid has no such effect.

8. Alcohol increases anaerobic disappearance of pyruvic acid by increasing lactic acid formation. The action of pyruvic acid as a carrier is discussed.

9. The influence of cyanide, iodoacetate, fluoride, arsenate, malonate, phlorhidzin and oxalate has been studied.

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