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EFFECT OF TWO DIFFERENT TYPES OF MALNUTRITION ON THE RATE OF ELIMINATION OF ETHANOL IN RATS

VILMA A. DA SILVA* and ANDRE E. M. MCLEAN

Laboratory of Toxicology, Department of Clinical Pharmacology, The Rayne Institute, University College London, 5 University Street, London WC1E 6JJ, U.K., and *Instituto Biomedico, Universidade Federal Fluminense, Niteroi/RJ, Brasil

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Abstract—Rats were fed "3% casein" or a "calorie deficient" diet, in the form of commercial pellet diet (SDS) at 50% of the amount consumed by the control group, which was fed SDS pellets *ad libitum*. Both of the deficient groups showed failure of weight gain in comparison with the control group. Blood levels of ethanol were measured for 3 hr after intraperitoneal injection of 1 or 1.5 g/kg at 15, 29 and 36 days after commencement of the diet. In addition the calorie deficient group was studied immediately after feeding as well as in the fasting state. Blood levels of ethanol were measured and the apparent volume of distribution and rate of removal of ethanol from the blood were calculated. A rate of ethanol metabolism/g of liver was derived.

The rate of removal of ethanol was markedly decreased in the 3% casein group to less than half of control values. Three hours after injection of ethanol circulating levels were less than 50 mg/100 ml in the control and calorie deficient groups but over 200 mg/100 ml in the group fed protein deficient diets.

There were no major changes in volume of distribution and the only explanation for the finding is that there is a failure of ethanol metabolism in the rats fed the low protein diet.

The implication is that protein deficient human populations who often consume considerable quantities of ethanol may have a high level of tissue exposure to ethanol though the rate of metabolite formation may be low.

Malnutrition is often found in association with high ethanol consumption. Sometimes it is a consequence of chronic ethanol intake, while in other cases, chronic malnutrition is present even before ethanol consumption starts. Surveys on alcoholism and alcohol effects in underdeveloped countries have shown high percentages of ethanol consumption among malnourished individuals [1]. In these counties malnutrition is a chronic problem, starting even before birth.

Malnutrition is thought to be a risk factor for the toxic effects of ethanol [2, 3]. Understanding ethanol pharmacokinetics in malnourished individuals is of importance if we are to interpret the mechanisms of interaction between nutritional status and ethanol effects. Malnutrition is most commonly observed as a consequence of reduced calorie intake (i.e. food deficiency as seen in famine conditions), or else following intake of a diet deficient in protein. These two form the extremes of the spectrum of protein-energy deficiency. Many studies have been carried out on ethanol pharmacokinetics in animals fed nutritionally imbalanced diets [4–9], but the results are often contradictory or inconclusive [4]. Often the type of malnutrition was not clearly defined or "protein deficiency" studies were undertaken with diets containing 12% or more protein [5] while 12% of protein is adequate for growth in rats [10, 11].

In other studies the results are expressed in units which do not inform us of the effects on the whole animals since ethanol metabolism *in vitro* can only be interpreted if liver weight and protein content are taken into account [4]. Some *in vitro* studies, using liver slices from rats fed a protein-deficient diet, have shown that ethanol metabolism/mg of fresh liver is reduced when compared to slices from well nourished controls [6]. Fasting and severe food restriction, leading to loss of 25% of body weight in 9 days, were also associated with a decrease of ethanol metabolism [7]. In other cases no effects of malnutrition on ethanol metabolism are reported. Allen *et al.* [9] studying blood ethanol elimination rates in mice with different body weights concluded that ethanol elimination rates were not affected by nutritional state. Vitale *et al.* [8] measured the rate of alcohol oxidation in food-deprived rats. Excretion of $^{14}CO_2$ in the expired air after administration of labelled alcohol was used as the index of alcohol oxidation. The authors suggested that the rate of alcohol oxidation decreases as the animals lose weight, but when they attain a stable weight the rate of alcohol metabolism returns to near normal. It is possible that differences of method of measuring alcohol elimination as well as the degree and type of malnutrition in the different studies could explain the discrepancies among the authors. We have studied blood levels of ethanol after a single injection of ethanol in control rats and two groups of rats with reduced body weights, arising from different models of malnutrition. We used a 3% casein diet fed *ad libitum* (previously used in studies of drug metabolism in this laboratory [12]) and also a calorie-deficient group whose food intake was restricted to 50% of daily food intake of the control group. In the 50% "calorie deficient" group ethanol elimination rate was studied both in the fasting state (20 hr after feed) and in the absorptive phase 2 hr

after feeding. Results were compared to a control group fed a commercial diet containing 14% protein and fed *ad libitum*.

MATERIALS AND METHODS

Fifteen male Wistar rats (body weight starting at 160–180 g) were allocated to single cages and divided in three experimental groups of five animals each, submitted to different diets:

SDS diet. SDS is a commercial diet (Special Diets Services Ltd, Witham, Essex, U.K., for maintenance of rats, mice and hamsters). This diet was offered *ad libitum* to a group of rats (group "SDS") and in restricted amounts (50% of the weight of diet eaten by the group fed *ad libitum* in the previous day) to another group (group "Calorie Deficient").

3% Casein. A diet containing 3% of protein (casein) previously used in this laboratory and published by McLean and Day [9] was offered *ad libitum* to a third group of rats.

Ethanol (1 or 1.5 g/kg) was injected intraperitoneally as a 10% v/v solution when animals had been on diet for 15, 29 and 36 days. In order to control feeding status at the time of ethanol injection, the calorie deficient group was injected 2 hr after feeding. One week after the last ethanol blood levels measurement (36 days) this group was injected before feeding (20 hr fasting). Blood samples (50 μl) were collected from the tail vein into heparinized capillary tubes at 30, 60, 90, 120 and 180 min after injection (day 36/42 or fewer times on days 15 and 29). Blood ethanol concentrations were determined with a Hewlett-Packard 573A gas-liquid chromatograph connected to a C-R 1B Shimadzu integrator. The glass columns were packed with 20% Carbowax 1500. The 50 μl sample of tail blood was put into a sealed 20 ml glass vial containing 50 μl of 50 mg per ml of propan-1-ol in saline as internal standard. The vial was incubated at 28° for at least 60 min and 100 μl head space samples were obtained from each vial for analysis. Standard curves were constructed from ethanol containing standards made up with blood. The peak height obtained for ethanol at the concentration of 50 mg/100 ml was equal to the peak height for the internal standard, and was taken as the limit of reliable measurement. The coefficient of variation for 80 mg/100 ml sample measured on different days was 12%.

Body weight and food consumption were assessed during the experiment. Livers were obtained after ethanol elimination rate studies and weighed. Ethanol elimination rate was calculated from the straight portion of the curve of concentration vs time. The rate of metabolism/g of liver was calculated from the volume of distribution and rate of elimination to give a rate of metabolism/100 g body weight and this was divided by the liver weight/100 g body weight to give a rate of ethanol metabolism/g of liver as calculated by Widmark's formula according to the procedure of Wilkinson [13].

Statistical analysis. Comparisons of the three groups were done by ANOVA followed by the Student's *t*-test. Ratios were compared by using the Kruskal Wallis test followed by the Mann Whitney test when $P < 0.05$. Analysis of data from the calorie-deficient group in the fed and fasted state was done by the Student's *t*-test for paired data or the Wilcoxon test.

RESULTS

Table 1 shows body weight throughout the experiment. Rats fed *ad libitum* with the SDS diet gained weight continuously, while both malnourished groups failed to grow normally and stayed at almost constant body weight, that is, the two malnourished groups were similar in terms of body weight. Food intake (mean per animal per day) during the experiment was 26 g (SDS); 13 g (calorie deficient) and 20 g (3% casein). Protein ingestion per day was estimated at 0.6 g (3% casein), 1.8 g (calorie deficient) and 3.6 g (SDS *ad libitum*). Liver weights were decreased in both malnourished groups when compared to SDS *ad libitum* (Table 1), but when liver weight was expressed as percentage of body weight, there were no differences between the 3% casein and "SDS *ad libitum*" groups, but the calorie-deficient group had a decreased ratio of liver to body weight.

The rate of ethanol elimination in the 3% casein group was far lower when compared to the SDS or calorie deficient groups after 36 days of feeding (Fig. 1, Table 2). Similar results were found when ethanol was injected after 15 and 29 days on diet (Table 3). Ethanol blood levels 180 min after the injection were still around 200 mg/100 ml in the protein deprived group but down to 50 mg/100 ml in the other groups. No significant differences were found between the SDS *ad libitum* group and the calorie deficient group, either in the "fed" or "fasting" state. Table 2 shows the effects of the different diets on the rate of ethanol

Table 1. Effects of food restriction and protein deficient diet on body weight and hepatic weight (mean ± SD)

Group	Days on diet: 1*	8	16	29	36	46	Liver weight (g)	Liver wt/body wt (g/100 g)
	Body weight (g)							
SDS	177 ± 7	228 ± 16	285 ± 22	330 ± 29	301 ± 38	373 ± 40	13.6 ± 1.7	3.6 ± 0.1
Calorie deficient (fasting)	175 ± 1	174 ± 10	182 ± 21	196 ± 14	202 ± 13	204 ± 17	5.9 ± 0.3	2.9[a] ± 0.1
3% Casein	175 ± 4	169 ± 7	175 ± 10	184 ± 11	183 ± 10	181 ± 14	6.5 ± 1.1	3.6 ± 0.5

* Day 1: Starting day.

[a] $P < 0.05$ when compared to SDS or 3% casein groups.

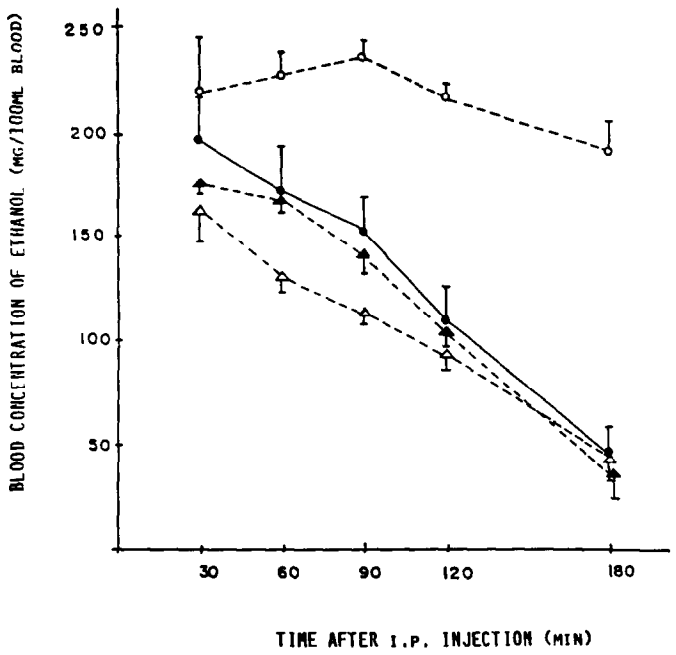

Fig. 1. Ethanol blood levels (mg/100 ml) after a 1.5 g/kg i.p. injection. Blood samples were collected from the tail at 30, 60, 90, 120 and 180 min after the injection. Three groups of rats submitted to different diets are represented by: SDS (●); 3% casein (○); calorie-deficient fed (▲), fasted (△). Groups SDS, 3% casein and calorie-deficient fed were injected after 36 days on diet. Group calorie-deficient fasted was injected after 43 days of daily food restriction. Means and standard errors for 5 rats per group.

elimination. Once again it is evident that ethanol elimination decreased in the 3% casein group. The volume of distribution increased and rate of change of ethanol concentration decreased in the calorie-deficient group injected in the fasting state. However, the rate of ethanol metabolism was not different when this group was compared to the group SDS fed *ad libitum*.

DISCUSSION

Our data indicate that ethanol elimination in malnourished animals can be affected in different ways which depend on the model of experimental malnutrition utilized. The calorie-deficient rats had a decreased liver weight to body weight ratio when compared to controls. However, ethanol elimination rates were not different when calorie deficient and *ad libitum* fed rats were compared. This finding suggests that in calorie-deficient rats there must be an increase in enzymatic activity per gram of liver which tends to compensate for the decrease of liver weight. Similar results were reported by Nakajima and Sato [14] for liver enzymes involved in the metabolism of aromatic and chlorinated hydrocarbons. They found a marked loss of liver weight in fasted rats but the total liver enzyme activity was still significantly higher than that measured in fed rats.

Table 2. Effect of diets fed for 36 days on the metabolism of ethanol after 1.5 g/kg dose

Groups	Conc. t_0[a] (mg/100 ml blood)	Vol. of dist.[b] (ml/g)	Rate of elimination[c] (mg/100 ml blood/hr)	Rate of[d] ethanol metabolism (mg/hr/g liver)
SDS	256 ± 52	0.61 ± 0.15	70 ± 11	11.5 ± 1.6
Calorie deficient (fed)	245 ± 15	0.61 ± 0.04	69 ± 5	—
Calorie deficient (fasting)	186 ± 12[e]	0.81 ± 0.05[e]	47 ± 9[e]	13.1 ± 1.1
3% Casein	279 ± 9	0.54 ± 0.02	29 ± 12[f]	4.5 ± 2.0[f]

Values are mean ± SD (N = 5).

[a] Conc. t_0 was measured by extrapolation back to 0 time, using data points from 90 to 180 min.

[b] Volume of distribution was measured as the dose divided by the concentration at t_0.

[c] Rate of elimination: rate of change of ethanol concentration. Rate of elimination was calculated from the straight part of the curve by taking points from 90 to 180 min.

[d] Rate of metabolism is corrected for liver weight by multiplying columns b × c to give the quantity of ethanol metabolised per 100 g body weight per hour and dividing the values by the liver weight per 100 g body weight, to give a calculated rate of ethanol metabolism by the liver.

[e] $P < 0.05$ when compared to SDS 50% after feeding.

[f] $P < 0.05$ when compared to SDS and SDS 50% fasting.

Table 3. Effect of deficient diets fed for 15 and 29 days on metabolism of ethanol

Group	15 days on diet ethanol 1.0 g/kg		29 days on diet ethanol 1.5 g/kg	
	(ethanol mg/100 ml blood)			
	30 min	60 min	30 min	60 min
SDS	98 ± 10	65 ± 13	207 ± 27	144 ± 38
Calorie deficient (fed)	104 ± 14	64 ± 11	237 ± 15	184 ± 12
3% Casein	128 ± 16	113 ± 13	242 ± 26	245 ± 33

Mean ± SD for N = 5 animals/group.
Rats were fed the diets as described in the section on methods and injected with 1 g/kg or 1.5 g/kg of ethanol.

In protein-deprived rats a different condition was observed. Despite the fact that liver weight expressed as a percentage of body weight did not differ from control animals, ethanol elimination was decreased significantly resulting in tissue exposure to high levels of ethanol for a much more prolonged time. It is interesting to notice that these differences between the two models of malnutrition occurred in our experiment even when body weight was affected similarly. Our results for food-deprived rats are in disagreement with those reported by Lumeng *et al.* [7]. These authors reported a significant decrease in ethanol elimination rates both in food-deprived and fasted rats. One possible explanation for the discrepancy between their results and ours is the higher degree of food deprivation imposed by Lumeng *et al.* [7]. They studied the effects of 24, 48 and 72 hr fasting following a previous feeding schedule of *ad libitum* access to stock diets. The food restricted group was given 5 g food per day. In our experiment the calorie-deficient group ate their food early each day (13 g) and were therefore partly fasted every day when they had finished eating their diet. The severe food restriction imposed by Lumeng *et al.* may also have decreased the effective protein intake very drastically. If this was the case, then data obtained by Lumeng *et al.* should be compared to those obtained in our experiments for protein-deprived rats.

In summary, this experiment indicates that in calorie-deficient rats a metabolic adaptation may occur which compensates for the decrease in liver weight, resulting in rates of ethanol elimination similar to those found in controls fed *ad libitum*. However, this adaption does not occur if the diet is protein deficient to the extent that growth is inhibited.

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