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HEPATIC MICROSOMAL ETHANOL OXIDIZING SYSTEM (MEOS): RESPECTIVE ROLES OF ETHANOL AND CARBOHYDRATES FOR THE ENHANCED ACTIVITY AFTER CHRONIC ALCOHOL CONSUMPTION

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Abstract—To study whether the inductive effect of alcohol containing diets is due to ethanol itself or to the decreased carbohydrate content of these diets, female rats were group-fed for five weeks liquid diets containing various amounts of carbohydrates and, when indicated, ethanol. Compared to the control diet, supplementation of the control diet by additional carbohydrates failed to alter MEOS activity, whereas a hypocaloric diet with a decreased carbohydrate content led to a significant increase of MEOS activity by 72% ($P < 0.001$). MEOS activity was also strikingly induced by 230% ($P < 0.001$) with a diet in which part of the carbohydrates (36% of total calories) was isocalorically replaced by ethanol. Moreover, supplementation of the control diet by alcohol to achieve an alcohol diet with a normal carbohydrate content resulted in a moderate increase of MEOS activity by 79% ($P < 0.01$). Conversely, the activities of other alcohol metabolizing enzymes such as alcohol dehydrogenase and catalase remained virtually unchanged under these various experimental conditions. It is therefore concluded that the induction of MEOS activity following chronic alcohol consumption can be ascribed primarily to the action of ethanol; however, a low carbohydrate content of the diet by itself increased MEOS activity to some extent suggesting that in addition to ethanol the carbohydrate content of the diet may contribute to the induction of MEOS activity.

The liver is generally considered as the main organ capable of oxidizing ethanol to acetaldehyde. The metabolism of ethanol can be catalysed *in vitro* by various hepatic enzymes including alcohol dehydrogenase [1, 2], the microsomal ethanol oxidizing system [2–6], and catalase [7]. Catalase appears to play no significant role in ethanol metabolism in the intact liver cell [2, 8–13]. There is, however, strong evidence that both alcohol dehydrogenase and the microsomal ethanol oxidizing system (MEOS) may quantitatively account for rates of ethanol metabolism *in vivo* [14–19], perfused liver [8], liver slices [2, 3, 20] and isolated parenchymal liver cells [21–23].

The microsomal ethanol oxidizing system has been separated from catalase and alcohol dehydrogenase by column chromatography [4, 23–26]. Recently, MEOS was reconstituted by the microsomal components cytochrome P-450 and NADPH–cytochrome *c* reductase in the presence of phospholipids [27, 28], characteristics which are common with other microsomal drug metabolizing enzymes [29]. There is now general agreement that hydroxyl radicals may be responsible for the activity of the microsomal ethanol oxidizing system [25, 30, 31], whereas superoxides are not involved in this reaction [31].

Chronic alcohol administration leads to a proliferation of the smooth endoplasmic reticulum in the hepatocytes [32]. This ultrastructural alteration is associated with an enhanced activity of the microsomal ethanol oxidizing system [3, 33] which in turn may be responsible for increased rates of alcohol metabolism commonly observed after prolonged ethanol administration [14, 18, 22, 23]. The adaptive increase of MEOS activity may be attributed to quantitative as well as qualitative changes of cytochrome P-450, since chronic intake of ethanol results not only in an increased content of cytochrome P-450 [34] but also in an enhanced content of a specifically inducible form of cytochrome P-450 [27].

Recent studies have shown that prolonged administration of carbohydrates significantly depresses the activities of hepatic microsomal enzymes [35, 36]. Based upon these findings it has been postulated that the administration of diets with a low carbohydrate content may lead to an increase of microsomal enzyme activities [37]. Indeed, the alcohol containing diets commonly used in experimental studies on the effects of chronic ethanol consumption exhibit a rather low carbohydrate content compared to the respective control diets, since parts of the carbohydrates are isocalorically replaced by ethanol [38]. The question therefore arose whether the observed inductive effect on MEOS activity by chronic alcohol consumption might be due to dietary imbalance with respect to carbohydrates or to the action of ethanol itself.

In the present study the specific effect of alcohol and carbohydrate administration on the activities of the microsomal ethanol oxidizing system and other alcohol metabolizing enzymes was therefore investigated.

MATERIALS AND METHODS

Materials. The chemicals and enzymes were obtained from the following sources: DL-sodium isocitrate and disodium-EDTA from Serva Corp., Hei-

delberg; NAD^+ (grade II), $NADP^+$-disodium salt, isocitric dehydrogenase (grade II) and NADH (grade II) from Boehringer Corp., Mannheim; semicarbazide hydrochloride, trichloroacetic acid, magnesium chloride, ethanol absolute, sodium azide, acetaldehyde, and sodium-dithionate from E. Merck Corp., Darmstadt, West Germany.

Animals. Female Sprague–Dawley rats were purchased from Zentral-Institut für Versuchstierzucht, Hannover, with a body weight of 130–150 g and fed Altrumin-R chow and tap water *ad libitum* until the start of the experiment. When the animals reached a body weight of 170–190 g they were housed in individual wired-bottom cages and group-fed for five weeks with various liquid diets in drinking tubes as the only source of food and water.

Composition of the diets. The various liquid diets were prepared essentially as described by DeCarli and Lieber [38]. The nutritionally adequate control diet contains protein (18% of total calories), fat (35% of total calories), dextrin-maltose as carbohydrates (47% of total calories), vitamins and trace elements. The corresponding alcohol diet (regular alcohol diet) was of the same composition as the control diet, except that parts of carbohydrates (36% of total calories) were isocalorically replaced by ethanol (Fig. 1). The control diet and regular alcohol diet contained 1.0 kcal/ml corresponding to 4.2 kJ/ml. The third diet contained all the nutrients as the control diet but less carbohydrates (11% rather than 47% of total calories) and no alcohol. Since this particular diet exhibited only 64% of the calories of the control diet it was designed as hypocaloric diet (Fig. 1). The fourth diet was a hypercaloric alcohol diet. It contained in addition to all nutrients of the control diet the same amount of alcohol as described for the regular alcohol diet (Fig. 1). Finally, the fifth diet was again hypercaloric and exhibited the same composition as the hypercaloric alcohol diet except that the alcohol was isocalorically replaced by carbohydrates (Fig. 1).

Feeding procedure. The animals were divided into five groups each consisting of 10 rats. One of each group received one of the diets described above. Preliminary studies have indicated that the animals fed the regular alcohol diet generally had the lowest dietary intake with respect to volume. Therefore, to ensure that each animal of one group received the same volume of the particular liquid diet as the corresponding animals of the other groups, the volume of these diets was determined according to the dietary volume consumed the day before by the animal fed the regular alcohol diet. This procedure ensured that the animals in all groups have been pair-fed to the animals fed the regular alcohol diet. During the 24 hr preceding killing of the rats, the diets were given in three divided doses at approximately 8-hr intervals.

Subcellular fractionation. Following the administration of the diets for five weeks the rats were decapitated, their livers *in situ* perfused with ice-cold 0.15 M KCl through the portal vein, excised, chilled, and a 25% liver homogenate was prepared with the same solution. The following steps were carried out at 4°C [25]. The liver homogenate was centrifuged at 10,000 *g* for 30 min. The resulting supernatant was then spun at 105,000 *g* for another 30 min, and washed microsomes and cytosol were prepared. The supernatant corresponds to the cytosolic fraction and was used for the determination of alcohol dehydrogenase. The pellet was resuspended and again centrifuged at 105,000 *g* for another 30 min. The resulting pellet consisted of washed microsomes and was used as enzyme source for the microsomal ethanol oxidizing system and cytochrome P-450 after resuspension.

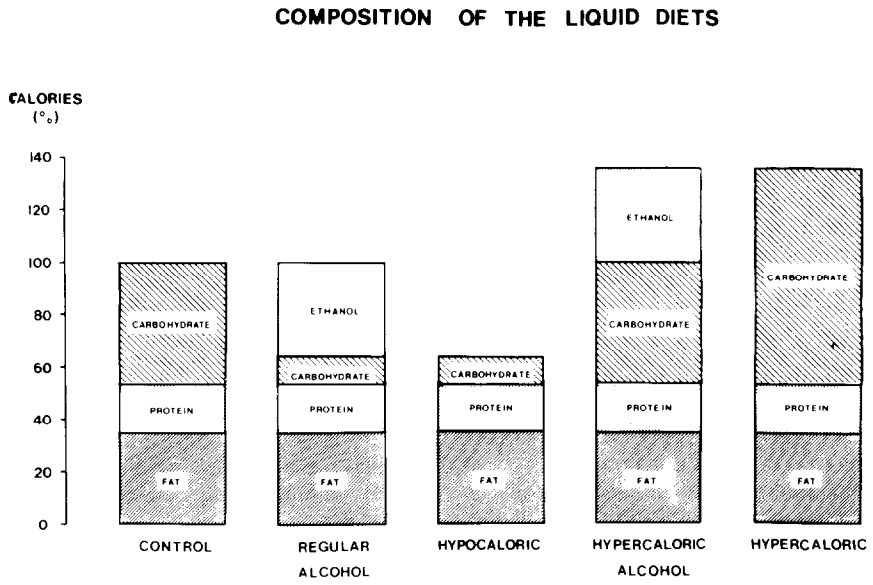

Fig. 1. Composition of the liquid diets. The control diet contained all essential nutrients in sufficient amounts and is considered as nutritionally adequate. The caloric value of the control diet was 1.0 kcal/ml (4.2 kJ/ml) and set as 100%. All diets exhibited the same amount of vitamins, trace elements, protein and fat, but differed in the composition regarding carbohydrates (dextrin-maltose) and/or ethanol.

Biochemical determinations

Alcohol dehydrogenase (ADH). The activity was measured in the hepatic cytosol following the reduction of NAD^+ at 340 nm with 0.1 M phosphate buffer (pH 7.4) and 50 mM ethanol as described before [2].

Microsomal ethanol oxidizing system. The activity was determined in the main chamber of 50 ml Erlenmeyer flasks with center wells containing 0.6 ml of 15 mM semicarbazide hydrochloride [3, 25]. Washed microsomes (3 mg of microsomal protein per flask) were preincubated with 50 mM ethanol for 5 min at 37°. The reaction was initiated by adding the NADPH generating system (0.4 mM $NADP^+$, 8 mM sodium isocitrate and 0.34 unit/ml of isocitric dehydrogenase). The incubations were carried out in a final volume of 3.0 ml containing 1.0 mM disodium-EDTA, 5.0 mM $MgCl_2$ and 0.1 mM sodium azide in 0.1 M phosphate buffer (pH 7.4) and terminated after 0, 5 and 10 min of incubation time by adding 0.5 ml of 70% (w/v) trichloroacetic acid. After an overnight diffusion period, the acetaldehyde formed by the oxidation of ethanol was measured as its semicarbazide derivative in a Zeiss DM 4 spectrophotometer.

Cytochrome P-450. The measurement was carried out in washed microsomes of the liver [39] and in liver homogenates [40]. Microsomal recovery was estimated from the amount of cytochrome P-450 recovered in the microsomal fraction compared with that in the homogenate of the same weight of liver [40].

Catalase. Catalatic activity of catalase was determined in the 25% liver homogenate by measuring the decrease in absorption at 240 nm following addition of H_2O_2 [41].

Protein. The determination of protein was performed according to the method of Lowry *et al.* [42] using crystalline bovine albumine as standard.

Statistical analysis. Each measurement was carried out at least in duplicate. The results obtained are expressed as means (± S.E.M.), and the significance of the differences was assessed by Student's *t* test for groups.

RESULTS

Caloric intake and weight gain

In all experimental groups the volume of dietary intake was similar with respect to total as well as average daily consumption (Table 1). The caloric value of the control and regular alcohol diet amounted to 1.0 kcal/ml corresponding to 4.2 kJ/ml. The respective values for the hypocaloric diet were considerably lower, and those for the hypercaloric diets substantially higher than for the control or regular alcohol diets (Table 1). The total as well as average daily alcohol intake was similar with both the regular alcohol diet and the hypercaloric alcohol diet (Table 1).

The animals fed the control diet showed an average weight gain of 0.81 ± 0.01 g/day, a value which was much higher than the corresponding one of 0.28 ± 0.01 g/day for the animals fed the regular alcohol diet (Table 2). Actually, the latter value was similar to the one obtained with the hypocaloric diet suggesting that calories derived from alcohol may be incapable of causing weight gain at the usual extent. As expected, a major weight gain was achieved with the hypercaloric diet but not with the hypercaloric alcohol diet (Table 2).

The liver weight was only slightly altered following the application of the various diets (Table 2). Moreover, the protein content per g of liver wet weight was considerably higher with the regular alcohol diet and the hypercaloric alcohol diet (Table 2).

Microsomal ethanol oxidizing system (MEOS)

Compared to the control diet the regular alcohol diet resulted in a significant increase of MEOS activity by 230 per cent ($P < 0.001$) when expressed per mg microsomal protein (Table 3). The increase of MEOS activity persisted when the activity was calculated per g of liver wet weight after correction

Table 1. Dietary intake during the feeding period*

	Diets				
	Control	Regular alcohol	Hypocaloric	Hypercaloric alcohol	Hypercaloric
Total dietary intake (ml)	1795 ± 12	1725 ± 55	1804 ± 21	1736 ± 44	1787 ± 17
Daily dietary intake (ml/day)	51.3 ± 0.3	49.3 ± 1.6	51.6 ± 0.6	49.6 ± 1.3	51.1 ± 0.5
Caloric value of diet (kcal/ml)	1.0	1.0	0.64	1.36	1.36
Total caloric intake (kcal)	1795 ± 12	1725 ± 55	1154 ± 13	2360 ± 60	2430 ± 23
Daily caloric intake (kcal/day)	51.3 ± 0.3	49.3 ± 1.6	33.0 ± 0.4	67.5 ± 1.7	69.5 ± 0.7
Total ethanol intake (g)	—	86.3 ± 2.8	—	88.8 ± 2.2	—
Daily ethanol intake (g/day)	—	2.6 ± 0.1	—	2.5 ± 0.1	—

* Female Sprague–Dawley rats with a body weight of 170–190 g were group-fed for five weeks with various liquid diets as the only source of food and water. The composition of the individual diets are given in Fig. 1. Each experimental group consisted of 10 animals. The caloric value of the control diet was 1.0 kcal/ml corresponding to 4.2 kJ/ml. The data are given as means ± S.E.M.

Table 2. Effect of various liquid diets on body weight gain, liver protein and microsomal protein*

Diet	Body weight gain (g/day)	Liver weight (g/100 g body wt)	Liver protein (mg/g liver wet wt)	Microsomal protein (mg/g liver wet wt)
Control	0.81 ± 0.01	3.38 ± 0.06	142.8 ± 14.4	18.4 ± 1.0
Regular alcohol	0.28 ± 0.01	3.55 ± 0.11	167.3 ± 24.5	21.2 ± 1.3
Hypocaloric	0.20 ± 0.01	3.14 ± 0.05	138.4 ± 7.4	20.2 ± 1.2
Hypercaloric alcohol	0.78 ± 0.01	3.42 ± 0.07	156.6 ± 8.2	21.0 ± 1.7
Hypercaloric	0.97 ± 0.01	3.72 ± 0.10	144.6 ± 15.5	23.0 ± 2.2

* The liquid diets contained various amounts of carbohydrates (dextrin–maltose) and, when indicated, ethanol. The rats were fed for a total of 5 weeks. The experimental groups consisted of 10 animals each. The data are given as means ± S.E.M.

for microsomal losses during the preparation of the microsomal fraction (Table 3). Similarly, there was a striking enhancement of MEOS activity when expressed per 100 g of body weight. These findings show that isocaloric replacement of carbohydrates by ethanol (36% of total calories) leads to a significant induction of MEOS activity (Table 3), raising the question whether the inductive effect is due to ethanol *per se* or to the decreased carbohydrate content of the regular alcohol diet.

To study this, MEOS activity was measured following chronic administration of a hypocaloric diet which has the same constituents as the regular alcohol diet with 11 per cent of total calories as carbohydrates except that alcohol was deleted (Fig. 1). With this hypocaloric diet MEOS activity was strikingly decreased by 48 per cent ($P < 0.001$) compared to the regular alcohol diet with the same amount of carbohydrates (Table 3). This finding clearly shows that the inductive effect on MEOS activity observed with the regular alcohol diet can indeed be ascribed to a major extent to the action of ethanol rather than to the decreased content of carbohydrates. Compared to the control diet, however, the hypocaloric diet led to a significant increase of MEOS activity by 72 per cent ($P < 0.001$), suggesting that a diet with a carbohydrate content as low as 11 per cent in the absence of ethanol also results in an induction of MEOS activity. It is therefore evident from these experiments that the induction of MEOS activity can be ascribed primarily to ethanol but a low carbohydrate content may also be of some importance. That ethanol *per se* exerts an inductive effect on MEOS activity could be confirmed with the control diet which contained additional ethanol. Under these experimental conditions, there was a significant increase of MEOS activity by 79 per cent ($P < 0.001$) when the hypercaloric alcohol diet was compared with the control diet (Table 3). However, the inductive effect of the hypercaloric alcohol diet on MEOS activity was much less than the one observed with the regular alcohol diet which contained less carbohydrates than the former one.

Moreover, whereas the feeding of the hypocaloric diet with a low content of carbohydrates resulted in a striking increase of MEOS activity compared to the control diet, supplementation of the control diet with carbohydrates to obtain a hypercaloric diet (Fig. 1) failed to significantly alter MEOS activity compared to the control diet (Table 3). However, when the hypercaloric diets containing either ethanol or additional carbohydrates were compared, an inductive effect on MEOS activity was still observed with alcohol, but the induction was much less than the one observed with the control diet and the regular alcohol diet.

Since changes of the diet with respect to ethanol and carbohydrate content result in striking alterations of MEOS activity when expressed per mg of microsomal protein, the question arose whether similar data could be obtained when the results were calculated per unit of liver wet weight or per unit of body weight. The calculation shows that the various diets exhibit similar results for MEOS activity irrespectively whether the activity was assessed per mg of microsomal protein, per g of liver wet weight or per 100 g of body weight (Table 3).

Table 3. Effect of alcohol and carbohydrates on the hepatic activity of the microsomal ethanol oxidizing system (MEOS)*

Diet	Microsomal ethanol oxidizing system (MEOS) nmoles acetaldehyde/min/ mg microsomal protein	nmoles/min/ g liver wet weight	nmoles/min/ g liver protein	nmoles/min/ 100 g body weight
Control	6.2 ± 0.5	119 ± 13	832 ± 90	402 ± 42
Regular alcohol	20.4 ± 1.9†	446 ± 46†	2670 ± 188†	1586 ± 162†
Hypocaloric	10.7 ± 0.8†	225 ± 20†	1630 ± 27†	696 ± 64†
Hypercaloric alcohol	11.1 ± 1.5‡	229 ± 22†	1468 ± 26†	774 ± 70†
Hypercaloric	5.7 ± 0.6¶	131 ± 14¶	909 ± 90¶	478 ± 53¶

* The various diets were administered for 5 weeks to female Sprague–Dawley rats. MEOS activity was determined in washed liver microsomes and expressed per mg of microsomal protein. After correction for microsomal losses during subcellular preparation the data are also given per g of liver wet weight and per 100 g of body weight. The results are derived from 10 animals of each group (mean ± S.E.M.).

† $P < 0.001$; ‡ $P < 0.01$; ¶ N.S.

Cytochrome P-450

To study whether other microsomal parameters may show a similar dependency upon alcohol and carbohydrate administration, cytochrome P-450 was determined under these experimental conditions both in liver homogenate and microsomes. In hepatic microsomes there was a striking increase of cytochrome P-450 content following the administration of the regular alcohol diet compared to the control diet (Table 4). However, part of the increase of cytochrome P-450 can be attributed to the low carbohydrate content of the regular alcohol diet since the hypocaloric diet by itself significantly increases the content of cytochrome P-450 compared to the control diet.

On the other hand, the inductive effect of alcohol was lost when a hypercaloric diet with a high carbohydrate content was employed compared to the respective hypercaloric diet (Table 4). Similar results were obtained when the content of cytochrome P-450 was measured in the liver homogenate and expressed per g of liver wet weight or per 100 g of body weight (Table 4). These data therefore suggest that an alcohol containing diet is capable of increasing the microsomal content of cytochrome P-450, provided the carbohydrate content of the alcohol diet is low.

Alcohol dehydrogenase (ADH)

Compared to the control diet, there was no significant alteration of hepatic alcohol dehydrogenase activity whether the regular alcohol diet was employed or the hypercaloric alcohol diet. Similarly, changes of the carbohydrate content of the diets failed to significantly alter ADH activity when expressed per g of liver wet weight, per g of hepatic protein or per 100 g of body weight. These data therefore indicate that neither alcohol nor the carbohydrate composition of the diet had any significant effect on ADH activity.

Catalase

When compared to the control diet, chronic administration of the regular alcohol diet failed to significantly alter catalase activity in the hepatic homogenate whether expressed per g of liver wet weight of per mg of hepatic protein. Moreover, alterations of hepatic catalase activity could not be demonstrated neither with the hypercaloric alcohol diet nor with the hypercaloric or hypocaloric diets. These results therefore suggest that both alcohol and carbohydrates are incable of significantly influencing hepatic catalase activity.

DISCUSSION

In the present study the long-term effect of alcohol and carbohydrates on the activities of various hepatic alcohol metabolizing enzymes was investigated. It could be shown that the prolonged administration of diets in which parts of the carbohydrates were isocalorically replaced by ethanol resulted in a striking elevation of MEOS activity (Table 3) without altering the activities of alcohol dehydrogenase or catalase. Under a variety of experimental conditions it was demonstrated that the inductive effect on MEOS activity can be ascribed primarily to the action of alcohol itself. However, an additional inductive property may be attributed also to dietary imbalance with respect to a low carbohydrate content of the diet.

It has been previously reported that carbohydrates are capable of suppressing various hepatic functions [43, 44] including microsomal ones [35, 36]. This observation led to the hypothesis that restriction of carbohydrates could result in an induction of hepatic microsomal enzyme activities [37]. That this is indeed the case was shown in the present study. Compared to the control diet with 47 per cent of total calories as carbohydrates, a hypocaloric diet restricted with respect to carbohydrates at the extent of 36 per cent of total calories led to a significant increase of MEOS activity by 56, 88 and 73 per cent, when expressed per mg of microsomal protein, per g of liver wet weight or per 100 g of body weight, respectively (Table 3). Under these experimental conditions there was also a pronounced increase of the microsomal content cytochrome P-450 (Table 4). Moreover, the addition of carbohydrates to the control diet in order to achieve a hypercaloric carbohydrate-rich diet failed to suppress MEOS activity (Table 3) as well as cytochrome P-450 content (Table 4). This may be explained on the basis that the control diets exhibit a carbohydrate content high enough to achieve a near maximal suppression of microsomal enzyme activities. The data of these studies led to the conclusion that carbohydrates are important in determining the levels of microsomal

Table 4. Effect of alcohol and carbohydrates on the hepatic content of cytochrome P-450*

Diet	Cytochrome P-450 nmoles/mg microsomal protein	nmoles/g liver wet weight	nmoles/g liver protein	nmoles/100 g body weight
Control	1.02 ± 0.05	20.0 ± 1.4	14.9 ± 1.4	68.1 ± 4.4
Regular alcohol	1.62 ± 0.11†	33.5 ± 2.2‡	18.9 ± 1.7¶	122.7 ± 6.4†
Hypocaloric	1.23 ± 0.05‡	25.5 ± 1.3‡	23.3 ± 2.9‡	80.0 ± 4.0¶
Hypercaloric alcohol	1.11 ± 0.05‖	24.1 ± 1.4¶	15.8 ± 1.3‖	82.3 ± 4.6‡
Hypercaloric	1.00 ± 0.04‖	22.3 ± 1.0¶	16.7 ± 2.0‖	82.3 ± 3.2‡

* The diets as indicated were administered to female Sprague–Dawley rats for 5 weeks. The content of cytochrome P-450 was determined in the liver homogenate and in washed liver microsomes. The data are expressed per mg of microsomal protein, per g of liver wet weight, per g of liver protein and per 100 g of body weight. The results are derived from 10 animals of each group (means ± S.E.M.)

† $P < 0.001$; ‡ $P < 0.01$; ¶ $P < 0.05$; ‖ N.S.

enzyme activities; in particular, a low carbohydrate content of the diet is capable of increasing microsomal functions when compared to diets rich in carbohydrates.

The mechanisms by which carbohydrates influence hepatic microsomal enzyme activities are not yet established. Preliminary studies have indicated that neither blood insulin levels nor hepatic cyclic AMP levels may initiate alterations of microsomal enzyme activities [35], and further studies are therefore necessary to elucidate the mechanism.

The commonly used alcohol diets contain low amounts of carbohydrates [38], and the increase of microsomal enzyme activities [3, 24, 33, 34] observed under these conditions was therefore attributed exclusively to the low content of carbohydrates rather than to ethanol itself [37]. While demonstrating that a low carbohydrate content of the diet increases to some extent microsomal functions such as the activity of MEOS (Table 3) and the content of cytochrome P-450 (Table 4), the present study nevertheless provides strong evidence that upon chronic intake ethanol itself has a much greater effect on the degree of microsomal induction. This is evident from the increases of MEOS activity (Table 3) as well as cytochrome P-450 content (Table 4) observed with the regular alcohol diet which are much higher compared to the control diet or the hypocaloric diet. This study therefore failed to support the concept proposed by some [37] that ethanol itself may lack inductive properties with respect to microsomal functions.

Compared to values noted in rats fed high or low carbohydrate diets, induction of MEOS activity was observed in animals on a high carbohydrate diet containing ethanol, although the inductive effect was much less pronounced than with the regular alcohol diet (Table 3). Of particular interest was the observation that this same pattern was not observed in the changes of hepatic cytochrome P-450 content (Table 4). This dissociation suggests in accord with other reports [24, 27] that qualitative changes of cytochrome P-450 rather than quantitative ones may be more important for the induction of MEOS activity. The results of the present investigation also show that the inductive effect of alcohol on MEOS activity is much more striking when alcohol was administered in diets with a lower rather than with a higher content of carbohydrates (Table 3). The fat content of the alcohol diets has also been considered to play a significant role for the degree of alcohol-mediated microsomal enzyme induction [45]. In these studies a high fat alcohol diet with 35 per cent of total calories as fat and 36 per cent as alcohol provoked a much greater inducibility of various microsomal enzyme activities than a low fat alcohol diet with only 2 per cent of total calories as fat and 33 per cent of total calories as additional carbohydrates. An extremely high carbohydrate content was achieved both in the low fat alcohol diet as well as the control diet which amounted to 69 per cent and 80 per cent of total calories, respectively [45]. In view of the data obtained in the present study it is reasonable to assume that the small inductive effect of ethanol with the low fat and high carbohydrate alcohol diet [45] might be attributed not only to the low fat content but also to the extremely high carbohydrate content which itself suppresses microsomal enzyme activities.

Previous studies have clearly shown that hypocaloria itself exerts no inductive effect on MEOS activity [45]. Therefore, the inductive effect on MEOS activity observed in the present study with the hypocaloric diet (Table 3) may be ascribed primarily to the low carbohydrate content of these diets rather than to hypocaloria.

A variety of studies have suggested that the enhancement of MEOS activity observed following chronic alcohol consumption is responsible for increased rates of alcohol metabolism both *in vitro* [2, 3, 8, 20–23] and *in vivo* [14–19]. Since the degree of induction of MEOS activity by ethanol depends to a certain extent upon the carbohydrate content of the diets (Table 3), dietary manipulations with respect to carbohydrates may alter the rate of hepatic alcohol metabolism accordingly. These findings may explain at least in part the variation of reported rates of alcohol metabolism in man both in a control population as well as in alcoholics.

This investigation confirms and extends previous reports on the effect of alcohol on body weight gain [45, 47]. It has been demonstrated before that compared to the respective control diet the weight gain of experimental animals was much less with alcohol diets exhibiting 36 per cent of total calories as ethanol and 11 per cent as carbohydrates [45], a finding confirmed in this study (Table 2). Similar data have been reported for man [46, 47]. In addition, in the present report a weight gain lower than with the regular alcohol diet was observed in rats fed the hypocaloric diet (Table 2) which exhibits the same nutrients as the former one except that ethanol was omitted. These data clearly demonstrate that ethanol *per se* has little if any effect regarding body weight gain due to an ineffective utilization of calories derived from ethanol. This is substantiated by the failure of the hypercaloric alcohol diet to achieve a significant weight gain compared with the regular control diet which apart from an additional ethanol content exhibits the same nutrients as the former diet (Table 2). The failure of ethanol of energy conservation may be due at least in part to the increased microsomal oxidation of ethanol, a process which is not linked to ATP production [46, 47].

The protein content of the liver was considerably higher with the regular alcohol diet compared to the control diet (Table 2), agreeing thereby with previous studies [48]. Since the hepatic protein content was similar in rats fed the control diet or the hypocaloric diet, the increased protein content observed in the livers of animals fed the regular alcohol diet can therefore be attributed to the effect of ethanol itself rather than to dietary imbalance with respect to the low carbohydrate content of the alcohol diet. This view is supported by similar data obtained for the hypercaloric alcohol diet in comparison with both the hypercaloric diet and the regular control diet (Table 2).

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