Inhibition of Methanol Metabolism in Mice with Acetyl Salicylic Acid

The biological oxidation of methanol to carbon dioxide in mammals has been known since 1893 (POHL[1]). Contrary to its higher homologues (e.g. ethanol and propanol), methanol is not oxidized to formaldehyde by crystalline horse liver alcohol dehydrogenase (THEORELL and BONNICHSEN[2]). This revived the concept that methanol is mainly oxidized by the catalase-H_2O_2 system (KEILIN and HARTEE[3]). In fact, this view is accepted as the major mechanism of methanol oxidation in vitro. However, the metabolic pathway of the alcohol in vivo is not fully understood, and it is believed that the peroxidative reaction of catalase is involved. Several approaches have been attempted to uncover the biochemical chain involved in methanol oxidation; using specific catalase inhibitors, e.g. 3-amino-1, 2, 4-triazole, or substances inhibiting catalase biosynthesis, such as allyl-isopropylacetyl carbamide (AEBI et al.[4] and MANNERING and PARKS[5]).

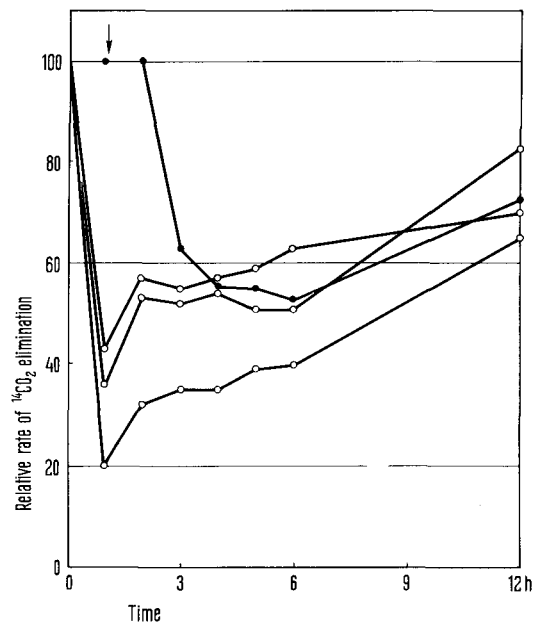
The present investigation is concerned with studying the effect of acetyl salicylic acid (ASA) on the in vivo metabolism of C^{14}-methanol in mice. The elimination of $C^{14}O_2$ in the expired air was followed as an index for the alcohol metabolism, since it is known that the main channel of the alcohol excretion is the lungs (BARTLETT[6]). Following the intraperitoneal administration of C^{14}-methanol (5% by volume in saline), the expired air was trapped in 1 *N* sodium hydroxide solution after different intervals, and the C^{14}-activity was determined as $BaC^{14}O_3$ in an end-window Geiger counter. It was found that over 70% of the administered dose (400 mg/kg/mouse) could be recovered as $C^{14}O_2$ during 24 h. The elimination of $C^{14}O_2$ followed a typical 2-phased curve. During the first 6 h (first phase), about 95% of the $C^{14}O_2$ was eliminated, then the elimination rate was remarkably decreased.

The excretion rate of $C^{14}O_2$ was studied after a single intraperitoneal injection of ASA, which was administered after neutralization with sodium carbonate solution. The Figure illustrates the inhibition of methanol oxidation during 12 h, and it could be seen that ASA (500 mg/kg per mouse) caused an inhibition as high as 40–80%. Even after the elapse of 24 h, a small degree of inhibition (10–20%) could be traced. A similar dose of salicylic acid (500 mg/kg) was less effective in inhibiting methanol metabolism.

The effect of ASA on liver catalase activity was studied, using 10% homogenates. The enzyme was assayed according to the method of FEINSTEIN[7]. Half inhibition was conferred by 15 m*M* of the acid. In a work aimed to correlate the antipyretic activity of ASA with catalase activity, an inhibition of similar magnitude has been reported (WILLIAMSON and RUDGE[8]). The effect of ASA on liver and blood catalase activity has also been investigated by injecting (i.p.) different doses of ASA ranging from 0.5 to 1.0 g/kg. Animals were sacrificed 1, 2, or 3 h after injecting ASA and the enzyme was immediately assayed. No inhibition of liver or blood catalase could be detected even after the administration of a dose as high as 1.0 g/kg where only few animals survived to 3 h.

In mice, LD_{100} for absolute methanol has been determined to be 8.0 g/kg for intraperitoneal injection. This dose was injected into 60 mice which were divided into 3 equal groups. The first group received (i.p.) a single dose of 0.5 g/kg/mouse 1 h before injecting the alcohol. The second group was similarly treated with 0.7 g ASA/kg per mouse. The third group was given only the alcohol to serve as a control. At the end of 24 h, 6 mice from the first group survived, but only to die after 48–72 h. From the second group, 6 mice survived and recovered completely. All animals belonging to the third group died within 24 h.

It is believed that the serious symptoms of methanol poisoning are due to its immediate metabolites, e.g. the effect on vision (amblyopia) is thought to be due to inhibition of retinal glycolysis by formaldehyde (POTTS and JOHNSON[9]). Also, the localized toxic effects of methanol may be explained by the formation of methyl-formate from methanol and formaldehyde (KENDAL and RAMANTHAN[10]). In other words, the accumulation of formaldehyde is apparently responsible for most serious symptoms of methanol poisoning. Therefore, substances which inhibit methanol oxidation should eliminate – at least partly – the toxic effects. As an antidote, ethanol has so far been used for treating methanol poisoning, since it inhibits methanol oxidation (AEBI et al.[4] and BARTLETT[11]), and minimizes the toxic effects (RÖE[12]). On the other hand, GILGER et al.[13] denied that ethanol suppresses methanol toxicity and claimed that ethanol significantly increases it in mice and lessens the interval to death following methanol administration.



Inhibition of $C^{14}O_2$ elimination by acetyl salicylic acid (0.5 g/kg). Zero time is the time of C^{14}-methanol administration. 100% = elimination rate without ASA. (o) = ASA injected 1 h before methanol. (●) = ASA injected 1 h after methanol (mean of 3 experiments).

[1] J. POHL, Arch. exp. Path. Pharmak. *31*, 281 (1893).
[2] H. THEORELL and R. BONNICHSEN, Acta chem. scand. *5*, 1105 (1951).
[3] D. KEILIN and E. F. HARTEE, Proc. Roy. Soc. B *119*, 141 (1935).
[4] H. AEBI, H. KOBLET, and J. P. VON WARTBURG, Helv. physiol. Acta *15*, 384 (1957).
[5] G. J. MANNERING and R. E. PARKS, Science *126*, 1241 (1957).
[6] G. R. BARTLETT, Am. J. Physiol. *163*, 614 (1950).
[7] R. FEINSTEIN, J. biol. Chem. *180*, 1197 (1949).
[8] J. WILLIAMSON and E. A. RUDGE, Biochem. J. *43*, 15 (1948).
[9] A. M. POTTS and L. V. JOHNSON, Am. J. Ophthal. *35*, 107 (1952).
[10] L. P. KENDAL and A. N. RAMANTHAN, Biochem. J. *52*, 430 (1952).
[11] G. R. BARTLETT, Am. J. Physiol. *163*, 619 (1950).
[12] O. RÖE, Acta med. scand. *125*, Suppl. 182, 256 (1946).
[13] A. GILGER, A. M. POTTS, and L. V. JOHNSON, Am. J. Ophthal. *35*, 113 (1952).

From the data presented in this investigation, it is obvious that ASA significantly inhibits the metabolism of methanol in vivo, and provides protection against methanol poisoning in mice. Should the view of methanol oxidation in vivo (by catalase-H_2O_2 system) be accepted, it is suggested that it is the depletion of the peroxide pool rather than the reduction in catalase activity which causes inhibition of methanol oxidation. This may be achieved by inhibiting enzyme systems involved in H_2O_2-delivering reactions, e.g. xanthineoxidase and monoamineoxidase (AEBI et al.[14]). The suggested mechanism of inhibition receives support from the inhibition of C^{14}-formate metabolism in vivo by ASA, since formate is known to possess a specific requirement for catalase-H_2O_2 complex (KEILIN and HARTEE[15] and AEBI and HASSAN[16]). C^{14}-formate was administered intraperitoneally and the measurement of the expired $C^{14}O_2$ served as a test system. It was found that a dose of 0.5 g ASA per kg per mouse inhibited formate oxidation to almost the same extent as that of methanol.

It is not unlikely that the antipyretic activity of ASA is related in a way to the depletion of H_2O_2 sources rather than to reduction in catalatic decomposition of the peroxide by catalase[8].

From this work, the following may be concluded: (1) ASA significantly inhibits methanol and formate oxidation in vivo. (2) ASA does not inhibit catalase in vivo, but it does in vitro. (3) ASA reduces the toxic effects of methanol poisoning in mice. (4) The mechanism of inhibition of methanol oxidation by ASA is believed to be due to reduction of the peroxide pool rather than the inhibition of catalase.

Zusammenfassung. Acetylsalicylsäure hemmt die Oxydation von Methanol und Formiat in vivo erheblich, die Katalase nur in vitro, und vermindert den toxischen Effekt der Methanolvergiftung. Der Mechanismus der Methanoloxydationshemmung durch Acetylsalicylsäure könnte eher auf die Verminderung des Peroxyd-Pooles – als die Katalasehemmung – zurückzuführen sein.

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[14] H. AEBI, J. QUITT, and A. HASSAN, Helv. physiol. Acta *20*, 148 (1962).
[15] D. KEILIN and E. F. HARTEE, Biochem. J. *60*, 310 (1955).
[16] H. AEBI and A. HASSAN, Helv. chim. Acta *43*, 544 (1960).

Ouabain-Sensitive Fatty Acid Release from Isolated Fat Cells[1]

Although the control of free fatty acid (FFA) mobilization has been extensively studied in many laboratories[2], the detailed mechanism by which stored triglycerides are released from fat cells as fatty acids and glycerol is as yet incompletely understood. In particular, it is not known whether FFA released from triglycerides simply diffuse through the cell membrane, whether they are actively transported, or indeed whether they are released within or close to the cell membrane itself. The recent finding[3] that FFA release induced by lipolytic hormones such as ACTH and epinephrine is inhibited by ouabain suggests that the release mechanism may involve an active transport step, since it has previously been shown that this agent can be used to differentiate between active and passive transport processes[4]. This preliminary report establishes the ouabain-sensitive nature of the FFA releasing system in isolated fat cells.

Material and methods. Male albino rats weighing 130 to 170 g and fed ad libitum were used throughout. Free adipose cells were prepared from epididymal fat pads using the technique of RODBELL[5] and incubated in bicarbonate buffer. FFA were measured using the method of DOLE and MEINERTZ[6].

Results and discussion. As illustrated in the Figure, the addition of ouabain to the incubation medium was associated with a decrease in the net change in FFA concentration during incubation in the presence of epinephrine. At an epinephrine concentration of 0.11 μg/ml, the minimum effective dose of ouabain was $9 \cdot 10^{-6} M$. The inhibition reached maximum at an ouabain concentration around $2.5 \cdot 10^{-4} M$. At this concentration the net FFA increase observed during incubation in the absence of ouabain was inhibited to the extent of between 60 and 70%. The concentration range of this dose response curve for ouabain was comparable to that which obtains in the studies of ouabain effects on the active transport of sodium and potassium.

Although ouabain increased the uptake and metabolism of glucose by fat cells, it is noteworthy that the inhibition of FFA release demonstrated in the Figure occurred in the total absence of glucose. Furthermore, it was found that ouabain did not increase the uptake and esterification of labelled FFA added to the incubation medium. These observations are interpreted to mean that the ouabain-induced inhibition of FFA release was not the result of an increase in the re-esterification process. Accordingly, the metabolic effect of the agent must be related either to the transport of FFA across the cell membrane, or to lipolysis, or both.

The inhibitory effect of ouabain on FFA release was not limited to epinephrine-stimulated lipolysis, but applied equally to the lipolysis induced by the presence of

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[2] A. E. RENOLD and G. F. CAHILL Ed., *Handbook of Physiology*, Section 5: *Adipose Tissue* (American physiol. Society, Washington 1965).
[3] S. J. Ho, R. J. Ho, and H. C. MENG, unpublished data.
[4] T. Z. CSÁKY, Ann. Rev. Physiology *27*, 415 (1965).
[5] M. RODBELL, J. biol. Chem. *239*, 375 (1964).
[6] V. P. DOLE and H. MEINERTZ, J. biol. Chem. *235*, 2595 (1960).