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INTERACTION OF ETHANOL WITH ACETAMINOPHEN METABOLISM IN THE BABOON

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Abstract—To evaluate the effects of ethanol on acetaminophen metabolism and toxicity, twelve female baboons were studied using three experimental designs. In the first one, animals fed ethanol chronically and their pair-fed controls received acetaminophen intravenously (40 mg/kg), and drug metabolism was studied for 6 hr in blood and urine. Elimination of acetaminophen from plasma was accelerated significantly in baboons fed alcohol chronically, and urinary excretion of mercapturic acid conjugate was increased. In the second, the experiments were repeated with the addition of ethanol infusion (120–160 mg/kg/hr). During ethanol infusion, elimination of acetaminophen from plasma was still accelerated significantly in baboons fed alcohol chronically. In the third experimental design, four pairs of baboons fed an alcohol or an isocaloric control liquid diet received, in addition, acetaminophen (85 mg/kg/day) in their respective liquid diets for 2 weeks. Liver histology was studied before and after acetaminophen feeding; SGPT, SGOT, SGDH, acetaminophen blood levels and acetaminophen urinary metabolites were also assessed. No morphological or functional liver alterations were found after chronic acetaminophen treatment, and urinary excretion of mercapturic acid conjugate was not increased in baboons fed alcohol chronically. Thus, our results in primates confirm that chronic ethanol consumption increases, whereas acute ethanol administration decreases, the excretion of mercapturic acid conjugate. When acute and chronic ethanol administration were combined, the effects tended to cancel each other out. A dose of acetaminophen which maintained blood levels similar to those recommended for humans did not produce deleterious effects in baboons drinking alcohol.

There have been a number of case reports suggesting that alcoholics may have increased susceptibility to the hepatotoxicity of an overdose of acetaminophen [1–3]. Furthermore, in rats, our group [4, 5] and others [6] have shown that chronic alcohol consumption results in increased hepatotoxicity of acetaminophen overdosage. The question still remained, however, whether in amounts recommended therapeutically in man hepatotoxicity is produced by acetaminophen when it is associated with chronic alcohol consumption. This question is particularly relevant in view of the observation that unlike chronic pretreatment with ethanol, which exacerbates acetaminophen toxicity [5], the presence of ethanol may, in fact, have the opposite effect [7]. The present study was undertaken to determine whether in subhuman primates, an amount of acetaminophen that results in blood levels therapeutically recommended in man produces untoward liver effects when associated with chronic and/or acute ethanol administration.

MATERIALS AND METHODS

Materials

Acetaminophen (*N*-acetyl-*p*-aminophenol) was obtained from the Sigma Chemical Co. (St. Louis, MO). All other chemicals used represented the best available commercial grades.

Animals

Twelve baboons (females) were pair-fed either an ethanol-containing (50% of total calories) liquid diet or a diet in which ethanol was replaced isocalorically by carbohydrate [8] for a period of 18 months (four pairs) and 5 years (two pairs). In addition, two chow-fed baboons were studied. The animals and feeding procedures were as described elsewhere [8].

EXPERIMENTAL DESIGN

Kinetics of acetaminophen elimination

Effect of chronic ethanol consumption on elimination of a single dose of acetaminophen. On the eve of each experiment, the baboons were fasted at 4:00 p.m. to allow for the elimination of ethanol. The experiments were started at 8:00 a.m. preceded by slight ketamine anesthesia during which a urinary catheter and two indwelling intravenous catheters were inserted, one for infusion and the other for blood sampling. During the experiments, a 5% dextrose solution was infused to meet the caloric needs of the animals. A saline solution of acetaminophen (40 mg/kg) was infused after ketamine anesthesia. Blood samples were taken every 30 min, and urine was collected for 6 hr to evaluate acetaminophen blood levels and acetaminophen urinary excretion.

Effect of an acute dose of ethanol administration, following chronic ethanol consumption, on elim-

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ination of a single dose of acetaminophen. In the second experimental design, the same pair-fed baboons were used after an interval of 2–3 months; ethanol was infused 30 min before the acetaminophen injection at a dose of 1.5 g/kg. After acetaminophen injection (40 mg/kg), ethanol infusion was maintained with a Harvard apparatus infusion pump, model 975, at a constant rate of 120–160 mg/kg/hr [9]. Remaining procedures were the same as in the first experimental design. Blood samples were taken at 60-min intervals into 0.6 N perchloric acid [10] for blood ethanol determination by head space gas chromatography. The same experiments were also carried out in two chow-fed baboons, with, in addition, evaluation of renal function, body temperature, heart rate and blood pressure.

Effect of 2 weeks of acetaminophen feeding

Four pairs of animals were used in each group; one baboon was fed a liquid diet containing ethanol (50% of total calories) for 18 months (ethanol-fed baboon) whereas the pair-fed control received the same amount of a diet in which ethanol was replaced isocalorically by carbohydrate (pair-fed baboons as previously described) [8]. The alcohol intake was about 4.6 ± 1 g/kg/day, resulting in blood alcohol levels of about 40 mM. These animals had a negative history of recent drug/xenobiotic exposure except for ethanol consumption as stated above. Acetaminophen (85 mg/kg/day) was administered daily in the liquid diet for 2 weeks. The diet was given in excess to the alcohol-fed animals and, therefore, was available throughout the entire study period; the control animals received an amount of diet equal to the quantity consumed by the alcohol-fed animals the day before. In these experiments animals with approximately the same weight were chosen, and they consumed about 800 ml of their respective diets. The amount of drug consumed every day by the animals was calculated by measuring the liquid diet consumed every day and was the same in the two groups of animals. The stability and availability of the drug were shown to be adequate by measurement of acetaminophen in the diet [by high performance liquid chromatography (HPLC)] and by measurement of blood acetaminophen levels. In all animals, blood samples for acetaminophen determination were taken on days 3, 7 and 14 of the experiment, at 9:00 a.m., after withdrawal of the diet for 1 hr. Urines were collected over dry ice, and the pH values, when checked, were found to be higher than 4. No significant difference was found in urine volumes between the two groups of animals. Needle liver biopsies were taken at the beginning and at the end of each experiment. Two pairs of animals underwent a liver biopsy after an overnight fast and two after their regular feeding. Acetaminophen was administered continuously in all. For light microscopy, paraffin sections were prepared after fixation of a portion of the biopsy with 10% buffered formalin and staining with hematoxylin and eosin. For electron microscopy a fragment of liver biopsy was immersed in 2.5% glutaraldehyde in cacodylate buffer, pH 7.4, followed by fixation in 2% osmium tetroxide. After dehydration the specimen was embedded in Epon 812. Acetaminophen (in blood and urine) and its urinary metabolites were separated by HPLC according to Howie *et al.* [11] using a Hewlett Packard 1048 B liquid chromatograph, with a Bondapak C18 column. The acetaminophen conjugates were identified according to Buckpitt *et al.* [12]. Serum glutamic oxaloacetic (SGOT) activity was measured according to Karmen [13]; serum glutamate dehydrogenase (SGDH) was assessed by the method of Ellis and Goldberg [14]; serum glutamic pyruvic transaminase (SGPT) was measured according to Wróblewski and LaDue [15].

Statistical analysis

All measurements were done at least in duplicate and expressed as the mean ± standard error of the mean (S.E.M.). Differences were analyzed by Student's *t*-test unless otherwise stated [16].

RESULTS

Kinetics of acetaminophen elimination: Effect of chronic and acute ethanol administration on the elimination of a single dose of acetaminophen

In baboons fed alcohol chronically, the plasma disappearance of acetaminophen was faster than in pair-fed controls (Fig. 1 and Table 1). Acetaminophen half-life was significantly shorter in the alcohol-fed group than in controls (Table 1). Ethanol infusion resulted in blood ethanol levels of 20–30 mM. During the ethanol infusion, the differences

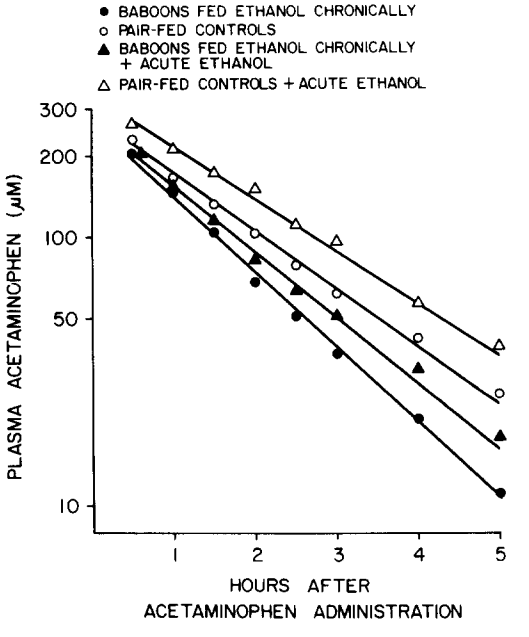

Fig. 1. Elimination of acetaminophen from plasma after i.v. injection of 40 mg/kg of the drug in baboons fed alcohol chronically (●) and pair-fed controls (○). In another experiment, the same baboons fed alcohol chronically (▲) and pair-fed controls (△) received 1.5 g/kg of ethanol i.v., 30 min before acetaminophen injection (40 mg/kg), and the ethanol infusion was continued during the experiment at the rate of 120–160 mg/kg/hr. Blood samples were taken at 30-min intervals for acetaminophen determination by HPLC. Values are means ± S.E.M. for six animals. Slopes were calculated by linear regression.

Table 1. Kinetics of metabolism of acetaminophen (40 mg/kg i.v.) in baboons fed ethanol chronically, with or without acute ethanol administration*

	Plasma disappearance (K/hr)		Renal clearance [(ml/min)/kg]		Acetaminophen half-life (min)	
	Ethanol	Saline	Ethanol	Saline	Ethanol	Saline
Ethanol-fed Baboons (N = 6)	0.50 ± 0.04	0.65 ± 0.04	0.92 ± 0.27	1.15 ± 0.1	81.7 ± 8.2	65 ± 4.6
Pair-fed Controls (N = 6)	0.42 ± 0.02	0.50 ± 0.05	0.61 ± 0.1	0.74 ± 0.1	98.5 ± 4.2	89 ± 6.8
P	<0.05	<0.05	NS	NS	<0.05	<0.01

* Blood samples were taken at 30-min intervals for 6 hr after acetaminophen injection, and urine was collected over the same period. Free acetaminophen in blood and urines was determined by HPLC as described in Experimental Design. The experiment was carried out with saline or ethanol infusions (120–160 mg/kg/hr).

between the alcohol-fed and control animals persisted, though in both groups the disappearance of acetaminophen was slowed. Ethanol infusion significantly increased acetaminophen half-life in both groups (Table 1). Thus, in the presence of ethanol, plasma disappearance of acetaminophen in baboons fed alcohol chronically was almost the same as in the controls which received 40 mg/kg of acetaminophen i.v. without ethanol infusion.

No difference was observed in the volume of distribution of the drug between baboons fed ethanol chronically and their pair-fed controls, either in the presence of an acute dose of ethanol (0.86 ± 0.14 vs 0.81 ± 0.12 l/kg, NS) or in its absence (0.88 ± 0.11 vs 0.83 ± 0.15 l/kg, NS); plasma clearance of the drug was increased in baboons fed ethanol chronically compared to pair-fed controls either in the presence of the acute ethanol (0.43 ± 0.05 vs 0.33 ± 0.03 l/hr/kg, $P<0.05$) or in its absence (0.57 ± 0.04 vs 0.41 ± 0.05 l/hr/kg, $P<0.05$).

The urinary excretion of acetaminophen and its metabolites was studied for 6 hr after the drug administration, and the results are expressed as percentage of injected dose. In the first experiment (effect of chronic ethanol feeding), the total excretion of acetaminophen and its conjugates, over 6 hr, was 66.0 ± 6% of injected dose in alcohol-fed animals and 66.7 ± 3% in the control group. No significant difference was found between the two groups of animals for total acetaminophen, free acetaminophen, or its sulfate or glucuronide conjugate, while the excretion of the acetaminophen mercapturate conjugate was significantly greater in baboons fed ethanol chronically than in controls (1.2 ± 0.20 vs 0.3 ± 0.08% of injected dose, $P<0.05$) (Fig. 2).

In the second experiment, when ethanol was infused 30 min before the drug administration, the urinary recovery of total acetaminophen was 65.5 ± 8% in alcohol-fed animals and 68.2 ± 8% of the injected dose in control animals; this difference was not statistically significant. No significant difference was found between the two groups of animals in the urinary excretion of unmetabolized, or the sulfate or glucuronide conjugate of acetaminophen as well as of the acetoaminophen mercapturate conjugate although the latter metabolite appeared to be

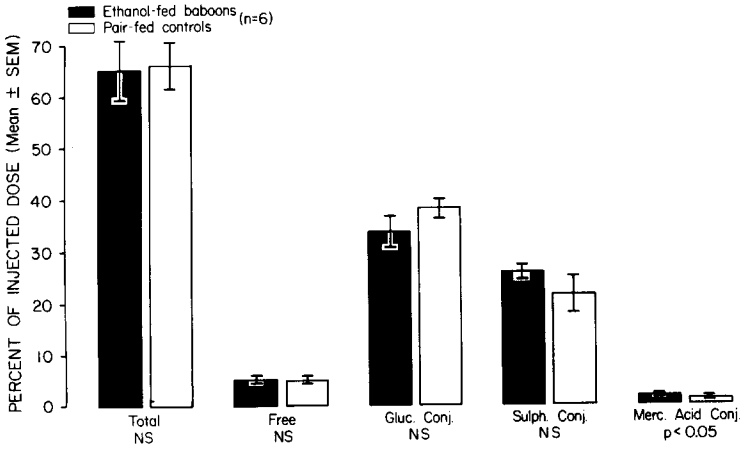

Fig. 2. Effect of chronic ethanol consumption on the urinary excretion of a single dose of acetaminophen. Baboons were paid fed diets containing 50% of total energy as either ethanol or isocaloric carbohydrate for a period of 18 months (four pairs) and 5 years (two pairs). On the eve of each experiment the animals were fasted at 4:00 p.m. to allow for the elimination of ethanol. The experiments were started at 8:00 a.m. Urines were collected for 6 hr. Acetaminophen and its metabolites were determined by HPLC as described in Experimental Design. Values are means ± S.E.M. for the six animals.

higher in the alcohol group when compared to its control group (Fig. 3). When ethanol was infused during the experiment, the urinary excretion of the acetaminophen mercapturate conjugate in both groups was significantly lower than in the experiment without ethanol infusion (0.2 ± 0.07 vs 1.2 ± 0.20 in alcohol-fed baboons, $P<0.05$, and 0.15 ± 0.04 vs 0.3 ± 0.08% of injected dose in control animals, $P<0.05$, in the presence or absence of the acute ethanol respectively) (Fig. 4).

In some experiments the urine was collected for 24 hr in order to evaluate possible differences between the rates of mercapturic acid excretion in alcohol-fed animals and pair-fed controls, both in the presence and absence of acute ethanol infusion. Under these conditions, drug recovery in the urines was about 85–90% of the injected dose in both alcohol and control groups, and the urinary mercapturate content (as a percentage of total urinary metabolites) was comparable to that observed in the 6-hr collection period; the percentage of this metabolite after the 24-hr collection period was: 1.1 ± 0.30 and 0.4 ± 0.10 in baboons fed ethanol chronically, and 0.5 ± 0.10 and 0.2 ± 0.07 in pair-fed controls, in the absence or presence of the acute ethanol respectively.

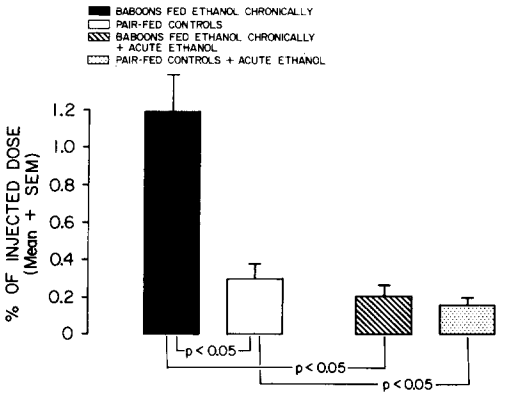

Fig. 4. Effects of chronic ethanol feeding on the urinary excretion of the mercapturic acid conjugate of acetaminophen during the 6 hr following the injection of acetaminophen (40 mg/kg, i.v.) with and without ethanol infusion (120–160 mg/kg/hr). Values are means ± S.E.M. for six animals.

Effect of 2 weeks acetaminophen administration

When acetaminophen was given orally at a dose of 85 mg/kg/day for 2 weeks, plasma levels of the drug, measured at days 3, 7 and 14 of the experiment, ranged between 10 and 20 μM (Fig. 5), concentrations similar to those used therapeutically in man [17].

Before acetaminophen treatment all control baboons, whether fasted or fed, had normal liver morphology by light microscopy. The alcohol-fed animals, however, showed various degrees of fat accumulation. As previously described [18], small and large fat droplets replaced the cytoplasm of numerous hepatocytes, predominantly in the perivenular area. No difference was seen between the fasted and fed animals. In three out of six alcohol-fed baboons, besides the fat accumulation, early perivenular and pericellular fibrosis was present, as previously reported [19]. After acetaminophen treatment, all control baboons, fasted or fed, remained normal, and no further changes were observed in the alcohol animals, again fasted or fed. By electron microscopy, the lesions previously described [20], such as proliferation of the endoplasmic reticulum and giant mitochondria, were present in all alcohol-fed animals when compared to the pair-fed controls. After 2 weeks of acetaminophen the liver ultrastructure of the control treated animals remained

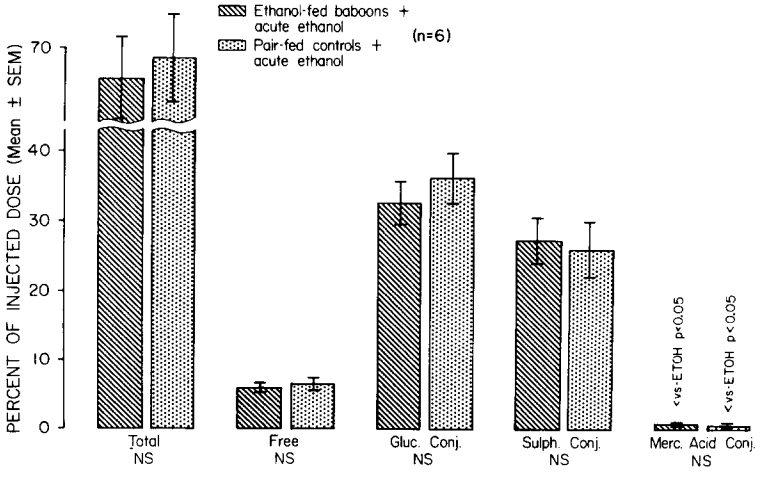

Fig. 3. Effects of chronic and acute ethanol administration on the urinary excretion of a single dose of acetaminophen. Animals were treated as indicated in the legend of Fig. 2. An acute dose of ethanol (1.5 g/kg) was infused i.v. 30 min before acetaminophen injection (40 mg/kg). After the drug administration, ethanol infusion was maintained at a constant rate of 120–160 mg/kg/hr. Urines were collected for 6 hr. The P values above the mercapturic acid conjugate refer to the difference in the excretion of this metabolite when compared to Fig. 2. Acetaminophen and its metabolites were determined by HPLC as described in Experimental Design. Values are means ± S.E.M. for six animals.

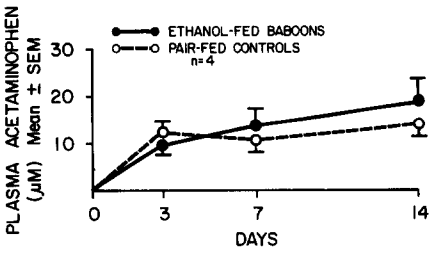

Fig. 5. Blood acetaminophen concentration during chronic intake of the drug (85 mg/kg/day). Acetaminophen was measured in blood by HPLC on days 3, 7 and 14 of the experiment. Values are means ± S.E.M. for four animals.

normal, and no further lesions were present in the alcohol-fed animals. The presence of abundant glycogen differentiated the fed from the fasted baboons. GPT, GOT, and GDH serum activities were studied before acetaminophen treatment and on days 3, 7 and 14 of the experiment. No difference was found in the alcohol-fed animals and pair-fed controls before and during all experimental periods. The urinary excretion of free acetaminophen, and its glucuronide and sulfate conjugates, studied at days 3, 7 and 14 of the experiment in 24 hr urines, did not show any difference between baboons fed alcohol chronically and their pair-fed controls; even the urinary excretion of the acetaminophen mercapturate conjugate was not significantly different in alcohol animals when compared to controls (0.6 ± 0.1 vs 1.2 ± 0.6% of injected dose respectively, NS).

The experiments on two chow-fed baboons confirmed the results obtained in baboons fed the control liquid diet; furthermore, they did not show any alteration of renal function, body temperature or blood pressure during all the experimental manipulations.

DISCUSSION

This study shows the interactions between chronic and/or acute ethanol administration and acetaminophen disposition in the baboon. It was found that chronic intake of acetaminophen in amounts that maintained the blood concentration at levels similar to those used therapeutically in humans did not produce deleterious effects in baboons fed ethanol chronically.

In the acute acetaminophen study, plasma disappearance of the drug (given i.v.) was faster in baboons fed ethanol chronically than in pair-fed controls; when an acute dose of ethanol was infused in both ethanol-fed baboons and controls, we observed again a faster plasma disappearance of acetaminophen in baboons fed ethanol chronically than in their pair-fed controls, even though the acute ethanol administration resulted in a decrease in the rate of the drug elimination in both ethanol and control animals.

The excretion of the mercapturic acid conjugate of acetaminophen was found to be increased significantly in baboons fed ethanol chronically when compared to their pair-fed controls, but the difference was not significant when an acute dose of ethanol was injected, most likely because the acutely administered ethanol competed with acetaminophen metabolism in the microsomes.

The interaction of acetaminophen metabolism with ethanol or other drugs has been extensively studied recently. Wong *et al.* [21] have shown in mice that a single dose of ethanol protects against acetaminophen-induced hepatotoxicity as assessed by plasma transaminases. Our group demonstrated that chronic ethanol feeding in rats enhances the hepatotoxicity of acetaminophen [22] and the acute ethanol administration in rats fed alcohol chronically decreases acetaminophen-induced hepatotoxicity [23].

Other studies have suggested that L-ascorbic acid protects, in mice and hamsters, against acetaminophen-induced hepatotoxicity by the inhibition of covalent binding of acetaminophen metabolites to hepatic microsomes [24]. It has also been reported that propylthiouracil protects against acetaminophen hepatotoxicity in rats and mice [25, 26] most likely through its direct binding of the reactive metabolite of acetaminophen. Mitchell *et al.* [27] observed that cimetidine inhibits the activities of microsomal enzymes, thereby also protecting against acetaminophen hepatotoxicity. Our group has shown previously that ethanol itself is metabolized by a microsomal cytochrome P-450 containing enzyme system [28–30] and that ethanol inhibits drug oxidation *in vivo* and *in vitro* [31, 32], that chronic alcohol consumption increases covalent binding of reactive metabolite(s) of acetaminophen in rats [5], and that acute ethanol inhibits the production of reactive metabolite(s) of acetaminophen in rats [7]. The interactions of acute and/or chronic ethanol administration with acetaminophen metabolism were investigated in the present study in the baboon. When acetaminophen was given i.v. at a dose of 40 mg/kg, the rate of plasma disappearance of the drug was increased in baboons fed alcohol chronically when compared to pair-fed controls. This was most likely due to accelerated metabolism. This finding was supported by an increased excretion of mercapturic acid conjugate in urines. When acute ethanol was perfused during the experiment, the rate of plasma disappearance of acetaminophen decreased in baboons fed alcohol chronically, but it was still faster than in pair-fed controls. Also the urinary excretion of mercapturic acid conjugate decreased when ethanol was infused but still remained higher than in pair-fed controls. Since urinary excretion of the mercapturic acid conjugate is a commonly used variable for the production of reactive metabolite(s) of acetaminophen *in vivo* [33], our data suggest that chronic consumption of alcohol increases the formation of reactive metabolite(s) of acetaminophen, whereas acute ethanol administration inhibits this formation both in baboons fed alcohol chronically and in pair-fed controls.

When acetaminophen was given orally for 2 weeks at a dose of 85 mg/kg/day, the plasma concentrations of the drug ranged between 10 and 20 μM, values similar to human therapeutic plasma concentrations of acetaminophen [17], and the urinary excretion of the mercapturic acid conjugate in baboons drinking alcohol was decreased when compared to pair-fed controls, confirming the results obtained during

ethanol infusion. At the dosage used, chronic acetaminophen intake did not produce deleterious effects as assessed by serum enzyme activities and light and electron microscopy both in control baboons and in animals drinking alcohol chronically.

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