IS ACETALDEHYDE AN INTERMEDIARY PRODUCT IN NORMAL METABOLISM?

by

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Mainly through the work of MEYERHOF, PARNAS, EMBDEN, and CORI, their collaborators and pupils, the intermediary products of the first part of carbohydrate metabolism are well known. The intermediary products have been isolated and the enzymes involved thoroughly studied. It is now generally accepted that glycogen or glucose is broken down to pyruvate through a series of phosphorylated compounds. Pyruvate forms a "natural dividing point" between the anaerobic and the aerobic phases of carbohydrate metabolism. It has, however, been extremely difficult to study the intermediary products and the corresponding enzymes in volved in the further oxidation of this substance. Several hypotheses concerning this part of carbohydrate metabolism have been proposed. The experimental facts hitherto obtained seem to be best explained by KREBS' citric acid cycle-theory. The individual processes are well known and need no further description (KREBS, 1943). Nevertheless it is not known whether other processes are also involved in the oxidation of pyruvate and alternative schemes have been proposed. The early theory of THUNBERG (1920) and KNOOP (1923) suggests that pyruvic acid is decarboxylated to acetaldehyde which is then oxidized to acetic acid. This compound is in turn condensed to succinic acid. Their theory has now been abandoned, mainly because it has been impossible to demonstrate any formation of succinic acid from acetic acid in living cells or cell extracts. It has, however, been shown by several authors that acetaldehyde can be formed during tissue metabolism. In *in vitro* experiments with minced tissues acetaldehyde has been trapped by means of aldehyde fixatures following the technique of NEUBERG. HIRSCH (1923) identified acetaldehyde formed in muscles of frogs or fishes. NEUBERG AND GOTTSCHALK (1924) showed the formation of acetaldehyde in different tissues of warm-blooded animals and their results have been confirmed and enlarged by PALLADIN AND UTEWSKI (1929), GORR (1932), TANKÓ, MUNK, and ABONYI (1940) and others. Addition of pyruvate to the minced muscles increases the yield of acetaldehyde (UTEWSKI, 1929) and the formation of acetoin, a condensation product of acetaldehyde and pyruvic acid, from pyruvate has been shown by GREEN *et al.* (1941) and by STOTZ, WESTERFELD, and BERG (1944). In animal tissues acetate was identified as an oxidation product of pyruvic acid by KREBS AND JOHNSON (1937), WEIL-MALHERBE (1937) and LONG (1938). It was shown that pyruvate anaerobically dismutes into lactate + acetate + carbon dioxide. Although KREBS AND JOHNSON emphasize that this process in animal tissues differs from that of decarboxylation of pyruvic acid in microorganisms, it cannot be excluded with certainty that acetaldehyde even in this process acts as an intermediary product.

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Acetaldehyde is oxidized very rapidly *in vivo* (LUBIN AND WESTERFELD, 1945): and the acetoin formed *in vivo* also appears to be very rapidly metabolized.

Even if the citric acid cycle is the main path of the normal metabolism of pyruvic acid and some of the results showing a possible formation of acetaldehyde from pyruvic acid are due to artefacts in the sense that the biochemical processes only occur under more or less abnormal conditions, it is still possible that pyruvate in normal metabolism is partly broken down with acetaldehyde serving as an intermediary product. Hitherto no means have been available to decide to what extent this secondary path plays a rôle in the normal metabolic processes of the organism.

At the present experiments performed in this laboratory are able to throw a light on the question.

HALD, JACOBSEN, AND LARSEN (1948) have shown that individuals given tetraethylthiuramdisulphide (Antabuse) will give a series of symptoms after ingestion of minute amounts of alcohol. The occurrence of these symptoms is due to an increased formation of acetaldehyde from alcohol, resulting in an increased concentration of acetaldehyde in the blood (HALD AND JACOBSEN, 1948; ASMUSSEN, HALD, AND LARSEN, 1948; and LARSEN, 1948). If the metabolic rate of acetaldehyde is slowed after ingestion of Antabuse, the increased concentration of this substance in the organism is easily explained. Preliminary experiments in this laboratory showed, however, that no difference in the rate of acetaldehyde elimination in normal and Antabuse-treated animals could be seen when acetaldehyde was given during short periods and in such an amount that the final concentration of acetaldehyde in the blood was 20–25 mg/%. In collaboration with DR.'S JENS HALD AND VALDEMAR LARSEN I have made a series of further experiments showing that the metabolic rate of small concentrations of acetaldehyde is decreased in animals treated with Antabuse. These experiments will be published in detail by HALD, JACOBSEN, AND LARSEN.

A series of rabbits weighing from 2.0–2.5 kg were given 0.50 g Antabuse 48, 24 and 16 hours prior to the experiment. The animals were anesthetized with urethan. Blood samples were taken from a cannula inserted in the carotid artery. Coagulation was prevented by the injection of 1500 units of heparin intravenously. Acetaldehyde determinations were made by STOTZ'S method. A cannula was inserted into the jugular vein. Two to ten per cent solutions of acetaldehyde in TYRODE'S solution were infused through the cannula at a known constant rate. The infusing apparatus consisted of a 10–30 ml syringe, the piston of which was controlled by a screw driven mechanically by a gramophone motor. The experiments generally lasted 1 ½–2 ½ hours. During this period the infusion rate was maintained at a constant level which did not exceed the capacity of the rabbits to metabolize acetaldehyde. There was no accumulation of acetaldehyde in the tissues during the experiment.

An average sized rabbit is usually capable of eliminating 7–8 mg acetaldehyde per minute. The concentration of acetaldehyde in the blood was determined 30 minutes after the beginning of the infusion and at intervals of $^1/_2$–$^3/_4$ hours. The levels of acetaldehyde in blood corresponding to a fixed infusion rate of acetaldehyde varying between 0.75 mg and 9 mg per minute were determined in two series of rabbits: one normal series, and one consisting of rabbits treated with Antabuse in the manner described above. A considerable variation of the blood acetaldehyde is noted from time to time although the infusion rate was kept as constant as possible. The results of the experiments are tabulated in Fig. 1. A clear difference between the concentration of acetaldehyde in

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blood in the two series is shown. When the same amount of acetaldehyde is metabolized, the level of acetaldehyde in blood is higher in the Antabuse-treated animals than in the untreated ones. The smaller the amounts of acetaldehyde metabolized per minute, the greater is the relative difference between the two groups. When 0.75–2.0 mg is infused per minute, the acetaldehyde level in blood of the Antabuse-treated rabbits is 5–10 times that of the normal animals, whereas it is less than twice when 8–9 mg are infused per minute. The same results are obtained in perfusion experiments with isolated liver and hind limbs. An account of these experiments will be published at a later date.

If acetaldehyde is found as a normal split product in metabolism, the experiments described here show that this will result in an increased concentration of acetaldehyde in the blood of rabbits treated with Antabuse. Acetaldehyde in blood was determined in normal and Antabuse-treated rabbits. The results are given in Table I. No significant statistical difference between the two groups is seen.

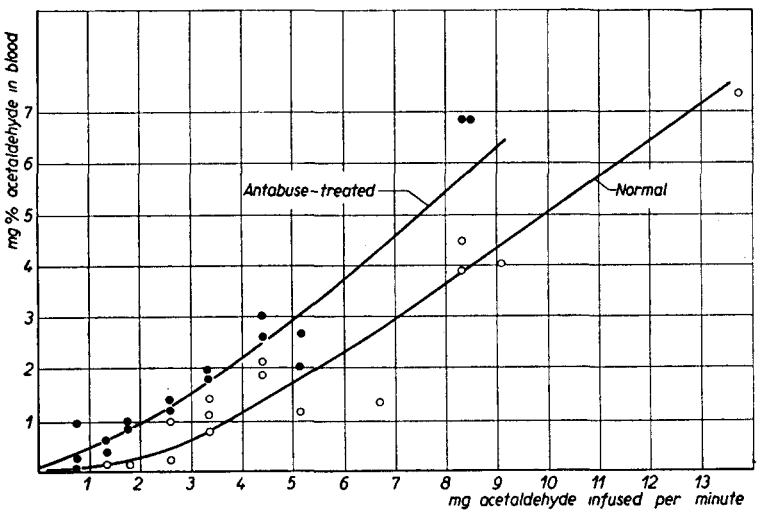

Fig. 1. Correlation between infusion rate of acetaldehyde into the jugular vein and mg acetaldehyde per 100 ml blood in normal rabbits and rabbits treated with Antabuse (tetraethylthiuramdisulphide)

Similar results are obtained in perfusion experiments. A series of livers and hind limbs from normal rabbits and rabbits treated with Antabuse were artificially perfused with blood as described by NIELSEN (1933). On an average the livers weighed about 80 g, and the muscles of the hind limbs 430 g. The average oxygen uptake per minute was 1–3 ml per minute in the livers and 2–4 ml per minute in the hind limbs. When acetaldehyde was added to the perfusion blood, the blood which passed through the livers or muscles from Antabuse-treated animals showed a considerably higher concentration of acetaldehyde than blood that passed through organs of normal animals. From the amount of blood perfused per minute and the difference in acetaldehyde concentrations in the blood before and after the perfusion it is possible to calculate the amount of acetaldehyde passing into the perfusion blood per minute. If any substantial quantity of acetaldehyde is formed during normal metabolism, a difference should be seen between the perfusion experiments made with normal animals and with Antabuse-treated animals. As seen in Table II this is not the case. At times the acetaldehyde

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TABLE I

	Antabuse treated rabbits	Normal rabbits
Number of animals	28	19
Range of acetaldehyde concentration in blood	0.01 to 0.25 mg %	0.00 to 0.30 mg %
Average and standard deviation of average	0.104 ± 0.012 mg %	0.085 ± 0.017 mg %
σ = Standard deviation of single determinations	0.021 mg %	0.023 mg %

Acetaldehyde in mg 100 ml blood in rabbits treated with Antabuse (tetraethylthiuramdisulphide) and in normal rabbits

TABLE II

	Antabuse treated rabbits	Normal rabbits	
Number of experiments	16	17	liver
Range of mg acetaldehyde formed per minute	—0.04 to + 0.15	— 0.02 ± 0.11	liver
Average and standard deviation of average	0.032 ± 0.013	0.030 ± 0.011	liver
σ = Standard deviation of single determinations	0.053	0.047	liver
Number of experiments	9	12	hind limbs
Range of mg acetaldehyde formed per minute	—0.02 to + 0.12	—0.07 to + 0.07	hind limbs
Average and standard deviation of average	0.012 ± 0.006	0.008 ± 0.001	hind limbs
σ = Standard deviation of single determinations	0.017	0.030	hind limbs

Acetaldehyde formation per minute in isolated organs from rabbits treated with Antabuse and from normal rabbits

formation is negative. This indicates that the concentration of acetaldehyde is lower in the blood which has been perfused through the organ than in the blood which enters the organ. Of course the analytical error is rather high when determining small concentrations of acetaldehyde and so will influence the results considerably. Furthermore substances other than acetaldehyde may give reactions which influence the determinations to a considerable degree when small concentrations of acetaldehyde are found in the blood. Nevertheless the production of acetaldehyde under the above mentioned conditions appears to be of very little importance.

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Thus it may be concluded that very little, if any, acetaldehyde can be formed during normal metabolism and that the alternative paths in metabolism in which acetaldehyde is supposed to be an intermediary product, do not play a significant rôle.

SUMMARY

It has been shown that acetaldehyde metabolism is delayed in animals treated with tetraethylthiuramdisulphide (Antabuse).

No increase of acetaldehyde formation can be seen in total organisms and in isolated livers and muscles from rabbits treated with Antabuse.

From these observations it is concluded that acetaldehyde plays a very insignificant rôle as an intermediary product in normal metabolic processes.

RÉSUMÉ

On montre que le métabolisme de l'acetaldehyde est retardé dans les animaux traités au tetraéthylthiuramdisulfide (Antabuse).

Aucune augmentation de la formation d'acetaldehyde n'a pu être observée dans les organismes entiers et dans les foies et les muscles de lapins traités à l'Antabuse.

De ces observations nous concluons que l'acetaldehyde joue un rôle très peu important dans les processus métaboliques normaux.

ZUSAMMENFASSUNG

Es wird gezeigt, dass der Metabolismus des Acetaldehyds in mit Tetraäthylthiuramdisulfid (Antabuse) behandelten Tieren verzögert ist.

Eine Zunahme der Acetaldehydbildung in ganzen Organismen oder in isolierten Lebern und Muskeln von mit "Antabuse" behandelten Kaninchen wurde nicht beobachtet.

Aus diesen Beobachtungen wird geschlossen, dass das Acetaldehyd eine sehr unbedeutende Rolle als Zwischenprodukt der normalen metabolischen Prozesse spielt.

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