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Effect of ethanol on the disappearance of acutely administered acetate from the blood in rats

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The involvement of acetate in the metabolic processes of plants and animals has been recognized for a long time. In ruminants, acetate and other short chain aliphatic fatty acids are derived from the microbial fermentation of ingested carbohydrates, whereas in primates acetate is derived almost exclusively from endogenous sources. Administration of ethanol or alcoholic beverages is a typical and interesting example of a greatly increased supply of acetate which is produced from an exogenously administered substrate.

Oxidation of ethanol in the liver results in the formation of acetaldehyde which is virtually instantaneously oxidized one step further to acetate. After administration of alcoholic beverages, the formation of acetate in the liver exceeds its capacity to utilize it metabolically [1, 2]. Thus, most of the acetate formed is released into the blood stream and is distributed among other organs of the body and muscle where it is utilized for a variety of metabolic processes. For the most part acetate is oxidized to carbon dioxide, water and energy.

Our earlier studies *in vitro* which indicated that ethanol severely inhibits the metabolism of acetate in liver slices [3, 4] have a number of implications concerning the biological disposition of both endogenous and administered acetate in the presence of ethanol in intact animals and possibly in man. One possibility is that the disappearance of administered acetate from the blood might be inhibited by ethanol. The results reported here tend to support such a possibility.

Male Sprague–Dawley rats, 200–300 g, were fasted overnight. The animals were divided into four groups and received one of the following substrates by intragastric intubation: 3 g/kg of ethanol; 1.93 g/kg of acetate; 3 g/kg of ethanol and 1.93 g/kg of acetate or water (controls). Ethanol was used as a 20% (w/v) solution and acetate as 12.8% (w/v) sodium acetate. In a separate experiment, ethanol and acetate were administered intraperitoneally to fed rats at doses of 2 g/kg of ethanol, 1.28 g/kg of acetate or 2 g/kg of ethanol and 1.28 g/kg of acetate. Blood samples were taken from a dorsal tail vein [5] at hourly intervals for 8–12 hr after the administration of the substrates. Both ethanol and acetate were determined in duplicate by gas chromatography as described by Mahadevan and Stenros [6] and Roach and Creaven [7].

When ethanol was administered by intragastric intubation, blood acetate concentrations reached a plateau at approximately 8–10 mg/dl within 1 hr after treatment and remained at this level as long as ethanol was present in the blood (Fig. 1). Blood acetate concentrations after administering half equivalent amounts of acetate were about 12 mg/dl within 1 hr and decreased to control levels (0.5 to 1.5 mg/dl) within 6–7 hr. When acetate was given simul-

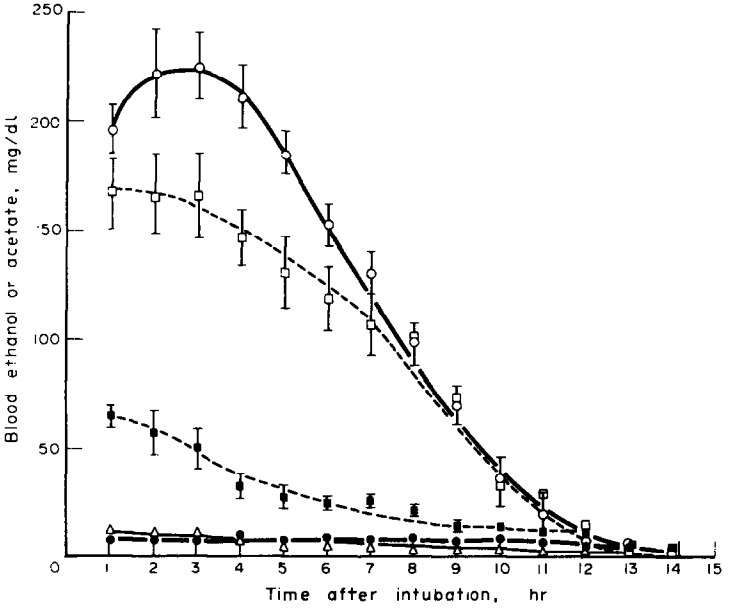

Fig. 1. Blood ethanol and acetate concentrations after a single oral dose of ethanol. Key: (O——O) ethanol after ethanol alone; (□----□) ethanol after ethanol and acetate; (■----■) acetate after ethanol and acetate; (Δ——Δ) acetate after acetate alone; and (●——●) acetate after ethanol alone. Each point represents the mean ± S.E. of 11–13 survivals.

taneously with ethanol, blood acetate concentrations were substantially elevated to 71 mg/dl 1 hr after administration and required 10–11 hr to be eliminated from the blood.

The question arose whether an accelerated absorption of acetate from the stomach could explain the high concentrations of acetate when acetate and ethanol were given together. The experiments were repeated, administering both substrates by the intraperitoneal route, but at somewhat lower doses than those by the intragastric route because the same dose intra-peritoneally was lethal. The pattern of disappearance of both, acetate and ethanol, was similar to that observed when the substrates were given by intragastric route (Fig. 2). The only difference appears at the end of the first hour after the administration of acetate alone, when blood acetate concentrations were considerably higher than in previous experiments.

The observed acetate levels after the administration of both ethanol and acetate could result from the addition of the acetate derived from ethanol and the exogenous acetate. Table 1, however, demonstrates that the observed acetate levels are 2 to 3.5 times greater than the expected values during the first 8 hr after oral administration and the first 4 hr after intraperitoneal treatment.

The administration of ethanol to rats, either orally or intraperitoneally, leads to an elevation of blood acetate concentrations approximately 10–15 times higher than those found in untreated animals. However, the amount of acetate observed appears to be independent of blood ethanol concentrations and is similar to what occurs for acetaldehyde [8]. Lundquist *et al.* [2] found the same lack of correlation between blood ethanol and acetate concentrations in humans. These findings presumably reflect saturation of alcohol dehydrogenase, the rate-limiting step in the metabolism of high concentrations of ethanol.

Exogenous acetate is eliminated fairly rapidly apparently by first order kinetics (Fig. 2). However, in the presence

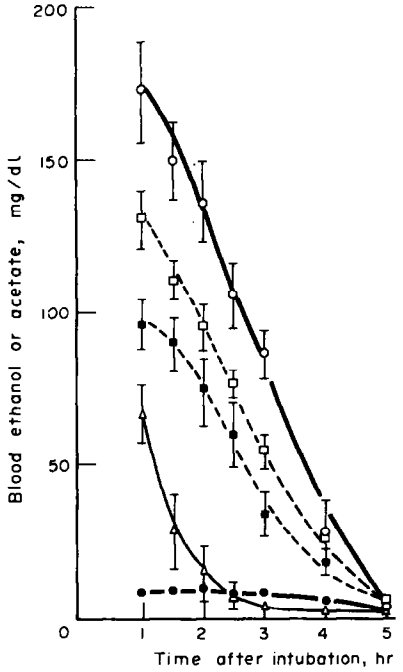

Fig. 2. Blood ethanol and acetate concentrations after a single intra-peritoneal dose of ethanol. Key: (○——○) ethanol after ethanol alone; (□- - - -□) ethanol after ethanol and acetate; (■- - - -■) acetate after ethanol and acetate; (△——△) acetate after acetate alone; and (●- - - -●) acetate after ethanol alone. Each point represents the mean ± S. E. of 7–12 survivals.

Table 1. Acetate levels after both ethanol and acetate administration*

Time after treatment (hr)	Oral		Intraperitoneal	
	Expected†	Observed	Expected†	Observed
1	20.4	71	75.6	96
1.5			37.4	90
2	18.3	69	24.9	78
2.5			13.6	60
3	19.4	54	9.8	34
4	15.8	39	6.9	18
5	12.6	33		
6	12.5	23		
7	12.8	23		
8	12.0	20		

* Acetate levels are expressed as mg/dl.

† Expected values are the sum of those from acetate alone and derived from ethanol.

of ethanol the rate of elimination of acetate is considerably reduced and cannot be explained by the additional acetate derived from ethanol (Table 1). The removal of acetate from the blood could be accomplished by several means: excretion in the urine and uptake and subsequent metabolism in various organs of the body. The concentration of acetate in the urine after administration of ethanol is quite low, indicating that renal excretion plays a minor role in acetate elimination [9]. After a high dose of acetate, the kidney could play a greater role in acetate elimination, but since ethanol induces diuresis, the retardation of acetate disappearance by ethanol is not likely to be explained by this mechanism.

Acetate appears to be taken up by various organs and metabolized to carbon dioxide and water or used for other biosynthetic processes. Since the liver uses acetate as a precursor for a number of metabolic pathways, it would seem that it should be an important site of metabolism of administered acetate. However, the amount of acetate derived from ethanol, which is predominantly metabolized by the liver, apparently exceeds the capacity of the liver to utilize it and is consequently released into the blood [1, 2]. Therefore, administered acetate would be metabolized mostly by extrahepatic systems. The mechanism of the inhibition of acetate disappearance by ethanol probably involves a disruption of either the uptake and/or the subsequent metabolism of acetate in various parts of the body.

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Prostaglandins: effect of prostaglandin E_1 on brain, stomach and intestinal serotonin in rat

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Prostaglandins (PGs) have been found in brain, gastro-intestinal tract and other mammalian organs, together with complete systems capable of synthesizing and metabolizing distinct PG types [1–5]. PGs of E series have been shown to produce sedation, stupor, catatonia and inhibit electrical and chemically-induced convulsions [6, 7]. Recent studies from this laboratory have shown that PGE_1-induced potentiation of morphine analgesia, antinociceptive effect of PGE_1, *per se*, and PGE_1-induced potentiation of hexobarbitone hypnosis in rat are probably serotonin mediated. PGE_1-induced inhibition of gastric secretion was also shown to be a serotonin mediated response. In addition, PGE_1 potentiated the inhibitory effect of serotonin on gastric secretion and the smooth muscle contractility of serotonin [8–12]. Haubrich *et al.* [13] have demonstrated that PGE_1-induced sedation in rat was associated with increased brain serotonin turnover. It thus seemed possible that an inter-relationship exists between PGs, more specifically PGE_1, and serotonin in the brain and the gastro-intestinal tract. It was therefore thought worthwhile to study the effect of PGE_1 on the level, rate of synthesis (accumulation) and decline of serotonin in brain, stomach and proximal intestine.

MATERIAL AND METHODS

The study was conducted on male Wistar albino rats (100–120 g). The rats were maintained on standard Hind Lever diet and were housed in an air cooled room (25°) in colony cages. Animals were fasted overnight but water was allowed *ad lib.* before experimentation. PGE_1 (0.4 mg/kg s.c.) was administered and the rats were killed by decapitation at different time intervals (30, 60, 120 and 240 min) after drug administration. The time of the experiments was kept constant between 9 and 11 a.m. Tissue serotonin were done by the method of Snyder *et al.* [14]. The rate of accumulation and the rate of decline of serotonin, after administration of pargyline (75 mg/kg i.p.) and *p*-chlorophenylalanine (PCPA, 316 mg/kg i.p.), respectively, were done with the help of the methods adopted by Neff *et al.* [15] and Neff and Tozer [16]. For studying the rate of accumulation, PGE_1 was administered along with pargyline and the animals were sacrificed after 30, 60 and 90 min whereas for the study of rate of decline, PGE_1 was administered 6 hr after PCPA and animals were sacrificed at 6, 7, 8, 9 and 10 hr after PCPA treatment. Statistical significance was done by Student's *t* test.

The choice of the dose and route of administration of PGE_1 was based on earlier reports from this laboratory [8, 9, 11].

RESULTS

The results are summarised in Tables 1 and 2. After administration of PGE_1, the brain serotonin level was maximally increased within 60 min and tended to normalise within 240 min. However, serotonin levels of both stomach and intestine were not significantly affected. PGE_1 increased only the rate of accumulation of brain serotonin but enhanced both rates of accumulation and decline of stomach serotonin. There was no significant effect on either the rate of accumulation or rate of decline of intestinal serotonin.

DISCUSSION

The results indicate that serotonin metabolism is strongly affected by PGE_1 in both brain and stomach of rat but not in the intestine. There was a marked increase in brain serotonin level after PGE_1 administration, an effect not noted in either stomach or intestine. However, when the results are analysed after pargyline and PCPA treatments, it is apparent that PGE_1 markedly accelerated both the rate of accumulation (4-fold) and rate of decline (1.5-fold) of serotonin in the stomach whereas only the rate of accumulation was increased in the brain (1-fold) with no appreciable change in rate of decline. There was no significant effect on either rates in the intestine.

Our results support the observations of Haubrich *et al.* [13], who have shown that the sedative effect of PGE_1,

Table 1. Effect of PGE_1 (0.4 mg/kg s.c) on serotonin levels in brain, stomach and intestine of Wistar albino rat

Time (min)	Serotonin μg/g wet tissue		
	Brain	Stomach	Intestine
0	0.41 ± 0.07	3.65 ± 0.36	2.07 ± 0.18
30	0.57 ± 0.06	4.01 ± 0.42	1.84 ± 0.22
60	1.20 ± 0.15†	3.36 + 0.31	2.42 ± 0.24
120	0.73 ± 0.08*	3.76 ± 0.19	2.53 ± 0.31
240	0.36 ± 0.08	3.34 ± 0.21	2.90 ± 0.36

Results expressed as Mean ± S.E.M. of five different determinations.
$n = 5$ in each group.
* and † indicate statistical significance in comparison to 0 min level as $P < 0.05$ and $P < 0.01$ respectively.