chlorotic disease were recognized, and certain types of symptom such as split leaf blotch and fluffy tip were suspected as being probably caused by viruses. Later, the virus origin of both nettlehead and split leaf blotch were confirmed by graft-transmission, but, with the exception of chlorotic disease, no natural method of plant-to-plant transmission has yet been conclusively determined for any hop virus disease. That mosaic and nettlehead do spread by means other than indiscriminate vegetative propagation has been shown by year-to-year surveys, and during the past four years the work has been mainly concentrated in determining whether insects are the natural agents of such spread.

As a preliminary to such a study, records were made of all insects visiting the hop and, in particular, hops where virus diseases were known to be spreading. Initial attempts to transmit mosaic disease by insects included such methods as: (1) mass periodic transfers of insects from plantations where the disease was known to be spreading, to indicator varieties in an insect-proof glasshouse; (2) periodic transfer of indicator plants in pots to such a plantation and subsequent restoration to the insect-proof glasshouse; and (3) the mass infection with suspected vector species of infector plants in pots provided also with indicator plants and kept in an insect-proof glasshouse. Positive results by such mass infections have been analysed in further experiments using individual insects, and to date a line-pattern type of symptom has been transmitted to an indicator hop variety by the common hop damson aphid, *Phorodon humuli* Schr. Probably owing to a long incubation period in the indicators used and very irregular and easily masked expression, vector determinations for nettlehead have not yet yielded results. But certain peculiarities in the incidence and spread of this disease recorded in the early years of this investigation are being studied, namely, the association of outbreaks with cleared hedgerows and old pasture sites. Split leaf blotch as a frequent precursor of nettlehead is also being studied from the point of view of a possible virological relationship of the two diseases.

In addition to such studies in diagnosis, modes of natural transmission, and virus analysis, a study of varietal reaction has shown that certain varieties are symptomless carriers of mosaic, including the widely grown fuggle hop and certain male hops. Work is being directed to indexing the new seedling varieties in this respect.

Propagation of Healthy Planting Stock

One important step in the control of both *Verticillium* wilt and virus diseases of the hop is the production of healthy planting stock. New intensive propagation methods now make it possible for such stock to be produced in areas far away from commercial hops. In such areas the chances of retaining the initial health of selected material are greater than in the hop districts, and the establishment of special nurseries is thus being encouraged. Such nurseries are especially necessary in the case of varieties resistant to wilt, since plants of these varieties grown on an infected farm may carry wilt disease to other farms, although they appear outwardly healthy. Provision is also needed for maintaining a small basic supply of healthy stocks of the commercial varieties and new seedlings from which such nurseries can periodically be renewed.

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THE METABOLISM OF ETHYL ALCOHOL*

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THE principal factors relating to the distribution and the elimination of alcohol in the organism have been known since the publication of the investigations commenced in 1919 by Mellanby and followed up by Widmark in his comprehensive publications during 1922–35. It is possible to follow the rate of disappearance of alcohol in the organism by determination of the concentration of alcohol in the blood. Both Mellanby and Widmark found the curve for the elimination of alcohol from the organism to be rectilinear, and in man an average of 15 mgm. per cent alcohol disappears from the blood per hour, the range being 10–20 mgm. per cent (Widmark's β-factor). For the same individual this rate of elimination was found to be constant and independent of many factors, especially of the alcohol concentration in the blood (and tissues), of the habituation to alcohol of the individual, and of the general metabolism.

The total amount of alcohol present in the organism can be calculated from the blood-alcohol concentration by multiplying this by the body-weight and a factor (Widmark's r-factor). In man, r is fairly constant within the same individual, but can vary somewhat from individual to individual, between 0·50 and 0·90 with an average of 0·66. These facts are generally known and form the basis for practical calculations, especially in medico-forensic practice, where it is of importance to estimate the amount of alcohol consumed from the blood-alcohol concentration or vice versa.

Nevertheless, a long series of reliable experiments have shown that the above principles are not valid in all circumstances[1]. Some investigators have found an increase of alcohol metabolism up to 100 per cent or even more after administration of dinitrophenol, insulin and glucose, pyruvate or alanine; but the accelerating effect seems only to take place if the initial elimination-rate of alcohol is low, and investigators who have worked with animals having a high initial elimination-rate have failed to observe any effect with these substances. During recent years, evidence has also been collected showing that the metabolic rate of alcohol is not completely independent of the alcohol concentration in the tissues. Not only is the elimination curve of alcohol in cats, rabbits and sometimes in dogs found to be more-or-less hyperbolic with a higher rate at greater concentrations of alcohol, but also, in dogs, Newman and his asssociates have observed a peculiar phenomenon, termed by them 'conditioning'[2]. If a moderate dose of alcohol is administered to a dog, resulting in a concentration of about 100 mgm. per cent in the blood, a rectilinear elimination curve with a certain slope will be found. If a higher dose is given, resulting, for example, in a maximum blood-alcohol concentration of 300 mgm. per cent, a rectilinear elimination curve is also found, but the slope is now much steeper than that seen after administration of the smaller dose. When, however, a small dose is given shortly after the elimination of a high dose, the elimination of this smaller dose proceeds at a rate

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not corresponding to the first small dose given, but at the same rate as obtained after the high dose just eliminated. Apparently the high dose has 'conditioned' the organism to a high capacity of elimination of alcohol. Even in man some recent investigations have shown that high doses really are eliminated at a somewhat faster rate than smaller doses[3,4]; but the differences are so slight that many experiments are necessary in order to get statistically significant figures, and it is not possible to decide whether this phenomenon is represented by a slightly hyperbolic curve or is due to a conditioning. Finally, a third observation suggests a dependence of the rate at which alcohol is metabolized on its concentration. As mentioned later, the first step in the oxidation of alcohol in the organism is acetaldehyde. If the alcohol were oxidized at the same rate independently of the concentration in the organism, it would be expected that the amount of acetaldehyde formed per unit of time would be independent of the alcohol concentration. However, this is not the case. There is a definite increase of acetaldehyde concentration in the blood with increasing alcohol concentrations, both in normal organisms and in organisms in which the acetaldehyde metabolism has been inhibited[5,6]. This can only mean that alcohol is metabolized at a somewhat higher rate at higher compared with lower concentrations. However, these observations are of no practical importance for medico-forensic calculations in which the rule of a constant elimination-rate still can be applied; but nevertheless they are of great theoretical interest.

A maximum of 5 per cent of the ethyl alcohol in the organism is eliminated unchanged in the urine and sweat, both having the same concentration of alcohol as the water phase of the blood and of the expired breath, of which 2 litres contains the same amount of alcohol as found in 1 ml. of blood. The rest is completely oxidized to carbon dioxide and water. This was already known at the turn of the century; but in 1949 it was confirmed by radioactive tracers. Ninety per cent of the radioactive carbon-14 in ethyl alcohol administered to rats[7] was excreted as radioactive carbon dioxide over a period of ten hours in the expired breath.

The oxidation proceeds in steps: alcohol is first oxidized to acetaldehyde, which in turn is oxidized to acetic acid. The capacity of the organism to oxidize acetaldehyde to acetic acid is much greater than the capacity to oxidize alcohol to acetaldehyde. Therefore, in normal circumstances, the concentration of acetaldehyde in the organism during alcohol metabolism will be low. It is, however, possible to inhibit the oxidation of acetaldehyde by certain drugs. In this case the acetaldehyde concentration in the organism will be considerably increased during alcohol metabolism. This fact has recently gained some practical importance. Administration of appropriate doses of tetraethylthiuramdisulphide ('Antabuse') results in a higher concentration of acetaldehyde than is normally found in the organism during the metabolism of alcohol[8], and, as increased concentration of acetaldehyde causes some very marked and unpleasant symptoms[9,10], persons under the influence of 'Antabuse' will feel a changed and undesirable effect of alcohol. Experience has shown that people who have felt this effect will abstain from alcohol as long as they are under the influence of 'Antabuse', a fact which is utilized in the therapy of alcoholics[11]. 'Antabuse' inhibits the flavin-containing aldehyde oxidases[12,13], and the aldehyde dehydrogenase[14], of the organism. *In vitro* concentrations about $1/10^7$ give a marked inhibition of the enzymatic actions, and the degree of inhibition is diminished with increasing concentrations of the substrates, suggesting a competition between 'Antabuse' and aldehyde for the enzyme. With aldehyde dehydrogenase, which requires diphosphopyridine nucleotide as a coenzyme, the inhibition can be partly abolished by increased concentrations of the coenzyme, suggesting that 'Antabuse' also competes with the coenzyme for the active centres of the enzyme[14]. The competition between 'Antabuse' and substrate seen in the *in vitro* experiments conforms well with the experiments *in vivo* showing a higher inhibiting effect of 'Antabuse' with low concentrations of acetaldehyde than with high concentrations[15]; thus, 'Antabuse' does not completely block the oxidation of acetaldehyde in the organism, but a higher concentration of acetaldehyde is necessary in the tissues in order to oxidize the amount of acetaldehyde formed by the oxidation of alcohol. This explains readily the increased level of acetaldehyde during alcohol metabolism in persons under the influence of 'Antabuse' or compounds with a similar effect. Acetaldehyde is in its turn oxidized to acetic acid. Apart from some *in vitro* experiments, convincing evidence for this process was given by Bernhard, who showed that substantial amounts of deuterium were found in the acetyl group of acetylated sulphonamide in the urine from animals given C_2D_5OD together with a sulphonamide which is excreted in the acetylated state[16]. Acetic acid is a normal intermediate in the oxidation of fats and carbohydrates, and this fact explains why alcohol can replace the calorie-producing factors of normal nutrition.

The two first steps in the oxidation of alcohol take place mainly in the liver. Eviscerated animals have practically no capacity for oxidizing alcohol, and in partly hepatectomized animals the rate of metabolizing alcohol depends on the amount of liver tissue left in the organism[17]. Isolated surviving livers perfused artificially with aerated blood have a capacity for oxidizing alcohol of about five-sixths that of the whole organism[18–19]; but the alcohol is not completely oxidized in the liver, as a substantial amount of acetic acid is formed, the energy of which can be utilized by other tissues, preferably the muscles[19].

Two different enzyme systems are necessary for the oxidation of alcohol to acetic acid: one catalysing the oxidation of alcohol to acetaldehyde, and the other catalysing the oxidation of acetaldehyde to acetic acid. According to our present knowledge, only two enzymes play a part in the oxidation of alcohol to acetaldehyde: alcohol dehydrogenase and catalase. Both have been isolated in a crystalline state and their properties have been studied.

Alcohol dehydrogenase[20,21] catalyses the process:

$$\begin{array}{l} CH_3 \\ | \\ CH_2OH \end{array} + DPN \xrightleftharpoons{\text{alcohol dehydrogenase}} \begin{array}{l} CH_3 \\ | \\ CHO \end{array} + DPNH + H^+$$

DPN represents diphosphopyridine nucleotide (cozymase).

Both in the oxidized and the reduced state the coenzyme is bound to the enzyme and reacts only with alcohol or acetaldehyde when bound to the enzyme[21]. As indicated in the equation, the process

is reversible—at the *p*H of the organism, sixty molecules of alcohol are in equilibrium with one molecule of acetaldehyde. The process proceeds from left to right in the organism because acetaldehyde is readily further oxidized and thus removed from the system.

In the presence of catalase, hydrogen peroxide is able to oxidize alcohol to acetaldehyde[22]. Generally the substrates forming hydrogen peroxide are not found in any substantial amount in the organism, but the acetaldehyde formed by the oxidation of alcohol can be further oxidized in the organism by the flavin-containing enzymes, xanthine (or aldehyde) oxidases, with the formation of hydrogen peroxide. In this way a circular process will proceed:

$$CH_3.CH_2.OH + H_2O_2 \xrightarrow{\text{catalase}} CH_3.CHO + 2H_2O$$

$$CH_3.CHO + H_2O + O_2 \rightarrow CH_3.COOH + H_2O_2$$

Actually, this process takes place *in vitro* with pure enzymes[23].

The kinetic data found in experiments with the pure enzymes can give considerable information as to the possible importance *in vivo* of the two processes already mentioned. There is sufficient alcohol dehydrogenase present in the liver to explain the rate of oxidation of alcohol in the intact organism. Bonnichsen's yield of crystalline alcohol dehydrogenase was 1 gm. per kgm. horse liver[20], and, provided that the same concentration is found in human liver, at least 1·5 gm. on an average should be present there. When the enzyme is fully saturated with the coenzyme, the turnover number of the enzyme is 150, which means that, the molecular weight of the enzyme being taken as 73,000, then 1·5 gm. enzyme can oxidize 8–9 gm. alcohol per hour, which is about the rate of oxidation actually found in man[20]. In addition, the kinetic data show that the rate of alcohol oxidation catalysed by alcohol dehydrogenase is independent of the alcohol concentration down to concentrations so low that they cannot be determined by the usual clinical methods[20].

In this way the function of the alcohol dehydrogenase explains both the oxidation-rate and the rectilinear course of ethanol oxidation in the organism; but the fact that methanol can be oxidized in the organism shows that other enzymatic pathways must also be considered. There seems to be an intimate connexion between the oxidation of methanol and ethanol in the organism, as high concentrations of the latter are able to inhibit the rate of metabolizing methanol[24,25,26]. This can be utilized in cases of methanol poisoning, where high and continued doses of ethanol given to the patients cause a delay in the oxidation-rate of methanol so that formic acid, in concentrations dangerous to sight or life, are not formed[27]. Alcohol dehydrogenase is unable to catalyse the oxidation of methanol[21], but catalase can[28]. Moreover, the slope and the shape of the elimination curve of methanol *in vivo*, definitely depending on the concentration of methanol, conform with the kinetic data of the binding of catalase with methanol, which supports the assumption that the oxidation of methanol is catalysed by the catalase systems[28]. *In vitro* the oxidation of methanol and ethanol by catalase – hydrogen peroxide proceeds at approximately the same rate, and if the same is the case *in vivo* we have an indication of the relative part played by the catalase system in alcohol metabolism. As the oxidation-rate of methanol in the human organism is about a quarter that of ethanol, about 80 per cent of the ethanol can be expected to be oxidized by the alcohol dehydrogenase system and the rest by the catalase – acetaldehyde oxidase system. The possibility that four-fifths of the alcohol elimination in man is due to alcohol dehydrogenase, the action of which is independent of the concentration of alcohol, explains why the alcohol elimination curve does not deviate from the straight line within the error of the alcohol determination; and if a fifth of the oxidation is due to the catalase system, the effect of which is dependent on the concentration of the substrate, it explains why we find indications of a dependence on alcohol concentration in certain circumstances.

However, the phenomenon of conditioning cannot be explained in this way, and there are other possibilities which may offer an explanation. The capacity of the alcohol dehydrogenase to oxidize alcohol depends on the amount of coenzyme available in the system, and the calculation discussed in the previous paragraph is based on the assumption that the enzyme is fully saturated with the coenzyme. As cozymase also is bound to other enzymes, it is not certain that the alcohol dehydrogenase is in all circumstances fully saturated[21], and an increase in the coenzyme available will increase the capacity of the enzyme. If the oxidation of high concentrations of alcohol in some way or another is able to release coenzyme from other enzymes and make it available for the alcohol dehydrogenase, a higher oxidation-rate will be the result. Nothing in the literature is contrary to this suggestion; but it must be emphasized that it has to be tested experimentally before it can be regarded as more than a working hypothesis.

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