Biochemical Pharmacology, Vol. 20, pp. 3269–3279. Pergamon Press, 1971. Printed in Great Britain

EFFECT OF CARBON TETRACHLORIDE TREATMENT ON ETHANOL METABOLISM

J. M. KHANNA, H. KALANT, G. LIN and G. O. BUSTOS

Department of Pharmacology, University of Toronto, Toronto 181, Ontario, Canada

(*Received* 14 *November* 1970; *accepted* 9 *April* 1971)

Abstract—Administration of a single dose (2·5 ml/kg body weight, p.o.) of carbon tetrachloride to rats was found to cause a marked decrease in activity of the hepatic microsomal ethanol oxidizing system (MEOS). As early as 6 hr after CCl_4 administration 50 per cent decrease of MEOS activity was observed; this decrease amounted to 58 and 63 per cent at 10 and 20 hr respectively. With identical CCl_4 treatment, there was no change in hepatic alcohol dehydrogenase activity. At 20 hr, when the reduction of MEOS was greatest, there was no significant effect of the CCl_4 on the rate of ethanol uptake by liver slices or on the rate of ethanol metabolism *in vivo* as measured in the whole body or as estimated from the rate of decrease of blood ethanol concentration. It is, therefore, suggested that MEOS does not play a significant role in ethanol metabolism *in vivo* in the rat.

HEPATIC alcohol dehydrogenase (ADH) is generally considered to be the principal enzyme responsible for oxidizing ethanol to acetaldehyde.[1–3] It has recently been found that hepatic microsomes can also catalyze the oxidation of ethanol to acetaldehyde *in vitro*.[4–8] This so-called microsomal ethanol oxidizing system (MEOS) has been claimed to have a potentially important role in ethanol metabolism *in vivo*,[5,9–11] but the results of several investigations are inconsistent with such a role.[12–15]

It is also well known that administration or exposure of animals to CCl_4 causes ultrastructural alterations of the endoplasmic reticulum, associated with a decreased activity of microsomal enzymes that catalyze the oxidation of drugs.[16–21] The administration of CCl_4 has been shown to decrease the metabolism of aminopyrine,[16,19] hexobarbital,[17,19] strychnine,[17] meprobamate,[17] ethylmorphine[20,21] and dimethylaniline.[18]

The present report deals with an investigation of the effect of CCl_4 on MEOS activity and on ethanol metabolism in liver slices and in the whole animal. The effect of CCl_4 on ADH activity was also studied under the same experimental conditions. It was hoped that this approach would shed further light on the relative roles of ADH and of MEOS in ethanol metabolism *in vivo*.

MATERIALS AND METHODS

Animals. Male Wistar rats of about 200–300 g body weight, obtained from Canadian Breeding Laboratories, were used in these experiments. The animals were allowed Purina chow and tap water *ad lib*. Food was withheld for 20 hr before sacrifice in all experiments, but water was available at all times.

Treatment. Carbon tetrachloride (2·5 ml/kg body wt.) was administered by gastric

tube. The control rats received an equal volume of water to balance the effect of stress due to intubation.

MEOS activity. The animals were killed by decapitation at 6, 10 or 20 hr after administration of CCl_4. The livers were quickly removed, weighed, washed and homogenized in 1·15% KCl solution. Microsomes were prepared by centrifugation at 105,000 *g* as described previously.[7] MEOS activity was determined as described by Lieber and DeCarli.[5] Protein content was determined by a biuret method slightly modified from the technique of Gornall, Bardawill and David, as described elsewhere.[22]

Liver alcohol dehydrogenase activity. The preparation of liver homogenates and assay of ADH activity were carried out as described previously.[23]

Ethanol metabolism in vivo. Ethanol metabolism was studied in CCl_4-treated and control rats, after intraperitoneal injection of a test dose (2·1–2·5 g/kg) of ethanol, given 15 hr after the CCl_4. Hourly samples of 0·05 ml of blood were taken from the tail of each animal for 5 hr after ethanol administration. Each sample was deproteinized and used for measurement of ethanol by the internal standard technique of gas–liquid chromatography.[24] The disappearance rates of blood ethanol (Widmark's β) were calculated from the slope of the linear descending portion of each curve, and the values of Widmark's C_0 and *r* factor as well as the rate of metabolism in milligrams per kilogram per hour were calculated as described previously.[14]

The disappearance of ethanol from the whole body was also studied in CCl_4-treated and control rats after intraperitoneal injection of a test dose (3 g/kg) of ethanol, given 17 hr after the CCl_4. Three hr after the ethanol injection, the rats were killed with a blow on the head, immediately dropped into a pre-cooled industrial Waring blendor and homogenized with 2000 ml of cold distilled water in a room at 4°. Two 50-ml portions of homogenate were centrifuged at 8000 *g* for 10 min and 1 ml of the supernatant fraction from each was used for analysis of ethanol as described previously. Recovery of ethanol added to homogenates was 100 per cent. The difference between the administered dose and the total recovered amount was used to calculate the mean hourly rate of ethanol oxidation over the 3-hr period.

Ethanol metabolism in vitro *by liver slices.* Rats were killed by decapitation. The livers were rapidly removed, weighed and chilled in ice. The tissue was cut into slices about 0·3 mm thick by the use of the recessed guide described by McIlwain.[25] Four liver slices weighing a total of 250–300 mg were transferred to each 50-ml ice-cooled Erlenmeyer flask, which contained 4 mg of ethanol in 5 ml of oxygenated Krebs–Ringer phosphate buffer (KRP), pH 7·4.[26] The flasks were flushed with oxygen again, stoppered, and immediately incubated at 37·5° in a shaking water bath for 30, 60, 90 and 120 min.

At the end of the respective incubation periods, the reaction was stopped by the addition of 0·5 ml of 50% trichloroacetic acid to each flask. After chilling, the contents of the flask were transferred to a cold centrifuge tube. To ensure complete transfer, the flask was washed three times with 2 ml of KRP. The combined contents and washings of each flask were then centrifuged for 10 min at 8000 *g* in a Servall RC-2B. The supernatant fraction was transferred to a 100-ml volumetric flask. The centrifuge tube was refilled with KRP and centrifuged again. The supernatants were combined and diluted to a total volume of 100 ml with KRP. One ml of the above dilution was used for alcohol measurement by the yeast alcohol dehydrogenase method as described

previously.[23] Flasks containing ethanol without liver slices, and others with liver slices but no ethanol, were treated as above and used for standard and blank determinations respectively. All analyses were carried out in duplicate.

Determination of body water. Rats of 250 g body wt. were divided into two groups. One group received 2·5 ml/kg CCl_4 by intubation, while the control group received an equivalent amount of water. Food was withdrawn after intubation but water was available *ad lib.* After 20 hr, the rats were killed with a blow on the head without bleeding. The fresh body weights were recorded to the nearest milligram. The rats were then placed in an oven at 105° until a constant dry weight was obtained.

In a separate experiment, the livers were removed from six controls and six animals treated with CCl_4 20 hr previously. Fresh and dry weights of the livers were measured, and the water contents calculated by difference.

RESULTS

Liver weight, microsomal protein and MEOS activity. Table 1 shows the effect of CCl_4, administered 20 hr earlier, on liver weight, microsomal protein and MEOS activity. Two separate experiments were performed. Pretreatment with CCl_4 produced a significant increase in liver weight and a significant decrease in the concentration of microsomal protein. There was an increase in liver weight of approximately 42 per cent in Experiment I and 53 per cent in Experiment II. A concomitant decrease in microsomal protein of approximately the same magnitude was also observed.

The MEOS activity, expressed as nanomoles of acetaldehyde produced per milligram of protein per minute, decreased by over 50 per cent ($P < 0{\cdot}001$) in CCl_4-treated rats, compared to controls. The decrease was even more pronounced, when MEOS activity was expressed per gram of liver (73 per cent in Experiment I, 78 per cent in Experiment II) or in total liver per 100 g body weight (61·4 per cent in Experiment I, 66 per cent in Experiment II). Somewhat less, but still highly significant, reduction of MEOS activity was observed as early as 6 and 10 hr after CCl_4 treatment (Table 2).

Liver alcohol dehydrogenase activity. The results on liver ADH activity are shown in Table 3. For determination of ADH activity, the animals were sacrificed 15–20 hr after administration of CCl_4. ADH activity per gram of liver decreased by approximately 25 per cent. However, treatment with CCl_4 increased the liver weight by about 40 per cent, so that total ADH was not reduced. The increase in liver weight is evidently attributable to rapid accumulation of non-protein constituents. Therefore expression of activity per gram of liver is not a true reflection of the rate of ethanol metabolism *in vivo*, and reliance should be based on activity calculated either per milligram of soluble protein or in total liver per 100 g body weight. Calculations on the latter two bases showed no significant differences between the two groups.

Ethanol metabolism in vivo. Figure 1 shows the curves for mean blood ethanol concentration after a test dose of ethanol in two separate experiments with controls and rats treated with CCl_4. As is evident from Fig. 1, the mean blood ethanol concentrations are lower in the treated animals than in the controls. In Fig. 1a, this was significant at 1 and 3 hr ($P < 0{\cdot}025$ and 0·02 respectively), whereas in Fig. 1b this was significant at 2 and 3 hr ($P < 0{\cdot}02$ and 0·05 respectively). In both experiments, the Widmark C_0 values[14] (blood alcohol concentration at zero time) were significantly lower in CCl_4-treated rats than in controls ($P < 0{\cdot}025$ in both experiments, Table 4).

TABLE 1. EFFECT OF CARBON TETRACHLORIDE TREATMENT 20 hr BEFORE SACRIFICE ON LIVER WT., MICROSOMAL PROTEIN AND ACTIVITY OF NADPH-DEPENDENT MICROSOMAL ETHANOL OXIDIZING SYSTEM (MEOS)*

	Body wt. (g)	No. of rats	Liver wt./body wt. (g/100 g)	Microsomal protein (mg/g liver)	MEOS activity (mμmoles acetaldehyde produced/min)		
					Per mg of microsomal protein	Per g of fresh liver	In total wt. of fresh liver per 100 g body wt.
Experiment I							
Controls	273 ± 5	7	3·30 ± 0·14	21·5 ± 0·9	4·6 ± 0·2	100 ± 7	329 ± 23
CCl_4-treated	292 ± 12	8	4·74 ± 0·02	12·9 ± 0·8	2·1 ± 0·1	27 ± 3	127 ± 8
P	NS		< 0·001	< 0·001	< 0·001	< 0·001	< 0·001
Experiment II							
Controls	222 ± 12	6	3·15 ± 0·09	24·6 ± 1·4	4·7 ± 0·1	115 ± 7	363 ± 24
CCl_4-treated	239 ± 4	7	4·90 ± 0·06	11·2 ± 0·6	2·2 ± 0·1	25 ± 1	123 ± 6
P	NS		< 0·001	< 0·001	< 0·001	< 0·001	< 0·001

* Results are expressed as mean ± standard error. P values are for the difference between control and CCl_4 group. NS = not significant ($P > 0·05$).

TABLE 2. EFFECT OF CARBON TETRACHLORIDE TREATMENT 6 AND 10 hr BEFORE SACRIFICE ON LIVER WT., MICROSOMAL PROTEIN AND ACTIVITY OF NADPH-DEPENDENT MICROSOMAL ETHANOL OXIDIZING SYSTEM (MEOS)*

	Body wt. (g)	No. of rats	Liver wt./body wt. (g/100 g)	Microsomal protein (mg/g liver)	MEOS activity (mμmoles acetaldehyde produced/min)		
					Per mg of microsomal protein	Per g of fresh liver	In total wt. of fresh liver per 100 g body wt.
Control	261 ± 34	4	3·18 ± 0·18	31·6 ± 2·6	4·5 ± 0·1	141 ± 13	443 ± 26
6 hr	214 ± 6	4	3·45 ± 0·08	24·8 ± 0·9	2·6 ± 0·2	65 ± 6	224 ± 16
P	NS		NS	< 0·05	< 0·001	< 0·005	< 0·001
10 hr	224 ± 1	4	3·86 ± 0·13	20·9 ± 0·9	2·3 ± 0·1	48 ± 1·0	186 ± 6
P	NS		< 0·025	< 0·01	< 0·001	< 0·001	< 0·001

* Results are expressed as mean ± standard error. P values are for the difference between control and CCl_4 group. NS = not significant ($P > 0·05$).

TABLE 3. EFFECT OF CARBON TETRACHLORIDE TREATMENT 15–20 hr BEFORE SACRIFICE ON HEPATIC ALCOHOL DEHYDROGENASE (ADH) ACTIVITY*

Treatment	No. of rats	Body wt. (g)	Liver wt./100 g body wt.	ADH activity (mμmoles of NADH formed/min) Per mg of soluble protein	Per g of fresh liver	In total wt. of fresh liver per 100 g body wt.
Controls	20	245 ± 15	3·26 ± 0·09	4·14 ± 0·45	653 ± 50	2127 ± 163
CCl_4-treated	20	245 ± 14	4·55 ± 0·15	3·54 ± 0·37	490 ± 40	2247 ± 217
P		NS	< 0·001	NS	< 0·02	NS

* Results are expressed as mean ± standard error. P values are for the difference between control and CCl_4 group. NS = not significant (P > 0·05).

In keeping with this, the Widmark *r* factor[14] (an approximate substitute for the volume of distribution of alcohol in the body) was significantly increased in CCl_4-treated rats compared to controls. Since the administration of CCl_4 apparently altered the distribution of ethanol (as indicated by these changes in C_0 and *r*) and the true post-equilibration values were not reached until the third hour, the disappearance rates of

TABLE 4. EFFECTS OF CARBON TETRACHLORIDE TREATMENT 15–20 hr BEFORE SACRIFICE ON C_0, *r* AND ETHANOL METABOLISM *in vivo**

Treatment	No. of rats	Body wt. (g)	C_0† (mg/100 ml)	*r*‡	β§ (mg/100 ml/hr)	Ethanol metabolized (mg/kg/hr)
Experiment I						
Controls	7	273 ± 5	365 ± 11	0·57 ± 0·02	54·4 ± 2·1	306·9 ± 7·0
CCl_4-treated	8	296 ± 14	319 ± 13	0·65 ± 0·03	44·7 ± 3·9	285·5 ± 14·9
P		NS	< 0·025	< 0·05	NS	NS
Experiment II						
Controls	14	232 ± 8	359 ± 11	0·70 ± 0·02	36·7 ± 3·2	251·2 ± 17·7
CCl_4	12	248 ± 5	310 ± 17	0·83 ± 0·04	30·0 ± 3·6	234·4 ± 19·8
P		NS	< 0·025	< 0·02	NS	NS

* Results are expressed as mean ± standard error. P values are for the difference between control and CCl_4 group. NS = not significant (P > 0·05).

† C_0 = Blood alcohol concentration at zero time, estimated by extrapolation of the line fitted to 3–5-hr values.

‡ *r* = Widmark "reduktionsfaktor", obtained by dividing C_0 into administered dose. This is an approximate substitute for the volume of distribution of alcohol in the body.

§ β = Disappearance rate of ethanol from blood.

blood ethanol (β) were calculated only for the period from 3 to 5 hr. The mean β-values and the calculated rates of ethanol metabolism did not differ significantly in the two groups in both experiments (Table 4).

Disappearance of ethanol from the whole body is shown in Table 5. There was no difference between the two groups. The net rates of disappearance in milligrams per kilogram per hour are in good agreement with those obtained by the Widmark cal-

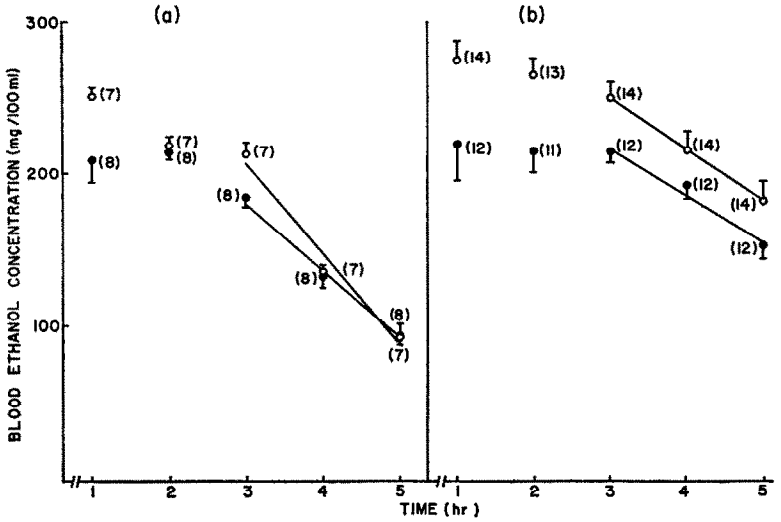

FIG. 1. Concentration of ethanol in blood of CCl_4 treated (●) and control (○) rats at various times after intraperitoneal injection of ethanol. CCl_4 (2·5 ml/kg body wt.) was administered 15 hr before the alcohol. (a) Experiment 1, ethanol dose 2·1 g/kg. (b) Experiment 2, ethanol dose 2·5 g/kg. Number of animals for each point is shown in parentheses. Vertical lines indicate positive or negative half of standard error. Lines showing descending slope of blood alcohol curve were calculated for best fit of 3–5-hr values.

TABLE 5. EFFECT OF CARBON TETRACHLORIDE TREATMENT 20 hr BEFORE SACRIFICE ON DISAPPEARANCE OF ETHANOL FROM THE WHOLE CARCASS*

Treatment	No. of rats	Body wt. (g)	Ethanol metabolized (mg/kg/hr)
Controls	8	198 ± 9	267 ± 33
CCl_4-treated	8	200 ± 10	281 ± 35
P		NS	NS

* Results are expressed as mean ± standard error. P values are for the difference between control and CCl_4 group. NS = not significant ($P > 0·05$).

culation from serial measurements of blood ethanol level (Table 4), and with those obtained by others in fasted animals.

Ethanol metabolism in vitro. The results pertaining to ethanol metabolism with rat liver slices from CCl_4-treated and control rats are presented in Fig. 2. In comparison with control rats of comparable weight and age, treated rats showed a significant decrease in ethanol uptake, when this was expressed as μg per 100 mg of liver tissue (Fig. 2a). However, this resulted from the marked increase in liver weight produced by the administration of CCl_4, rather than from a decrease in enzyme activity. When the results were calculated on the basis of milligrams of ethanol taken up by total liver per 100 g body weight (Fig. 2b), the two groups did not differ. Although ethanol

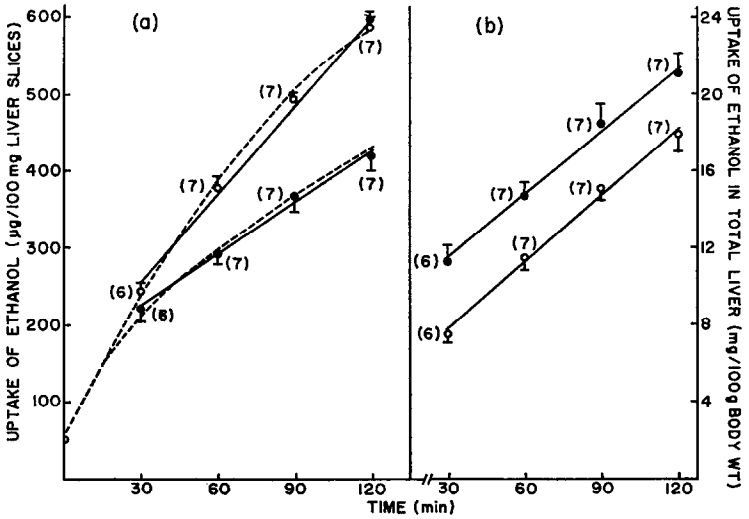

FIG. 2. Uptake of ethanol *in vitro* by liver slices from CCl_4 treated (●) and control rats (○). CCl_4 (2·5 ml/kg body wt.) was administered 20 hr before the uptake experiments. (a) Uptake of ethanol expressed as μg/100 mg liver slices. (b) Uptake of ethanol calculated in total liver (mg/100 g body wt.). Number of animals for each point shown in parentheses. Vertical lines indicate positive or negative half of the standard errors. Regression lines were calculated by the method of least mean squares. For explanation of the broken lines, see Discussion.

uptake appears significantly higher at all time periods in slices from the treated rats than in those of controls, the slopes are not significantly different. Since part of the total uptake by slices corresponds to equilibration of ethanol between the incubation medium and the water contained in the tissue slices, only the slope of the uptake curve can be taken as a measure of the rate of metabolism. The principle has been validated elsewhere.[15] The calculated regression lines have slopes (mg ethanol/100 g/min) of 0·119 ± 0·007 for the CCl_4 group and 0·113 ± 0·007 for the controls. Thus CCl_4 pre-treatment did not change the capacity *in vitro* of the liver cells to metabolize ethanol.

Whole body weight and water content. Measurements of fresh and dry weights of the whole carcasses were carried out in eight untreated rats and eight which had received CCl_4 20 hr earlier. The total water content as percentage of fresh weight was 68·35 ± 0·23 for the controls and 71·30 ± 0·59 for the CCl_4 group. The difference, though small, was clearly significant ($P < 0·001$). In contrast, the water content of the liver was 71·24 ± 0·39 per cent in the controls and 71·72 ± 0·31 per cent in the CCl_4 group, so that the mean values were not significantly different.

The loss of weight over a fasting period of 20 hr was also studied in this experiment, in treated and control rats. The mean loss of weight was 20·9 ± 1·4 g in controls, and 14·7 ± 1·4 g in CCl_4-treated animals, the difference being significant ($P < 0·01$).

DISCUSSION

The present work shows that pretreatment of animals with CCl_4 decreased MEOS activity by an average of 63 per cent, without having a significant effect upon liver alcohol dehydrogenase activity. This marked reduction in MEOS activity would be

expected to affect the rate of ethanol metabolism, if MEOS plays an important role in it. The evidence presented in this paper suggests that MEOS does not play such a role, as reduction of MEOS activity by 63 per cent did not have any influence on the calculated rate of ethanol metabolism. It is conceivable that MEOS might normally be active, and that impairment of MEOS activity in the CCl_4 group might be exactly offset by a stimulation of extrahepatic utilization of ethanol. However, this seems rather improbable, in view of the small role played by extrahepatic tissues in ethanol metabolism.

The lower blood alcohol levels (Fig. 1) and a significantly lower C_0 value (Table 4) in CCl_4-treated rats appear to be due to dilution of alcohol in a greater distribution volume in the body, as suggested by the higher value of r (Table 4). This is sufficient to explain the apparently lower β value in treated rats than in controls, in both experiments. The significantly higher body water content and lower loss of weight in the CCl_4 group as compared to controls over a fasting period of 20 hr suggest that the increase in r is at least partly the result of water retention. However, r was increased by 14–18 per cent in the CCl_4 group, while body water was only 5 per cent higher than in the controls. It is not clear what accounts for the bulk of the increase in r. Nevertheless, the close agreement between the rate of ethanol disappearance as calculated from the descending part of the blood ethanol curve, and that measured directly in the whole body, provides good validation for the time-honored Widmark calculation, even in the CCl_4-treated animals. The difference in the shape of the blood alcohol curves, with the 1-hr values being significantly lower in the CCl_4 group, suggests the possibility of some impairment of absorption of ethanol from the injection site. If this had any influence on the later part of the curve, it should be to make the descending slope less steep, so that the lack of significant difference in slope is all the more convincing.

The results of the studies *in vitro* also appear to be consistent with the results *in vivo*, in that no significant difference was found between the two groups. However, the rate of ethanol uptake calculated from the slope of the straight line of best fit (Fig. 2b) for the controls is approximately 70 mg/kg/hr. Although this is in reasonably good agreement with rates of uptake found by others using liver slices,[27–29] it is less than one-third of the rate *in vivo*. Moreover, if the calculated straight lines in Fig. 2(a) are extrapolated back to zero time the vertical intercept indicates a redistribution uptake of 140–150 μg/100 mg. Yet the water content of the liver was about 70 per cent in both groups, so that 300 mg of tissue should have contained only 0·210 ml of water. When added to 5·0 ml of medium containing 4 mg of ethanol this should have given a redistribution uptake of only 50–55 μg/100 mg of tissue.

This value is plotted on the vertical axis in Fig. 2(a), and connected to the other points by broken lines. On this basis the results appear to fit a hyperbolic function just as well as a linear one. The initial rate of uptake, derived from the initial slope of the broken line, appears to be of the order of 380 μg/100 mg/hr for the controls, corresponding to about 125 mg/kg/hr for the whole animal, and slightly less for the CCl_4 group. Undoubtedly, part of the discrepancy can be explained by the inevitable presence of at least one layer of cut cells on each surface of a liver slice, which may amount to 20 per cent or more of the mass, depending on slice thickness. If shear or compression forces damaged another layer, it is evident that the metabolic capability of a tissue slice could easily be reduced to half of its level *in vivo*. Another possible

explanation might be progressive leakage of soluble constituents from the tissue, such as alcohol dehydrogenase or its cofactors. Similar non-linearity of ethanol metabolism by the isolated perfused liver was reported to be corrected by addition of pyruvate or fructose to the perfusion fluid.[30]

These results suggest that liver slices, as used under these experimental conditions, do not provide an adequate indication of metabolism *in vivo* of ethanol in absolute terms. However, the lack of difference between slices from the two treatment groups under the same conditions is at least consistent with the results *in vivo*.

The results *in vivo* are in complete agreement with those of Sirnes,[31] who reported no change in ethanol disappearance in 175–250 g rats 24 hr after a single subcutaneous injection of 0·30 ml of CCl_4. In rabbits fasted for 15 hr and then given CCl_4 (2·5 ml/kg), Fischer[32] reported unchanged alcohol elimination at 4 hr, an increased rate of elimination at 8 and 12 hr and a reduced rate at 16 and 20 hr. The significance of altered ethanol elimination in Fischer's study cannot be assessed because he did not explain how the rate of alcohol elimination was determined and he did not give actual slopes of the ethanol disappearance curves in treated and control rabbits. Moreover, no information is available in Fischer's paper to permit evaluation of the significance of the reported difference. But even at earlier times, when he reported a higher rate of ethanol elimination,[32] we found a significant reduction of the MEOS. Thus, bearing in mind the possible significance of species differences, no parallelism appears to exist between MEOS activity and ethanol metabolism.

Repeated exposure to CCl_4 has also been found to have no effect on ethanol metabolism. In 12 rats having free access to H_2O and a 9·5% (v/v) ethanol solution, and exposed to CCl_4 for 1 min daily for 7 weeks, Campos *et al.*[33] reported increased voluntary alcohol intake and faster ethanol disappearance in 8 rats which developed liver cirrhosis, while the four noncirrhotic rats of this group did not change significantly with respect to the controls. A similar increase in voluntary intake of alcohol has been reported by Sirnes[34] in rats subjected to subcutaneous injections of CCl_4 twice a week for 4 months. If it can be assumed that repeated exposure to CCl_4 affects the MEOS system in the same way as a single dose, then these findings also suggest that MEOS does not play a role in ethanol metabolism *in vivo* even in animals receiving ethanol chronically.

Acknowledgements—This work was supported by a grant from the Alcoholism and Drug Addiction Research Foundation of Ontario. We are indebted to Mr. G. Donkin, Mr. G. Ho and Mrs. J. Loth for technical assistance.

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