THE METABOLISM OF ETHYL ALCOHOL

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HISTORICAL

The great *Liebig* assumed that alcohol was oxidized in the human and animal organism through aldehyde, lactic acid, oxalic acid and formic acid to carbonic acid, but so far as is known he had no experimental evidence for his assumption. About 1860 the first animal experiments showed that ingested alcohol could be recovered qualitatively from the urine and the expired air, which made these early investigators conclude that alcohol was excreted from the body and not metabolized at all. This was the state of affairs when the first period of modern study of alcohol metabolism began about 1870.

Dupré (1872) (44) and *Anstie* (1874) (5) showed definitely that the alcohol recovered from the excretions over a long period was only an insignificant part of the alcohol administered, and that the rest disappeared. The German and Italian investigators *Binz* (14), *Schmidt* (134) and *Albertoni* (3) independently found the same. As it was impossible to demonstrate the presence of any metabolic products of alcohol in the body or in the excretions, it was concluded that 95–98 per cent of the ingested alcohol was totally oxidized in the organism to carbon dioxide and water.

The chemical energy liberated by the total oxidation of 1 gram alcohol is 7.1 calories, and in the years following 1880 much work was devoted to the question whether ingested alcohol is metabolized independently of the general metabolism. Experiments by *Henrijean* (1883) (71) and *Bodländer* (1883) (17) showed clearly that the general metabolism was not increased by the ingestion of alcohol. This was later confirmed by extensive and convincing experiments by *Bjerre* (1899) (15), *Atwater and Benedict* (1902) (7) and others. Moreover *Strassmann* (1891) (139) demonstrated that the bodies of animals given alcohol over a long period show a higher content of fat tissue than control animals kept on quantitatively and qualitatively the same diet but without alcohol. There was some discussion about the protein-saving effect of alcohol, but when it was discovered that the toxic action of alcohol at first causes an increased

excretion of nitrogen in individuals not accustomed to alcohol, it was easy to show that alcohol spares protein to the same extent as isodynamic amounts of carbohydrate or fat. Thus the first period of the study of alcohol metabolism ended at the beginning of this century with the general acceptance of the very important fact that *alcohol can be utilized as a foodstuff by the organism.*

A little after 1900 the second period started with the study of the metabolic rate of alcohol. In 1903 *Gréhant* (61) analyzed whole animals at various times after the ingestion of alcohol. *Rosemann* (1909) (132) tried to calculate the metabolic rate in man from the depression of the respiratory quotient during alcohol oxidation, and in 1919 appeared *Mellanby's* (105) very commonly cited experiments in dogs. These and other investigators with their rather primitive analytical methods reached figures not deviating much from those found later. The literature up to 1904 is collected by *Abderhalden* (1) and the results of the studies up to 1920 are extensively reviewed by *Kochmann* (87) and *Rosemann* (133). *Widmark's* introduction of his micromethod for alcohol determination in 1922 (145) was an important step forward. During the following decade, stimulated by the increasing forensic importance of test on drunken drivers, by means of this and similar micromethods *Widmark* himself and other investigators cleared up the general facts about alcohol absorption, distribution and elimination in the human and animal body.

Our knowledge in 1935 can be summarized as follows: Alcohol is readily absorbed in the intestinal tract, but certain foodstuffs, especially protein and fat, are able to delay the absorption considerably. The distribution of alcohol in the body follows the equation given by *Widmark* (147):

$$A = p \times c \times r$$

A being the total amount of alcohol in the organism in grams, p the body weight in kg, and c the alcohol concentration in mg per gm blood. The factor r is the proportion of the body in which the alcohol is distributed; on the average it is 67% ± 20%, but it is relatively constant in the same individual from time to time. From the slope of the alcoholaemic curve and the formula it is possible to calculate that an average individual is able to oxidize about 1 gm alcohol per hour per 10 kg bodyweight. The results from these earlier periods are beyond dispute and well described in every textbook and will be omitted from this review.

Three general lines have been followed in the recent study of alcohol metabolism: (i) The factors that may influence the rate of alcohol metabolism and its connections with the metabolism of other compounds in the body; (ii) the sites of alcohol metabolism in the organism; (iii) the enzymes involved in alcohol metabolism. These lines will also be followed in the present review, not because they represent a chronological development or because they represent the most logical arrangement, but because the gap between the investigators who follow the different lines is often so wide that it is impossible to present the experimental results otherwise without imposing on authors opinions and interpretations that they never intended. The following earlier reviews can be recom-

mended which give different points of view on the question,—*Widmark's* (1932, in German) (146), *Kochmann's* (1936, in German) (88), *Le Breton's* (1936, in French) (94), *M. Schmidt's* (1937, in Danish, 135), *Jellinek and Jolliffe's* (1940, in English, (78), *Carpenter's* (1940, in English) (21), *Derobert and Duchène's* (1942, in French) (35), and *Newman's* (1941 and 1947, both in English) (111, 112).

ALCOHOL METABOLISM IN INTACT ORGANISMS

Most investigators working on this problem have measured the rate of disappearance of alcohol from the blood after the administration of alcohol, either intravenously or more commonly by mouth to the experimental subjects. On many points there is much disagreement. One reason is that the technique of many of the investigators has not been beyond criticism. Far too many have disregarded the rather high variation in the absorption of alcohol from the intestinal tract and have not realized that the time required to obtain complete equilibrium in the distribution of alcohol in the tissues is rather long even when the alcohol is given intravenously. The reviewer regrets that only too few of the numerous published experiments fulfill the requirements of a technique which cannot be criticized; the best to say about the rest is that they sometimes are able to support the results obtained by better methods, but not always. In any case they will never be able to contradict them.

The Slope and Shape of the Alcoholaemic Curve in the Postabsorptive Period: Since the work of *Mellanby* (105) and *Widmark* (146) it has generally been accepted that the decrease of the alcohol content in the organism, as measured by the concentration in blood, follows a straight line from the point where the absorption is ended and a diffusion equilibrium between the tissue fluids and the tissues has been established until all measurable remnants of alcohol have disappeared from the blood. The most common expression of this slope is found in *Widmark's* factor, β, which indicates how many milligrams of alcohol disappear from each gram (or ml) blood per minute or per hour. The β factor is independent of the concentration of alcohol in the blood. Since then numerous observations in many species have confirmed these fundamental observations, sometimes even called "laws."

In spite of this, the conclusion that the metabolic rate of alcohol is independent of the concentration of alcohol in the organism has been repeatedly challenged, and convincing experimental evidence has been brought against it. Not a few investigators have failed to find a rectilinear curve of alcohol disappearance from the body and found a hyperbolic curve instead, the slope of which decreases with decreasing concentrations of alcohol. Even some who have found a rectilinear course of the curve claim a definite dependence of alcohol metabolism on alcohol concentration. *Newman et al.'s* (122) experiments have led to the astonishing conclusion that the metabolic rate of alcohol depends on the initial content of alcohol in the organism, but remains constant until all alcohol has disappeared. The experiments do show that a dog starting with a higher alcohol content in the organism has a steeper decline in the alcoholaemic curve than another

dog starting with a lower alcohol content, even when the decline is measured from a point where the two dogs have exactly the same concentration of alcohol in their blood. Moreover, if a dog is pretreated with a certain amount of alcohol, a renewed smaller dose given shortly after the first dose of alcohol has been metabolized will disappear at a constant rate equal to the rate of disappearance

TABLE 1

SPECIES	AUTHOR AND REFERENCE	METHOD OF INVESTIGATION	DEPENDENCE OF ALCOHOL METABOLIC RATE ON ALCOHOL CONCENTRATION
Dog	Mellanby (105)	alcoholaemic curve	no
	Widmark (149)	alcoholaemic curve	no
	Newman and Lehman (118)	alcoholaemic curve	no
	Ewing (47)	alcoholaemic curve	yes, hyperbolic curve
	Cutting, Newman & Lee (32)	alcoholaemic curve + infusion experiments	yes, rectilinear decline, of alcoholaemic curve, but "conditioning"
	Loomis (100)	Same	no
Cat	Newman and Lehman (118)	alcoholaemic curve	no
	Eggleton (46)	alcoholaemic curve +infusion experiments	yes, hyperbolic curve
Mice	Nicloux (123)	analysis of total animal	yes, hyperbolic curve
Rat	Le Breton (91)	analysis of total animal	no
Guinea pig	Le Breton (91)	analysis of total animal	yes, hyperbolic curve
Rabbit	Newman and Lehman (118)	alcoholaemic curve	no
	Le Breton (89, 91)	analysis of total animal	yes, hyperbolic curve

Results of some experiments on the dependence of alcohol metabolic rate on alcohol concentration in the tissues. All these experiments seem to have been performed with equally reliable techniques.

of the first dose (122). *Newman* says that the high dose of alcohol has "conditioned" the organism to a higher rate of alcohol metabolism (111, 112). The phenomenon is difficult to explain in the term of our present knowledge of the mechanism of alcohol metabolism and of the nature of enzymatic processes. Some of the reliable experimental results of the dependence of alcohol metabolic rate on alcohol concentration are assembled in table 1. It is seen here that they differ widely from each other, not only from investigator to investigator but

in some cases investigators from the same group have obtained different results from time to time. These contradictions have naturally inspired some investigators to vary their experimental technique in order to check their findings. The most commonly used variation in the technique has been to infuse alcohol diluted with saline slowly at a constant rate for hours in an experimental animal or a person. If the infusion rate exceeds the metabolic rate, an increased concentration of alcohol in the blood will result and *vice versa*. Such experiments have been made with dogs by *Newman* and his group (111). Here it was found that a higher infusion rate was necessary to maintain a high alcohol concentration in the blood than to maintain a low level. *Eggleton* (46) obtained the same results in cats. The experiments were repeated by *Loomis* (100) in dogs, but he found that the same infusion rate (within ± 30%) was able to keep the blood alcohol level constant for several hours independently of the concentration of alcohol in the blood. All three series of experiments seem to be beyond criticism, and the only conclusion which can be drawn is that the conditions of alcohol metabolism can vary from time to time, even in the same species.

As to man, until recently experiments on several hundred persons had invariably shown no signs of a dependence of the alcohol metabolic rate on the concentration of alcohol, either in form of an asymptotic approach of the alcoholaemic curve to the zero line or a steeper slope in persons with an initial high alcohol concentration in the blood. From a practical point of view it seems well established that the human organism metabolizes alcohol at a constant rate independent of the concentration of alcohol. For low concentrations of alcohol this is confirmed by *Newman and Cutting* (115) who in experiments with man were able to maintain the blood alcohol concentration at a constant level with the same amount of alcohol slowly infused intravenously, whatever the initial blood alcohol concentration between 15 and 94 mg %. Yet even here some recent experiments show a dependence of alcohol metabolic rate on alcohol concentration. *Hjelt* (74) working for the Finnish government and *Goldberg* (54) working for the Swedish government both found a statistically significant increase of the β-factor with increased amounts of alcohol ingested. In *Goldberg's* material the β-factor was found increased about 40% when 1.5 grams alcohol per kg body weight was taken instead of 0.5 gram per kg. In both series of experiments the results were widely scattered, indicating that many experiments must be made in order to show the effect.

A quite different approach to the problem indicates that, at least in man and rabbits, the metabolic rate of alcohol is not completely independent of the concentration of alcohol. As mentioned later, tetraethylthiuramdisulphide (antabuse) is able to inhibit the second step in alcohol metabolism, the oxidation of acetaldehyde to acetate. Organisms pretreated with this substance will therefore accumulate acetaldehyde after the administration of alcohol and the concentration of acetaldehyde will depend on the rate at which acetaldehyde is formed, *i.e.*, the metabolic rate of the first step of alcohol oxidation. In rabbits (68) and in man (128) it is found that the concentration of acetaldehyde, both in untreated subjects and after antabuse and alcohol, is higher with higher

concentration of blood alcohol and *vice versa*. This suggests that the oxidation of alcohol to acetaldehyde proceeds at a higher rate with higher concentrations of alcohol. On the other hand, the metabolic rate of alcohol can be calculated from the concentration of acetaldehyde in the blood, and an elimination curve in the rabbit constructed from these calculations shows so little deviation from the straight line that it lies within the analytical error of the alcohol determinations (77). We have no information about the relation between acetaldehyde production and the blood acetaldehyde concentration in man, and it is at present impossible to make a similar calculation for the human organism; but, if the conditions are similar to those in rabbits, it will also here be impossible to distinguish between a rectilinear decline and the expected slightly curved decline in the blood alcohol.

Our present knowledge can probably be summarized in the following statement: The rate of oxidation of ethyl alcohol in the organism is somewhat increased with increasing concentrations of alcohol. Within the concentrations possible in the living organism this increase is so small that the elimination curve of alcohol generally follows a straight line, and for all practical forensic purposes no error is made if we assume a rectilinear elimination curve for alcohol. Under certain conditions which have not yet been defined the dependence of alcohol metabolism on alcohol concentration may be so pronounced that the blood alcohol curve deviates significantly from the straight line. An attempt to explain this is given in a later section.

Individual Variations: Widmark concluded from his experiments that the capacity to metabolize alcohol was subject to relatively little variation within the species, and to still less variation from time to time within the same individual. Taken as a whole, later investigations have confirmed his point of view, but in some experiments a slight, but statistically significant variation in β is observed in the same individual, even when measured under apparently the same conditions (135, 136). The observed variation is, however, not very high. In man it is not found to exceed 25% in any published experiment, which is so close to the exactitude with which β can be determined that many experiments are necessary in order to demonstrate this variation with statistical significance. In man all the experiments on the variation of the alcohol metabolic rate in the same individual have been made at relatively short intervals, and no experimental evidence is found in the literature as to the order of magnitude of the variations occurring during the whole life cycle. In rats, it is shown that young animals have a much higher metabolic capacity for alcohol per unit of weight than older rats, while the capacity in chickens is the same at all ages (*cit.* after 35). The problem of the variation within the single individual may have some forensic importance, but theoretically it must be considered a minor one.

The question of whether the continued use of alcohol can increase the capacity of the organism to metabolize alcohol has been the aim of extensive investigations and discussions in earlier papers. Comprehensive reviews are given by *Bernhard and Goldberg* (12) and *Carpenter* (21). The general opinion now is

that the metabolic rate of alcohol is not increased, even after long and continued use of alcohol, either in rabbits (80), dogs (114), rats (4), cats (46) or man (12, 135).

Effect of Body Temperature: Like all other chemical processes, the metabolic rate of alcohol depends on temperature. In experiments with frogs *Nicloux* (123) found that the rate was doubled for each 10° rise in temperature. *Dybing* (45) showed that β in rats with a body temperature of 20–25° was about a fourth of that found in rats with a body temperature of 38–39°. In rabbits *Fuhrman* (52) found an increase of 56% with an increase of body temperature from 25° to 37°. The variation of body temperature must, however, be rather high if any influence on the metabolic rate is to be demonstrated, and it has not been observed by all investigators. Thus *Le Breton* (94) could not demonstrate any effect of temperature in rats and *Ewing* (47) failed to show any difference in dogs after the temperature had been raised about 4° C. by means of short-wave radiothermy. In man *Fleming and Reynolds* (51) observed a slight, though not quite convincing, increase after diathermy and in a couple of experiments *Danopoulos* (34) was able to demonstrate a higher rate of disappearance of alcohol from blood in patients during attacks of malaria than in the fever-free periods, but the difference was not very high. The effect of fever induced by drugs, such as α-dinitrophenol, will be discussed later.

Influence of Metabolic Processes: From species to species there seems to be a rough relation between basal metabolism and capacity to metabolize alcohol (95); small animals with high metabolism per kg body weight have a higher metabolic rate of alcohol per kg body weight than bigger animals, but so far only mice, rats, guinea pigs and rabbits have been examined. Within the single individual, it is well established that the metabolic rate of alcohol does not follow the variations of the general metabolism. Even heavy muscular work sufficient to increase metabolism maximally is not able to increase the rate of disappearance of alcohol in the blood more than can be explained by the increased evaporation through perspiration and respiration (24, 91, 124). Less clear is the effect of increased metabolism caused by hyperfunction of the *thyroid gland* or by intake of *thyroid hormone*. Some have found an increase, some a decrease, and still others no effect (39, 70, 80, 152). *Starvation* decreases the metabolic rate of alcohol to $\frac{3}{4}$–$\frac{2}{3}$, and tissues from starving animals oxidize alcohol more slowly than normal tissues *in vitro* (97). The metabolism of fat seems to have no influence (40). The same is the case with protein metabolism. The possible influence of alanine metabolism (90) seems to be connected with the deamination of alanine to pyruvate, as will be mentioned later.

The *influence of carbohydrate* metabolism has been much discussed. *Dontcheff* (40) and *Berg, Stotz and Westerfeld* (10) showed that animals kept on a high carbohydrate diet, and consequently with a high metabolism of carbohydrate, had a higher metabolic rate than animals kept on a diet low in carbohydrates. *Dontcheff* was even able by means of measurements of the RQ to show that the metabolic rate of alcohol closely followed the amount of carbohydrate oxidized in rats (41). In mice, the alcohol metabolism is retarded after starving,

but rapidly brought back to normal range for this species after a single dose of glucose. In organisms with sufficient carbohydrate reserves, the additional administration of carbohydrate is without influence on the alcohol metabolism and the rate of alcohol metabolism is found to be independent of the level of the blood sugar (27, 75, 85, 100). Only *Carpenter and Lee* (22, 23) found an increase after glucose, but their technique of determining the rate of alcohol metabolism-disappearance of alcohol from the expired air is probably less reliable than the direct measurement of blood alcohol.

A long series of investigators have *examined the role of insulin in alcohol metabolism.* Depancreatized dogs and cats show a rate of alcohol metabolism only $\frac{1}{3}$–$\frac{1}{4}$ of that of normal animals. The effect of pancreatectomy on the alcohol metabolic rate starts about 24–48 hours after the operation and is first complete 72 hours after. *In vitro,* livers from pancreatectomized cats showed only a slight capacity to oxidize alcohol, about $\frac{1}{6}$ of that of livers from similar animals treated with insulin (30). Diabetic patients seem, however, to have the same capacity to metabolize alcohol as normal persons. The effect of pancreatectomy has not been observed by all investigators, (106) and is in any case not so intense as after hepatectomy (30).

The effect of the administration of insulin to normal organisms on alcohol metabolism has been the subject of numerous investigations. Some of the results are difficult to evaluate: in many cases it is impossible to calculate the β-factor from the experimental data, and where the authors draw their conclusions from a lower peak of the blood alcohol curve after insulin and oral administration of alcohol, the effect might have been due to a delayed absorption of alcohol, and not to an effect on alcohol metabolism in itself. But even the results of experiments made with a reliable technique differ from author to author. One group find a definite acceleration of alcohol metabolism in dogs after insulin. This was first shown by *Supniewsky* (139) in 1926 and later confirmed by *Widmark* (151), *Clark and coworkers* (28–30), *Greenberg* (58) and *Newman and Cutting* (117). About 1 unit of insulin per kg body weight is necessary to obtain an effect. In rats no effect could be seen with doses of insulin up to 15 units per kg; in rats kept on high carbohydrate diet, 40 and 500 units insulin per kg body weight caused a marked increase of the alcohol metabolic rate, especially when the insulin was given during several days (42). Some investigators claim to have observed an effect in man after single doses of 20, 40 or more units of insulin (137). The maximal increase of β after insulin in experiments with man or dogs has, however, never exceeded 50%.

On the other hand, not a few investigators have found no effect of insulin on alcohol metabolism, even with high doses of insulin and the addition of extra glucose (59, 70, 100). There can thus be little doubt that the effect of insulin in this respect does not occur under all circumstances. Perhaps *Widmark's* (152) observation that he only saw the effect of insulin in dogs with a low alcohol metabolic rate and not in animals with a high metabolic rate, gives a hint of a possible explanation.

The effect of alcohol on carbohydrate metabolism is very little known. On

the whole alcohol seems to have no effect on the normal or increased blood sugar. It has been claimed that the continuous intake of alcohol impairs glycogen formation in the liver, and the insulin-glucose treatment of chronic alcoholics is based on this conception. But the conditions in chronic clinical alcoholism involve a long series of other factors which have nothing directly to do with the metabolism of alcohol in these patients. All experiments on the connection between alcohol and carbohydrate metabolism have been rather primitive, as none of the experiments has given information of the fate of the carbohydrate administered or present in the organism, or about the oxidation, mobilization and formation of glycogen in the liver or the muscles, and it is difficult to suggest any explanation of the effect until further details about the carbohydrate metabolism in such experiments are available.

The experimental evidence puts it beyond doubt that there is some connection between carbohydrate and alcohol metabolism. As it is very unlikely that alcohol is metabolized by the same systems of enzymes and regulated by the same systems of hormones as carbohydrate metabolism, the hypothesis has been advanced that certain metabolites of carbohydrate are able to react with alcohol in the tissues, and here interest has been concentrated on pyruvic acid, which seems to have an effect like insulin on the alcohol metabolism. *Leloir and Muñoz* (97) observed an acceleration of alcohol oxidation by pyruvic acid and by oxalacetate in *in-vitro* experiments with liver slices. In experiments with dogs *in vivo*, *Westerfeld, Stotz and Berg* (143) found an average increase of the alcohol metabolic rate of 2–3 times after about 0.5 g to 1 g sodium pyruvate per kg body weight, and this finding is confirmed by other authors (58). An increased formation of acetaldehyde is also found after administration of pyruvate and alcohol (144). The effect of pyruvate does not seem to be found under all circumstances: *Hulpieu et al.* found no accelerating effect in dogs (75), neither could *Gregory and his coworkers* (60). The discrepancy between the results obtained in the same experimental animals with apparently reliable experimental methods is possibly due to the fact that *Westerfeld et al.* worked with animals with a much smaller initial metabolism of alcohol than *Hulpieu and coworkers.* There may be an analogy with the above mentioned observations of *Widmark*, who only found an accelerating effect after insulin in animals with a relatively slow initial metabolism of alcohol. *Bartlett* and *Barnet* found that pyruvate not only did not stimulate alcohol metabolism, but even inhibited it, both *in vivo* and *in vitro* (8). Their experimental technique differed, however, considerably from that of the other workers: Alcohol with C^{14} was given to rats, and the rate of alcohol metabolism was measured from the amount of radioactive CO_2 excreted per hour. Pyruvate delayed alcohol metabolism measured in this way considerably. A possible explanation of this will be discussed in a later section.

The accelerating effect of *dl-alanine* was first suggested by *Widmark* (148, 150), confirmed by *Le Breton* in rabbits (90), by *Eggleton* in cats (46), and most recently also found by *Westerfeld et al.* in dogs (143). The effect is of the same order of magnitude as the effect of pyruvate. *Westerfeld and his coworkers* showed

an increase of pyruvate and lactate in the blood after the injection of *dl*-alanine, and as the maximal effect on alcohol metabolism in some experiments was found delayed by one hour or more, in comparison with the effect of corresponding amounts of pyruvate, they concluded that the effect of alanine is merely due to pyruvate formed in the organism from alanine by deamination (143).

Vitamin Deficiencies: In spite of the important role vitamins are known to play in general metabolism, very few investigations have been made on alcohol metabolism during vitamin deficiencies. This is the more astonishing as it is known that at least two members of the vitamin B group are found in coenzymes necessary for the metabolism of alcohol and its metabolites in the tissues: nicotinic acid which is part of the diphosphopyridine nucleotide molecule, and riboflavin which is part of the flavoprotein.

In experiments with dogs and pigeons deficient in thiamine no effect on the metabolic rate of alcohol was seen (10). This conforms well to the fact that no enzyme containing thiamine is found to play a part in any known system in alcohol oxidation; the thiamine deficiencies observed in chronic alcoholism must probably be attributed to lack of thiamine from improper food intake.

Guinea pigs fed on a scorbutogenic diet showed an alcohol metabolism about half the rate than seen in normal control animals (79). The role of vitamin C in alcohol metabolism is unknown.

Effect of Drugs on Alcohol Metabolism: Of the many drugs so far examined only dinitrophenol and dinitrocresol have been found to *accelerate* alcohol metabolism. In dogs an increase of 100–200% was found in the first experiments with α-dinitrophenol or dinitro-o-cresol (70, 15). Such large effects were not found by other investigators who claimed that the increased alcohol elimination after dinitrophenol seen in their experiments could be explained by increased alcohol excretion due to increased respiration and perspiration caused by the increased general metabolism after ingestion of α-dinitrophenol (120). *Ewing* (47) found a definite but more moderate increase of alcohol metabolism in dogs after α-dinitrophenol, even when the amount of alcohol excreted in the urine, the expired air and the sweat was taken into consideration. However, very high and toxic doses of α-dinitrophenol are necessary to demonstrate the effect. The use of α-dinitrophenol to accelerate alcohol metabolism in intoxicated persons would therefore be too dangerous for practical use in patients. The addition of dinitrophenol to the blood of artificially perfused isolated livers has no effect on the rate of alcohol disappearance from the circulating blood (48); but, *in vitro*, dinitrophenol in a concentration of 1:5,000,000 has been found to increase alcohol oxidation in liver slices by 5–10%; higher concentrations gave a definite inhibition (121).

Other drugs seem to inhibit the alcohol metabolism. In dogs, *Clark and coworkers* found that phloridzine inhibited alcohol metabolism by about 25%. They interpret this effect as due to fatty infiltration of the livers and starvation of the animals (30).

Cyanamide and antabuse, two drugs known to have a strong inhibitory effect on the second step of alcohol oxidation (acetaldehyde to acetic acid) have also a

definite effect on the first step, although in doses much higher than those necessary to influence the acetaldehyde oxidation. *Gärtner* (53) found an almost complete inhibition of alcohol metabolism after the administration of cyanamide to rabbits. Also in rabbits, higher doses of antabuse (tetraethylthiuramdisulphide) result in a decrease of β to almost zero (66). A similar trend was seen in dogs (119). Smaller doses of antabuse, fully capable of inhibiting the acetaldehyde oxidation, have no effect on the rate of alcohol metabolism, either in man (65), rabbits (66, 89) or dogs (100, 113, 119).

From a practical point of view it is important whether or not it is possible to accelerate the alcohol metabolism in a person under the influence of alcohol. It will be understood that although some procedures seem to have an effect in some cases, their effect has been disputed and seems only to appear under certain conditions the nature of which has not yet been cleared up. Moreover, very few reliable experiments have been made on man. As mentioned the use of dinitrophenol is too dangerous. With insulin it is of no use to give a dose of less than 0.5 unit per kg body weight; with, for example, 40 units of insulin + glucose the effect presumably does not exceed 50%, *i.e.*, the elimination time of a certain concentration of alcohol is shortened by one third, but many patients will most likely not show any measurable effect even after this high dose.

THE SITES OF ALCOHOL METABOLISM IN THE ORGANISM

The *liver* is the most important organ capable of oxidizing alcohol. Liver slices and liver brei can oxidize alcohol *in vitro* (8, 9, 30, 97, 103), and liver forms the raw material for the isolation of alcohol-oxidizing enzymes (18, 19). More important is the fact that surviving livers, artificially perfused with blood containing alcohol, remove the alcohol from the blood at a rate corresponding to $\frac{1}{2}$ to $\frac{5}{6}$ of the rate expected in the whole organism. Such experiments have been made in dogs (48), cats (102) and rabbits (68). The earliest of these published experiments were made by *Fiessinger et al.* in 1936 (48), but the most comprehensive are the experiments of *Lundsgaard* (102) who followed not only the disappearance of alcohol but also the oxygen consumption and carbon dioxide production. He showed that the alcohol is only partially oxidized in the liver under these circumstances, presumably to acetic acid.

Some experiments seem to indicate that *muscles* can oxidize alcohol to a certain degree. *Fleischman* (50) found a lowering of the respiratory quotient when frog muscles were made to contract in a Ringer's solution containing alcohol. He also found a formation of aldehyde or ketone in muscle brei suspended in a Ringer's solution during 24 hours. He claimed that this was due to acetaldehyde. *Lehman* showed that horse muscle and alcohol are able to decolorize methylene blue in *Thunberg* experiments (96). Earlier *Hammill* (69) and *Fischer* (49) found the isolated heart capable of utilizing alcohol. Their experiments were extensive, but as they were made in 1910 and 1917, respectively, they ought to be repeated with more modern techniques. Nevertheless they fit well with the recent observation that rat diaphragm and rat heart muscle *in vitro* from $C^{14}O_2$ from $C_2^{14}H_5OH$ (8). It has already been mentioned that muscular work has no influence on the rate of alcohol metabolism in mammals.

Some investigators have detected the oxidation of alcohol by *kidney* tissue *in vitro*. In some experiments the effect was small (97), but in single paper reports an effect even exceeding that of liver tissue (8); but the role of the kidneys in alcohol metabolism in the intact organism cannot be very important.

It has been discussed whether or not *brain tissue* is capable of oxidizing and utilizing alcohol. *Himwich et al.* (72) have found that the respiratory quotient of cortex tissue of rats was 0.8–0.9 after intraperitoneal injections of alcohol as against 1.0 in untreated animals. They interpret this finding as evidence for the ability of cerebral cells to oxidize alcohol, as the respiratory quotient for alcohol (0.67) is much lower than the respiratory quotient for carbohydrate (1.00) which is the normal substrate for oxidation in the brain. No perfusion experiments have been made and the results of the *in-vitro* experiments vary considerably. *Dewan* (37) found ox brain *in vitro* capable of forming acetaldehyde and acetic acid from added alcohol and chemically identified both metabolites. The oxidation requires a nicotinic acid-containing catalyst, diphosphopyridine nucleotide (see later). He also found brains from dog, cat, pig, cow, guinea pig and rabbit (all the species examined) able to oxidize alcohol *in vitro* (36). In experiments with radioactive alcohol (C^{14}) *Bartlett and Barnet* found no formation of $C^{14}O_2$ by brain tissue from rats (8). As to the significance of the possible capacity of the brain tissue to metabolize alcohol, all investigators agree that it plays a very small role in the alcohol metabolism of the total organism, and that its significance as an energy spender in brain metabolism is of no practical importance. It has been suggested that the alcohol oxidases present in the brain may have a function as a possible "detoxicator" of alcohol, and thereby as a natural protector of the brain tissue against alcohol. However, until now there is no experimental evidence for this hypothesis.

As to the other tissues *Leloir and Muñoz* have examined *testes*, *spleen* and the *intestinal tract in vitro* and did not find any oxidizing effect of these tissues (99). For the sake of completeness it must be mentioned that *Lehman* (96) found that tissue from a sarcoma catalyzed the reaction of alcohol with methylene blue in *Thunberg* experiments.

Up to the end of the 1930s there was much discussion about which organ should be regarded as the *principal* one in alcohol metabolism (see the reviews by *Kochmann* (87), *Rosemann* (133) and *M. Schmidt* (135)). Now there can be little doubt that by far the largest part of alcohol metabolism takes place in the liver, and that the tissues outside the liver play only an insignificant role. As already mentioned, artificially perfused livers have shown a capacity to metabolize alcohol corresponding to a great part of what can be expected of the whole organism. More important is the fact that eviscerated animals metabolize alcohol very slowly (28, 29). In hepatectomized dogs *Loomis* infused alcohol at the rate of 3 mg per min. per kg. This resulted in progressively increasing blood alcohol levels, although the same amount infused in control dogs gave no increase in blood alcohol levels (100). The increase of blood alcohol in *Loomis'* experiments after 2 hours infusion was of an order of magnitude which indicates that very little alcohol, if any at all, had been metabolized in the hepatectomized dogs. In partly hepatectomized animals the metabolic rate of alcohol decreased

as more liver tissue was removed, to almost nil when the animals were totally hepatectomized (107, 108). These and similar results conform well with the fact that liver damage caused by arsenic, chloroform, phosphorus (125) and antimony (126) results in a lowered capacity of the organism to oxidize alcohol (4, 13, 26, 108). It is also shown that the alcohol metabolism is decreased in patients with liver cirrhosis (13). In rats *Lolli and Rubin* found that the alcohol metabolism had already fallen to $\frac{3}{5}$ of the normal value 24 hours after ligation of the bile duct (99). The variation from individual to individual is, however, too high and the effect too uncertain to allow the metabolic rate of alcohol to be used as a clinical liver function test (13).

THE INTERMEDIARY PRODUCTS IN ALCOHOL METABOLISM

There is little doubt that the oxidation of alcohol takes place in steps, and the following steps are by far the most likely:

1) $\underset{\text{ethyl alcohol}}{\overset{\displaystyle CH_3}{\underset{\displaystyle CH_2OH}{|}}} - 2H \rightarrow \underset{\text{acetaldehyde}}{\overset{\displaystyle CH_3}{\underset{\displaystyle CHO}{|}}}$

2) $\underset{\text{acetaldehyde}}{\overset{\displaystyle CH_3}{\underset{\displaystyle CHO}{|}}} + H_2O - 2H \rightarrow \underset{\text{acetic acid}}{\overset{\displaystyle CH_3}{\underset{\displaystyle COOH}{|}}}$

Acetaldehyde: There is much essential evidence that *acetyldehyde* is an intermediate in alcohol oxidation. In *in-vitro* experiments with tissue slices or with tissue brei, acetaldehyde can be isolated as a reaction product (103). It will later be shown that with all purified enzyme systems acetaldehyde appears as the primary oxidation product of alcohol. *In vivo*, acetaldehyde is found in the blood during alcohol metabolism, and when alcohol oxidation is accelerated by means of insulin or pyruvic acid, the concentration of acetaldehyde is raised (139, 144). Acetaldehyde does not seem to be an intermediate product of normal metabolism (76). Thus the presence of acetaldehyde is specifically linked with alcohol metabolism, and a description of acetaldehyde oxidation clearly falls within the scope of this review.

The liver is the principal site for the oxidation of acetaldehyde. Evisceration of cats delays the elimination of intravenously infused acetaldehyde 4–5 times (101), and in experiments with artificially perfused rabbit livers *Hald, Jacobsen and Larsen* (68) have found such livers able to metabolize about $\frac{3}{4}$–$\frac{4}{5}$ of the amount which would be expected to be metabolized by intact animals. Other tissues, especially muscles, are not completely unable to oxidize acetaldehyde but their action is much less than that of the liver, both per gram tissue and in the whole organism. In contrast to what is generally found with alcohol, the metabolic rate of acetaldehyde depends on the concentration of acetaldehyde in the tissues. In perfused rabbit livers an acetaldehyde concentration of 7–8 mg per 100 gram is necessary to get the maximal metabolic rate of this substance, while a concentration of 1–2 mg per 100 gram only results in a metabolic rate of about half of the maximum (68).

Acetyldehyde is very readily oxidized in the organism. *Lubin and Westerfeld* (101) found that 100 mg/kg was eliminated during 10–15 min. by a cat. *Hald*

and Larsen (64) found that rabbits could eliminate at least 3 mg per kg per min. and sometimes more for hours; in experiments with man, a person weighing about 70 kg was easily able to eliminate about 8 mg of acetaldehyde per minute, but the maximum rate is most probably considerably higher (6). From these figures it is seen that the oxidation rate of acetaldehyde to acetic acid is much faster than the oxidation rate of alcohol to acetic acid, and therefore the limiting factor is the first step, the oxidation of alcohol to acetaldehyde. Even a considerable inhibition of acetaldehyde oxidation has no influence on the rate of alcohol disappearance.

Inhibition of the oxidation of acetaldehyde: Certain drugs are able to inhibit the oxidation of acetaldehyde. Of those the effect of tetraethylthiuramdisulphide (antabuse) has been most studied. When rabbits premedicated with about 1 gram of antabuse per kg body weight are infused continuously with acetaldehyde, the concentration of acetaldehyde in the blood is considerably higher than in control rabbits infused with acetaldehyde at the same rate (64). Similar results have also been obtained by *Newman* (113) in experiments with dogs. The increase depends on the concentration of acetaldehyde and is the less pronounced the higher the infusion rate. At small infusion rates (*e.g.*, 1 mg per min.), the increase is several 100%, and near the maximum capacity of the organism to metabolize acetaldehyde the effect of antabuse is nil (64). The same can be seen in experiments with artificially perfused livers and to a less pronounced degree with artificially perfused hindlimbs (68). Antabuse interferes very little with the maximum capacity of an organism to metabolize acetaldehyde, but it increases the tissue concentration of acetaldehyde necessary to metabolize a certain amount of acetaldehyde per unit of time. For example, it may be mentioned that while livers from normal untreated rabbits require about 0.2–0.5 mg acetaldehyde per 100 gram in order to metabolize about 3 mg acetaldehyde per minute per 100 gram of liver, livers of rabbits treated with antabuse require 2–3 mg per 100 gram in order to metabolize the same amount. With concentrations of 15–20 mg per 100 gram, livers from both treated and untreated animals were able to metabolize the same maximum amount of acetaldehyde, 9–10 mg per minute per 100 gram of liver tissue (68). This fits in very well with the findings of the enzyme chemists.

Formation of acetaldehyde during alcohol metabolism: Acetaldehyde has repeatedly been found in the blood during alcohol metabolism in dogs (113), cats, rabbits (89) and man (63). As already mentioned the concentration of acetaldehyde obtained depends somewhat on the concentration of alcohol in the blood, and the theoretical consequences of this phenomenon have already been discussed. The concentration rarely exceeds about 1 mg per 100 cc with 200–300 mg of alcohol per 100 cc, and with small concentrations of alcohol only negligeable amounts of acetaldehyde are found in the blood in normal individuals. Alcohol given to animals *premedicated with antabuse* results in a much higher (2–10 times) concentration of acetaldehyde in the blood than that found with the same concentration of alcohol in normal non-treated animals (63, 66, 73, 77, 98, 104, 119). The highest concentration seen in any experiment is about

3 mg acetaldehyde per 100 cc blood. It is natural to relate this phenomenon with the effect of antabuse on acetaldehyde metabolism, and this assumption is confirmed by the close agreement between the expected concentration of acetaldehyde calculated from the rate of alcohol disappearance and the concentrations actually found (77).

Not only antabuse, but a number of other substances cause a similar increased concentration of acetaldehyde after the administration of alcohol. In experiments with rabbits this has been found to be the case with cyanamide (67), a substance which has long been known to provoke disagreeable symptoms in combination with alcohol, similar to those observed after antabuse. In hitherto unpublished experiments *Hald and Larsen* have found that tetraethylthiuram-monosulphide and diethyldithiocarbamic acid also cause the accumulation of acetaldehyde. Among the analogues to tetraethylthiuramdisulphide, thiuramdisulphide has no effect, and tetramethylthiuramdisulphide has an effect somewhat higher than tetraethylthiuramdisulphide but its general toxicity is much higher. The effect declines rapidly as we pass through tetrapropylthiuramdisulphide to tetrabutylthiuramdisulphide, the effect of which is nil.

A series of other substances elicit the same clinical symptoms as cyanamide and antabuse after alcohol intake. The most frequently mentioned are some hitherto unknown substances in the fungus *Coprinus atramentarius*, *carbontetrachloride* and *charcoal* (109), but so far no examination has been made of the influence of these substances on acetaldehyde metabolism.

Acetic Acid: Until recently acetic acid was merely a hypothetical step in alcohol metabolism, mainly because the determination of acetic acid in tissues is rather difficult. In an ingenious way *Bernhard* has brought experimental evidence for this hypothesis since $\frac{1}{6}$—$\frac{1}{3}$ of the deuterium given with the alcohol CD_3CD_2OD was recovered as the acetate of paraacetylbenzene sulphonamide excreted in the urine and derived from simultaneously administered sulphanilamide (11). The acetate formed during the oxidation of alcohol in the liver is not exclusively oxidized in this organ, but is carried with the blood to other tissues (*e.g.*, to the muscles) and is utilized there. In *Lundsgaard's* (102) perfusion experiments with isolated livers the amount of oxygen used was too small for the total oxidation of the alcohol which disappeared. He therefore assumed that the oxidation of some of the alcohol did not proceed further than to the acetic acid stage, and his assumption was confirmed by the fact that a considerable amount of acid was found in the perfusion blood during the experiment. Under normal circumstances the acetate must be rapidly oxidized further to carbon dioxide and water. *Bartlett and Barnet* (8) showed that more than 90% of the radioactive carbon can be recovered as $C^{14}O_2$ from the expired air within 10 hours after the administration of $C_2^{14}H_5OH$. When other metabolites were given together with alcohol (*e.g.*, pyruvate), the excretion of $C^{14}O_2$ was substantially delayed. This is not in contradiction to the findings that the first step of alcohol oxidation can be accelerated with pyruvate, but is rather to be explained as a delayed oxidation of acetate formed from alcohol. Acetate is also formed as an intermediate step in normal metabolism, not only in the process of

oxidation but also in the formation of fats and perhaps also glycogen, and when the general metabolic processes are increased, for instance after the administration of pyruvate, the acetate formed from alcohol will be pooled with the acetate formed from other metabolites and the oxidation thus delayed. The nutritional value of alcohol is easily explained by the fact that the second product of oxidation, acetic acid, is a compound also found during the metabolism of carbohydrate, fats and protein. The acetic acid from alcohol joins the "acetylic pool" of normal metabolism, and from this step on the metabolism of alcohol offers no special problem. The description of acetate metabolism is not within the scope of this review, and the interested reader is referred to special articles on this subject (16).

POSSIBLE BIOCHEMICAL PROCESSES OF ALCOHOL METABOLISM IN THE ANIMAL ORGANISM

In 1910 *Battelli and Stern* (9) showed that extracts from animal tissues are able to oxidize alcohol *in vitro*. In the 25 years following this basic observation practically no attempt was made to study the biochemical processes of alcohol metabolism in animal tissues, but much evidence has been gathered since the middle 1930s showing a number of possible mechanisms for the oxidation of alcohol to acetaldehyde and of acetaldehyde to acetic acid.

Alcohol → Acetaldehyde: Two widely different processes are possible as the first step of alcohol oxidation: dehydrogenation and a reaction with hydrogen peroxide. The first process is catalyzed by *alcohol dehydrogenase*, the latter is catalyzed by *catalase*.

Alcohol dehydrogenase: This enzyme catalyzes the transference of hydrogen from alcohol to the nicotinic acid-containing compound diphosphopyridine nucleotide, abbreviated DPN (also called cozymase in earlier nomenclature), according to the formula:

$$\text{Alcohol} + \text{DPN} \rightleftharpoons \text{Acetaldehyde} + \text{DPNH} + \text{H}^+$$

(Alcohol dehydrogenase)

The alcohol dehydrogenase from yeast was the first to be thoroughly examined It was crystallized by *Negelein and Wulff* (110) who also described it more closely. Animal alcohol dehydrogenase was studied by *Lehman* who first showed the dependence on cozymase (96). In 1938 *Lutwak-Man* purified alcohol dehydrogenase from liver and studied its properties more closely (103). About 10 years later *Bonnichsen and Wassén* crystallized the dehydrogenase from horse liver (18, 19) and this crystalline enzyme was comprehensively examined by *Theorell and Bonnichsen* (141). It differs from the yeast alcohol dehydrogenase in several respects, the most important of which are that the molecular weight of the yeast enzyme is about twice that of the liver enzyme (141) and that the yeast enzyme, unlike the liver enzyme, is inhibited by monoiodoacetate (103). Its most interesting chemical property is that it can be bound to diphosphopyridine nucleotide and to reduced diphosphopyridine nucleotide (141). The combination probably occurs between the pyridine group of the coenzyme and

some sulfhydryl group in the dehydrogenase molecule. As indicated by the equation, the processes are reversible. The constants found in the experiments shows that at pH 7 in a state of equilibrium the rates alcohol: acetaldehyde will be about 60. The process nevertheless usually proceeds from left to right in the tissues in spite of the rather "unfavorable" equilibrium point, and this is naturally due to the fact that acetaldehyde is very rapidly oxidized further and thus removed from the equilibrium. The equilibrium figure is greatly increased (to 10,000) when the concentration of dehydrogenase in the system is low. The coupling of the coenzyme to the enzyme greatly favours the oxidation of ethanol to acetaldehyde, and the reaction is rather improbable without this effect.

Two of the results obtained by *Theorell and Bonnichsen* (141) are of extreme practical interest. The *turnover number* (the number of molecules of alcohol oxidized by one molecule of enzyme per minute) is found to 140 at 20°. It is probably somewhat higher at 37°, but on the other hand this figure is determined under optimal conditions which probably are not found in the organism. Using the turnover number 150, 73 grams (1 millimol, as the molecular weight of the dehydrogenase is 73,000) enzyme is able to oxidize 150 × 46 mg alcohol = 6.9 grams alcohol per minute, or 414 grams per hour. In order to oxidize 10 grams alcohol per hour, 1.7 grams alcohol dehydrogenase must be present in the organism. This is not an astonishingly high figure. *Bonnichsen* (18) obtained a yield of 1 gram of crystalline dehydrogenase per kg of horse liver, and thus it does not seem incredible that a human liver weighing about 1.5 kg should contain 1.5 grams of alcohol dehydrogenase, enough to oxidize the amount of alcohol actually oxidized by the human organism per hour. Another interesting point is that the *Michaëlis constant* (an expression of the affinity between enzyme and substrate) is so low that the rectilinear course of the physiological alcohol elimination (down to tissue concentrations of alcohol too low to be determined by most methods available) found in human experiments is to be expected from the kinetic data of the *in-vitro* experiments with the pure enzyme.

The alcohol dehydrogenase is not specific for ethyl alcohol, since the oxidation of propyl alcohol, amyl alcohol and butyl alcohol is also catalyzed (103, 140). Only alcohols containing the group $-\overset{|}{\underset{|}{C}}-CH_2OH$ react; secondary and tertiary alcohols do not. Very remarkable is the fact that alcohol dehydrogenase also is able to catalyze the process

$$\text{vitamin A} \rightleftharpoons \text{retinene},$$

retinene being the aldehyde corresponding to vitamin A. It has been suggested that alcohol dehydrogenase may be present in the retina and that it exerts an important physiological function in the formation of visual purple. Especially it must be emphasized that the crystalline alcohol dehydrogenase does not react with methyl alcohol (20, 141), in contrast to more impure preparations (103); the theoretical consequences of this fact will be discussed later. The reduced

diphosphopyridine nucleotide transfers the hydrogen to other systems so that the rebuilt oxidized form is ready to accept hydrogen from other alcohol molecules. One of these hydrogen acceptors is the riboflavin-containing respiratory enzyme diaphorase (38) which in turn reacts with the cytochrome system and through cytochrome oxidase with free oxygen.

Leloir and Muñoz were first to demonstrate another possible acceptor for the hydrogen liberated from alcohol during its oxidation to acetaldehyde. This acceptor is pyruvic acid. The formula of the process is the following:

$$CH_3CH_2OH + CH_3CO\cdot COOH \rightarrow CH_3CHO + CH_3\cdot CHOH\cdot COOH.$$

In vitro, the addition of pyruvate to liver slices suspended in a solution containing alcohol results in an increase of the rate of disappearance of alcohol to two to three times that in controls not containing pyruvic acid; under these circumstances lactic acid is formed (97). The experiments of *Westerfeld et al.* (143) showing the accelerating effect of pyruvic acid on alcohol metabolism in the intact organism have already been mentioned; here also an increased concentration of lactic acid in the blood of the experimental animals was found. The process has not yet been studied in detail. Probably diphosphopyridine nucleotide is essential, and naturally some enzyme or enzymes. It would be interesting to see if oxidation would proceed in a system containing pure lacticodehydrogenase, pure alcohol dehydrogenase, diphosphopyridine nucleotide, alcohol and pyruvic acid.

Catalase: A second possible mechanism for the oxidation of alcohol to acetaldehyde is given by *Keilin and Hartree* (82, 83). When ethyl alcohol and catalase are added to an oxidation system which reacts directly with molecular oxygen with the formation of H_2O_2, alcohol is oxidized to acetaldehyde with the simultaneous reduction of H_2O_2 to H_2O. The general scheme of this process is, for example, as follows: Uric acid + (uricase) + O_2 → alloxantin + H_2O_2 H_2O_2 + (catalase) + CH_3CH_2OH → 2 H_2O + CH_3CHO. Hydrogen peroxide and alcohol are bound to the catalase molecule and presumably react in this combination. The kinetics have been studied by *Chance* (25) who finds the following processes the most likely:

$$\underbrace{\text{Catalase} + H_2O_2}_{\text{(very rapid combination)}} \longrightarrow \underbrace{\text{Catalase } (H_2O_2) + C_2H_5OH}_{\text{(rapid second order reaction)}} \longrightarrow$$

$$\underbrace{\text{Catalase } (H_2O_2)\,(C_2H_5OH)}_{\text{(relatively unstable compound)}} \longrightarrow \text{Catalase} + 2H_2O + CH_3CHO$$

Not only ethanol, but also other alcohols react in a similar way; ethyl and methyl alcohol have the same rates of combination with catalase-H_2O_2, but the relative rates of combination of *n*-propanol and *n*-butanol are only 1/60 and 1/500, respectively. Liver and kidney tissues are rich in catalase and in enzymes which, *in vitro*, are able to react directly with O_2, reducing it to H_2O_2 (*e.g.*, uricase, *d*-aminoacid oxidase and tyraminase). In general, however, it must be assumed that the substrates for these enzymes are not present in the organism

in concentrations high enough to explain the oxidation of 5–10 grams of alcohol per hour. The only possible exception is acetaldehyde. As will be mentioned below, acetaldehyde is oxidized by xanthine oxidase to acetic acid with the formation of H_2O_2 which in its turn oxidizes alcohol as described above. The newly formed acetaldehyde increases the supply of substrate for the primary oxidation reaction. In this way a "cyclic oxidation" of ethanol will take place. Actually this reaction occurs very readily *in vitro* in a system consisting of acetaldehyde and ethanol with highly purified xanthine oxidase and catalase. The process proceeds as follows (83):

$$\begin{cases} \rightarrow CH_3CHO + H_2O + O_2 \rightarrow CH_3COOH + H_2O_2 & \text{(xanthine oxidase)} \\ \quad\downarrow \\ H_2O_2 + CH_3CH_2OH \rightarrow CH_3CHO + 2H_2O & \text{(catalase)} \end{cases}$$

Acetaldehyde → Acetic Acid: A rather long series of possibilities have been mentioned in connection with the oxidation of acetaldehyde. The enzymes involved fall in two groups: the *flavoproteins* and the *dehydrogenases*. None of the known enzymes is specific for acetaldehyde; both groups catalyze the oxidation of other aldehydes, and the flavoproteins also catalyze the oxidation of cyclic compounds chemically little akin to aldehydes.

Flavoproteins: In these enzymes riboflavin is the active group that accepts hydrogen from the substrate and transfers it to free oxygen with the formation of hydrogen peroxide. The earliest known member of this group is *xanthine oxidase*, discovered in milk by *Schardinger* in 1902 and hence called the Schardinger enzyme. This enzyme catalyzes the oxidation of hypoxanthine and xanthine to uric acid, but aldehydes are also oxidized by the same enzyme. A flavoprotein with qualitatively the same properties has been isolated from pig liver, although not in a perfectly pure state (31, 55). The liver xanthine oxidase differs from the milk xanthine oxidase by having less action on aldehydes in comparison with its action on hypoxanthine and by its sensitivity to certain inhibitors. Antabuse inhibits liver xanthine oxidase but not milk xanthine oxidase (131), and liver xanthine oxidase is completely and irreversibly inactivated by 24-hours dialysis against distilled water (55).

Richert and Westerfeld have examined the capacity of tissues from several species to oxidize xanthine, and have found a high capacity in rat and mouse livers, a fairly high capacity in livers from cats, guinea pigs, cows and pigs, and little or no capacity in human livers or livers from rabbits and dogs (130). However, this does not mean that the tissues in question contain no flavoproteins able to oxidize acetaldehyde. Using the same procedure as was used for the isolation of xanthine oxidase from pig liver, *Knox* (86) has isolated a flavoprotein from rabbit liver which has no effect on xanthine; but, unlike the xanthine oxidase prepared from pig liver, it is able to oxidize cinchonidine and quinine. Besides, it has pronounced capacity to catalyze the oxidation of aldehydes. It is open to question whether or not *Knox's* enzyme can be regarded as a variant of the xanthine oxidase described above, but *Knox* (86) mentions that traces of xanthine oxidase from rabbit liver can be separated from his flavoprotein by

fractionation with ammonium sulphate. No flavoprotein able to oxidize cinchonidine or quinine can be isolated from pig liver.

Dehydrogenases: In 1939 *Corran and coworkers* found that pig liver contained enzymes which oxidized aldehydes but which were not flavoproteins (31); but it was 10 years later that *Racker* first isolated from beef liver an enzyme able to react with diphosphopyridine nucleotide and acetaldehyde (129). This enzyme is called *aldehyde dehydrogenase.* The mechanism of reaction is as follows:

$$CH_3CHO + DPN + H_2O \rightarrow CH_3COOH + DPNH_2$$

(aldehyde dehydrogenase)

Unlike the dehydrogenation of alcohol to acetaldehyde which is catalyzed by alcohol dehydrogenase, the dehydrogenation of acetaldehyde seems to be irreversible, and all attempts to make it reversible have so far failed (129). The hydrogen of the reduced diphosphopyridine nucleotide is transferred as described above *via* diaphorase and the cytochrome system to molecular oxygen.

A reaction with other molecules of acetaldehyde seems to be possible if alcohol dehydrogenase is present in the system:

$$DPNH_2 + CH_3CHO \rightarrow DPN + CH_3CH_2OH$$

(alcohol dehydrogenase)

This means that, in the presence of appropriate enzyme systems, one of two molecules of acetaldehyde is oxidized to acetic acid, while the other is reduced to alcohol. *In-vitro* experiments demonstrating this dismutation of acetaldehyde have been described in the literature, and it has been especially studied by *Dixon and Lutwak-Man* (39). These authors claim that the dismutation of acetaldehyde is catalyzed by a single enzyme which they call *aldehyde mutase. Racker* showed that the dismutation will occur in a system with crystalline alcohol dehydrogenase, highly purified aldehyde dehydrogenase and diphosphopyridine nucleotide. His results make it possible that the dismutation of aldehyde is not the effect of a single enzyme, but a combined effect of the two enzymes. However, some discrepancies still have to be cleared up. *Lutwak-Man* found marked inhibition of the alcohol dehydrogenase by methanol, oxalate, maleate or pyrophosphate, but no effect of these substances was seen on the mutase effect, as would be expected if the combination of the two systems were responsible for the mutase effect (103). In any case not all enzymes with an oxidizing effect on alcohol or acetaldehyde can take part in the dismutation; *Dixon and Lutwak-Man* showed for instance that xanthine oxidase was ineffective in a dismutation system.

Other ways of elimination of acetaldehyde are at least theoretically possible. One way is suggested by *Stotz et al.* (138) whose views are based on the fact that acetaldehyde and pyruvic acid in tissue preparations and in the presence of diphosphothiamin react with the formation of acetoin:

$$CH_3CHO + CH_3CO \cdot COOH \rightarrow CH_3CO \cdot CHOH \cdot CH_3 + CO_2$$

It will later be shown that this reaction plays a minor role in the elimination of acetaldehyde *in vivo* (58).

Aldolase is an enzyme acting in the carbohydrate metabolism, catalyzing the process:

$$1 \text{ Mol fructose diphosphate} \rightleftharpoons 2 \text{ Mol triosephosphate.}$$

Aldolase is able to catalyze a condensation of aldehydes (including acetaldehyde) with dioxyacetonephosphate. The role played by processes of this kind is at present unknown.

Inhibition of the enzyme systems acting on acetaldehyde: Both from a theoretical and practical point of view the inhibition exerted by tetraethylthiuramdisulphide (antabuse) is important. Three series of investigations have been made on this point. *Kjeldgaard* (84) examined the effect on *Knox's* aldehyde oxidase and found that antabuse in concentrations of about 1:10,000,000 definitely inhibited the oxidation of all the aldehydes tested, including acetaldehyde. The degree of inhibition is diminished with increasing concentrations of the substrates, which suggests a competition between antabuse and aldehyde for the enzyme. This is very much in conformity with the results of the *in-vivo* experiments where the inhibiting effect of antabuse on acetaldehyde metabolism was found higher with small concentrations of acetaldehyde in the blood (64, 68). *Graham* (56) has examined the effect on *Racker's* aldehyde dehydrogenase and found an inhibitory effect in concentrations of tetraethylthiuramdisulphide of the same order of magnitude as were used by *Kjeldgaard. Graham* demonstrated that the inhibition can be partly abolished by increased concentrations of diphosphopyridine nucleotide, and suggests that tetraethylthiuramdisulphide also competes with diphosphopyridine nucleotide for the active centres of the enzyme. Reduced gluthathione and relatively high concentrations of ascorbic acid are able to reverse the inhibitory effect. From his experimental data *Graham* calculates the relative affinities of the enzyme protein for the substances investigated, and finds that the affinity for tetraethylthiuramdisulphide is approximately 50 times the affinity for diphosphopyridine nucleotide, and 350 times the affinity for acetaldehyde. *Richert, Vanderlinde and Westerfeld* (131) have investigated the effect of antabuse on the xanthine oxidase of livers. About 50% inhibition was found at concentrations of 1:4000 and 1:40,000 using xanthine and *p*-hydroxybenzaldehyde, respectively, as substrates. These concentrations are much higher than those found effective in *Kjeldgaard's* and *Graham's* experiments, and about as high as is necessary to inhibit a long series of other enzymes; so it seems as if the inhibition of this flavoprotein is not very specific. It is, however, interesting that *Richert and his coworkers* found that the addition of methylene blue not only increased the oxidizing effect of xanthine oxidase considerably, but also abolished the inhibition due to added antabuse. When the system was heated to 56° for 5 minutes, little effect was seen on the rate of oxidation of xanthine, but the inhibitory effect of antabuse was abolished. The authors conclude that antabuse has no effect on the dehydrogenating effect of xanthine oxidase, but that it inhibits the reoxidation of the reduced enzyme with free oxygen.

DISCUSSION

Our knowledge of the enzymatic processes involved in alcohol oxidation is still limited, and leaves many phenomena observed during alcohol metabolism in the total organism unexplained. Yet so many advances have been made in recent years in the field of enzyme chemistry that we are able to descry a connection at some points.

It has already been mentioned that the amount of alcohol dehydrogenase present in the liver is large enough to explain the elimination rate of alcohol in man, provided that the content of alcohol dehydrogenase in the human liver is of the same order of magnitude as in horse liver. The rectilinear course of the alcohol combustion curve conforms well with the kinetic data found in studies of the isolated enzyme (141). In this way the presence of alcohol dehydrogenase is fully able to explain the shape and slope of the curve showing the disappearance of alcohol after the administration of alcohol.

However, there is some indication that the dehydrogenation of ethyl alcohol by means of alcohol dehydrogenase is not the *only* pathway of alcohol metabolism *in vivo*. Methanol is presumably oxidized to formaldehyde just as ethanol is oxidized to acetaldehyde (81). The oxidation of methanol is not, however, catalyzed by pure alcohol dehydrogenase, and obviously there must be some other way of oxidation in the organism. Ethyl alcohol interferes with the enzyme systems involved in this process, as the presence of ethanol in the tissues inhibits the metabolic rate of methanol considerably (2, 8a, 153). It is to be expected that ethanol is not only fixed to the enzyme system oxidizing methanol but that it is also oxidized, and in this indirect way we may conclude that alcohol dehydrogenase is not the only enzyme active in the first step of alcohol metabolism. According to *Lutwak-Man* impure preparations of alcohol dehydrogenase are active with methanol as a substrate (103), but instead of supposing an unknown enzyme, it will be more natural to assume that the oxidation by means of catalase and hydrogen peroxide is the pathway sought for. *In vitro*, methyl alcohol is oxidized readily by this system, and moreover *Agner and Belfrage's* (2) data for the elimination rate of methanol in rabbits conform well with the kinetic data of the enzymatic processes found *in vitro* by *Chance* (25). Hence we must conclude that the catalase oxidation also plays a role in alcohol metabolism. For two reasons this form of oxidation presumably is not so important as the oxidation *via* alcohol dehydrogenase. One reason is that the kinetics of the catalase-hydrogen-peroxide process suggest a hyperboloidal decline of the alcohol curve (25). Still more convincing is that in an *in-vitro* system of catalase and H_2O_2 methanol and ethanol are oxidized at the same rate, while *in vivo* ethanol is oxidized at least 4–5 times as rapidly as methanol.

If it is supposed that the first step of alcohol oxidation is a combination of the "dehydrogenase oxidation" and the "catalase oxidation," the shape of the disappearance curve of alcohol in the organism will depend on the ratio between the effects of these two systems. If the former prevails the curve will be rectilinear with no dependence on the concentration of alcohol in the tissues. If a high proportion of alcohol oxidation is due to the catalase reaction, we should expect the rate to depend on the alcohol concentration, but this dependence would be-

come less pronounced as the "dehydrogenase oxidation" became more predominant. On the assumption that the ratio between the two systems can vary from species to species or even under some conditions from individual to individual within the same species, it is easy to explain why some investigators have found the alcohol metabolic rate dependent on alcohol concentration, in contrast to the general opinion that such a dependence does not exist.

It is more difficult to explain the other discrepancies especially the much disputed effect of insulin and of pyruvate. This can not be done without experimental *in-vitro* data on the reaction between alcohol and carbohydrate metabolites, including pyruvate, and the kinetics of these processes. Apart from this the available concentration of diphosphopyridine nucleotide must also be taken into consideration. The turnover number of alcohol dehydrogenase depends on the concentration of coenzyme available for the formation of the enzyme-coenzyme complex. *Theorell and Bonnichsen* have expressed the opinion that, under natural conditions in the tissues, the concentration of free coenzyme can be so low that the enzyme is not fully saturated with coenzyme. In this case any process which will liberate diphosphopyridine nucleotide from other enzyme systems will result in a higher metabolic rate of alcohol. If the dehydrogenase is saturated with coenzyme the liberation of more coenzyme will naturally be without effect. It is possible that these factors might explain the conditioning of animals to a higher metabolic rate of alcohol (112) and perhaps also a part of the observed accelerating effect of carbohydrate metabolism. In any case they explain why the effect is only seen when the initial alcohol metabolic rate is low, but obviously this is merely a hypothesis which must be tested thoroughly before it can be accepted.

It is difficult to decide which of the many different possible mechanisms for the oxidation of acetaldehyde are important *in vivo*. Some of them are less probable than others. For several reasons the condensation of acetaldehyde with pyruvic acid with the formation of acetoin must be negligible. Dogs deprived of thiamin show a slightly decreased capacity to oxidize acetaldehyde (100), but no acetoin is found during alcohol metabolism (58). For the present no information is available to choose between the two types of aldehyde oxidizing enzymes, the dehydrogenase and flavoproteins. Both types are sensitive to tetraethylthiuramdisulphide, and the accumulation of acetaldehyde under the influence of this drug does not help to decide between the two possibilities. The dismutation of acetaldehyde to alcohol and acetic acid most certainly plays a minor role. *Westerfeld et al.* found no formation of alcohol in cats during the oxidation of acetaldehyde, and we have been able to confirm this in experiments with rabbits. The enzymes of acetaldehyde oxidation have not been studied as thoroughly as the enzymes of alcohol oxidation and, therefore, it is impossible for the present to give more than an outline of this second step in alcohol metabolism.

From this review it can be seen that the metabolism of few compounds has been studied so effectively as the metabolism of alcohol. Still our knowledge is deficient at several points easily found in the present review. As to the future,

the reviewer's opinion is that it is a waste of time to discuss the shape and slope of alcoholaemic curves further without paying due attention to the enzymatic processes behind the elimination of alcohol. Recent progress in enzyme chemistry has made this possible, and the most important task for the future will be to fill the gap which still exists between the enzyme studies on the one hand and the *in-vivo* observations and experiments on the other. When this is done, the many riddles and discrepancies between the results of various groups of investigators will be easily explained, and we will wonder why there was ever any room for discussion.

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