Br. J. exp. Path. (1977) **58**, 606

HEPATIC TRIGLYCERIDE ACCUMULATION AND THE ETHANOL PHYSICAL WITHDRAWAL SYNDROME IN MICE

C. ABU MURAD, S. J. BEGG, P. J. GRIFFITHS* AND J. M. LITTLETON

From the Department of Pharmacology, King's College, Strand, London WC2R 2LS

Received for publication April 1, 1977

Summary.—Physical dependence on ethanol was induced in TO strain mice by chronic administration of ethanol by inhalation. The severity of the behavioural syndrome of withdrawal from ethanol was quantified by a subjective scoring method. During the chronic administration of ethanol, triglycerides accumulated in livers of male or female mice with a time course similar to that of the induction of physical dependence. When ethanol was withdrawn from adult or weanling dependent mice, a relationship was observed between the decline of triglyceride concentrations in liver and the duration of the ethanol withdrawal syndrome.

The addition of DL-carnitine (7% w/w) to diet during the administration of ethanol markedly inhibited the accumulation of triglycerides, and significantly reduced the intensity of the ethanol withdrawal syndrome. Administration of carbon tetrachloride ((1·3 ml/kg i.p.), however, although augmenting hepatic triglyceride accumulation, had no significant effect on the withdrawal syndrome.

The results are interpreted as suggesting either that ethanol-induced liver dysfunction plays a part in dependence, or, more likely, that triglyceride accumulation reflects an ethanol-induced metabolic disorder which is itself related to the induction of dependence.

THE use of animal models to elucidate the mechanism of ethanol dependence has concentrated on ethanol-induced changes in central neurotransmitter metabolism (Littleton, 1975). A variety of changes have been reported, some of which seem to apply to most models, whereas others are reported comparatively rarely (*e.g.* Pohorecky, Jaffe and Berkeley, 1974; Hunt and Majchrowicz, 1974). It now seems very likely that some of the observed changes in neurotransmitter metabolism are important in determining the expression of the ethanol withdrawal syndrome (Littleton, 1975). However, the wide variety of neurotransmitter changes reported in ethanol-dependent animals suggests that it may be futile to pursue this approach, and that some more fundamental alteration of brain metabolism may be of primary importance in the induction of physical dependence. We have chosen to examine the possibility that an ethanol-induced alteration in hepatic function might alter brain metabolism in such a way as to predispose to physical dependence.

The recognition of an association between liver damage and alcoholism is by no means new, and fatty change in liver has long been recognized as a sign of excessive ethanol consumption in man (Lieber and Rubin, 1968). It is not, however, clear what part these pathological changes may play in the development of alcoholism, and whether they contribute in any way to the establishment of the addicted state. Such hypotheses would be very difficult to test in clinical alcoholism, where presentation is normally delayed until the condition is established. In animal models, however, the development of dependence and of ethanol-induced liver dysfunction can be studied together. For example, preliminary find-

* Present address: Battelle Institute, 7 rue de Drize, 1227 Carouge, Geneva, Switzerland.

ings (Abu Murad, Griffiths and Littleton, 1976) suggested that, in mice exposed to ethanol vapour, the time course of accumulation of triglycerides in liver (fatty change) was similar to the time course of induction of physical dependence. This paper explores this relationship further and demonstrates that, in many situations, there is an association between the degree of dependence (as shown by the severity of the ethanol withdrawal syndrome) and the amount of triglyceride accumulated by liver. It should be emphasized that this relationship applies only to the triglyceride accumulation produced by *chronic* administration of ethanol. The triglyceride accumulation in liver induced by single large doses of ethanol seems to be mediated by a different mechanism (Kalant *et al.*, 1975) and is unlikely to have any relationship to dependence.

MATERIALS AND METHODS

Induction of ethanol dependence.—Ethanol dependence was induced in groups of mice by administration of ethanol by inhalation (Griffiths, Littleton and Ortiz, 1973, 1974). Ethanol withdrawal severity was assessed by a subjective procedure in which the major signs of withdrawal (convulsions, tremor, piloerection and tail erection) are scored 0 to 4 for severity (Griffiths *et al.*, 1974). In most animals the withdrawal syndrome lasts about 10 h. This period has therefore been used for assessment of the syndrome, unless stated otherwise in the text.

Measurement of ethanol concentrations.—Ethanol concentrations in inspired air were measured by taking 1 ml of air from the inhalation chamber into a gas-tight syringe and injecting this sample directly on to the gas liquid chromatograph (glc) column described previously (Griffiths *et al.*, 1974). Blood ethanol concentrations were measured in the air of the head space above a protein-precipitated, centrifuged blood sample of mixed arterio–venous blood obtained after decapitation (Griffiths *et al.*, 1974). It has been found possible to obtain an accurate estimate of the blood ethanol concentration in the living mouse by measuring the concentration of ethanol in the expired air. This concentration was measured by forcing individual mice to rebreathe air in a 5-ml chamber for 30 sec. At the end of this period a 1-ml sample of air was withdrawn and injected on to the glc column. Several experiments, in which estimated blood ethanol concentrations of individual mice were verified by subsequent killing and direct measurement, established the validity and reproducibility of this method. It has been used routinely in this study.

Measurement of hepatic triglyceride concentration.—Mice were killed by whole-body immersion in liquid nitrogen. Livers were dissected in the cold, weighed and taken for triglyceride estimation. Total triglyceride content of each liver was measured by a modification of the method of van Handel and Zilversmit (1957) after extraction of lipids by the method of Folch, Lees and Sloane Stanley (1957).

Experimental design.—To investigate the relationship between the time course of accumulation of triglycerides in liver and the induction of ethanol dependence, 100 male TO mice, 20–25 g, were exposed to ethanol vapour in the usual way. On each day, 10 mice were removed from the inhalation chamber. Five were killed for estimation of hepatic triglycerides and 5 were taken for assessment of the severity of the ethanol withdrawal syndrome.

To further investigate any relationship between the concentration of triglycerides in liver and the severity of ethanol withdrawal a direct comparison was made between male and female mice. It has previously been reported that, when exposed to the same concentration of ethanol vapour, female mice show a significantly less severe ethanol withdrawal syndrome than males (Goldstein, 1973). It was therefore decided to compare also the time course of accumulation of triglycerides in liver with the time course of induction of ethanol dependence in female mice. This experiment was carried out at the same time and in exactly the same way as that described above.

The possibility that some relationship might exist between the time course of the fall of hepatic triglyceride concentrations after the withdrawal of ethanol and the time course of the withdrawal syndrome was also considered. This was tested by comparing the fall in triglyceride concentrations and the withdrawal syndrome in adult and weanling mice. It had previously been noted (unpublished) that when adult and weanling mice were exposed to the same ethanol concentration weanling mice showed a significantly shorter duration of the ethanol withdrawal syndrome, although severity at the peak of withdrawal was very similar. In the experiments reported here 50 male TO mice, 30–35 g, were compared with 50 male TO mice, 8–10 g. Each group was exposed to ethanol vapour in the same chamber for 10 days. Blood ethanol concentrations were estimated frequently by the expired air method. After 10 days, 5 mice of each group were removed from the inhalation chamber and immediately killed for hepatic triglyceride measurement. The remainder were used

to assess the time course and severity of the withdrawal syndrome. After 12 h 5 mice of each group were killed for estimation of hepatic triglyceride concentrations. Testing for withdrawal signs in the remaining animals continued until no evidence of ethanol withdrawal was apparent.

Some experiments were made to investigate the effects of drug-induced alteration of hepatic triglyceride accumulation on the ethanol withdrawal syndrome.

The administration of DL-carnitine has been reported previously to inhibit ethanol-induced triglyceride accumulation (Hosein and Bexton, 1975). We therefore compared two groups of 30 male TO mice, 20–25 g, in an experiment in which one group received DL-carnitine (7% w/w) in diet, while the other group received the normal laboratory diet. Both groups were exposed to ethanol vapour in the same inhalation chamber for 10 days. Ten mice of each group were killed for estimation of hepatic triglyceride concentration immediately before ethanol withdrawal. The remainder were used to assess the severity of the ethanol withdrawal syndrome. Frequent estimations of blood ethanol concentration were made throughout the period of the experiment.

The i.p. injection of carbon tetrachloride has been reported to potentiate ethanol-induced triglyceride accumulation in liver (Strubelt, Buettner and Siegers, 1975). The effects of potentiating triglyceride accumulation on the induction of ethanol dependence were therefore tested in experiments in which carbon tetrachloride (1·3 ml/kg i.p.) was administered to one group of mice (30 male TO, 20–25 g) and arachis oil, the vehicle for carbon tetrachloride, was administered to a similar group during the exposure of both groups to the same concentration of ethanol vapour. This experiment was performed in two ways: in one, the additional treatments were administered on the first day of ethanol administration; in the other, the additional treatments were given on the penultimate day of ethanol exposure. In both cases frequent estimations of blood ethanol concentrations were made. Ten animals from each group were killed for hepatic triglyceride measurement before ethanol withdrawal, and the remainder used for assessment of the withdrawal syndrome.

In all these experiments comparison of biochemical and behavioural parameters has been made between the experimental groups and control animals exposed to the same conditions, except for the absence of ethanol from the inspired air.

Animals and materials.—TO strain white mice were obtained from Animal Suppliers Ltd, Welwyn. Mice were maintained on Spillers Labsure 41B diet. Food and drinking water were freely available throughout every experiment.

All reagents used for biochemical analysis were Analar grade when these were commercially available. Ethanol (99·8%, A.R. grade) was obtained from James Burroughs Ltd, DL carnitine HCl (99%) was obtained from Aldrich Chemical Co Ltd, carbon tetrachloride (S.L.R. grade) from Fisons, and arachis oil (special for injections) from Boots Ltd.

RESULTS

Time course of accumulation of triglycerides in livers of male and female TO mice: relation to withdrawal syndrome

The results, shown in Figs 1a and 1b, indicate a very similar time course for the ethanol-induced accumulation of triglycerides in liver and the onset of a recognizable ethanol withdrawal syndrome. In male mice significant triglyceride accumulation does not occur until after 5 days of ethanol administration (Fig. 1a). The onset of a withdrawal syndrome follows closely so that, after 6 days of administration, a recognizable syndrome can be observed in some male animals. The appearance of significant changes in these parameters is slightly delayed in female mice, but, once again, there is close agreement between the time courses (Fig. 1b). The intensity of the withdrawal syndrome after 10 days confirms that female mice suffer a less severe withdrawal syndrome than males exposed to the same concentration of ethanol vapour. It is important to note that in this experiment no attempt was made to maintain the blood ethanol concentration at the same level in the two sexes. Female mice do, in fact, show significantly lower blood ethanol concentrations than males throughout this regime of ethanol administration.

Time course of reversal of ethanol-induced triglyceride accumulation in adult and weanling mice: relation to withdrawal syndrome

When weanling and mature adult mice were exposed to the same concentration of ethanol vapour, similar blood ethanol concentrations were obtained. Both groups showed accumulation of triglycerides in

liver after 10 days (Table I). The accumulation of triglycerides in liver of weanling animals is greater than that in adult animals; this difference is significant at the $P<0{\cdot}05$ level. The experiment was unusual in that the mean triglyceride concentration of livers of adult ethanol-dependent mice was lower than that found in other experiments (*e.g.*, *cf.* Fig. 1a). It should be remembered, however, that the adult animals used in this experiment were considerably older than those used in the other experiments reported here.

The severity of the ethanol withdrawal syndrome was very similar for both groups during the first 6 h after withdrawal

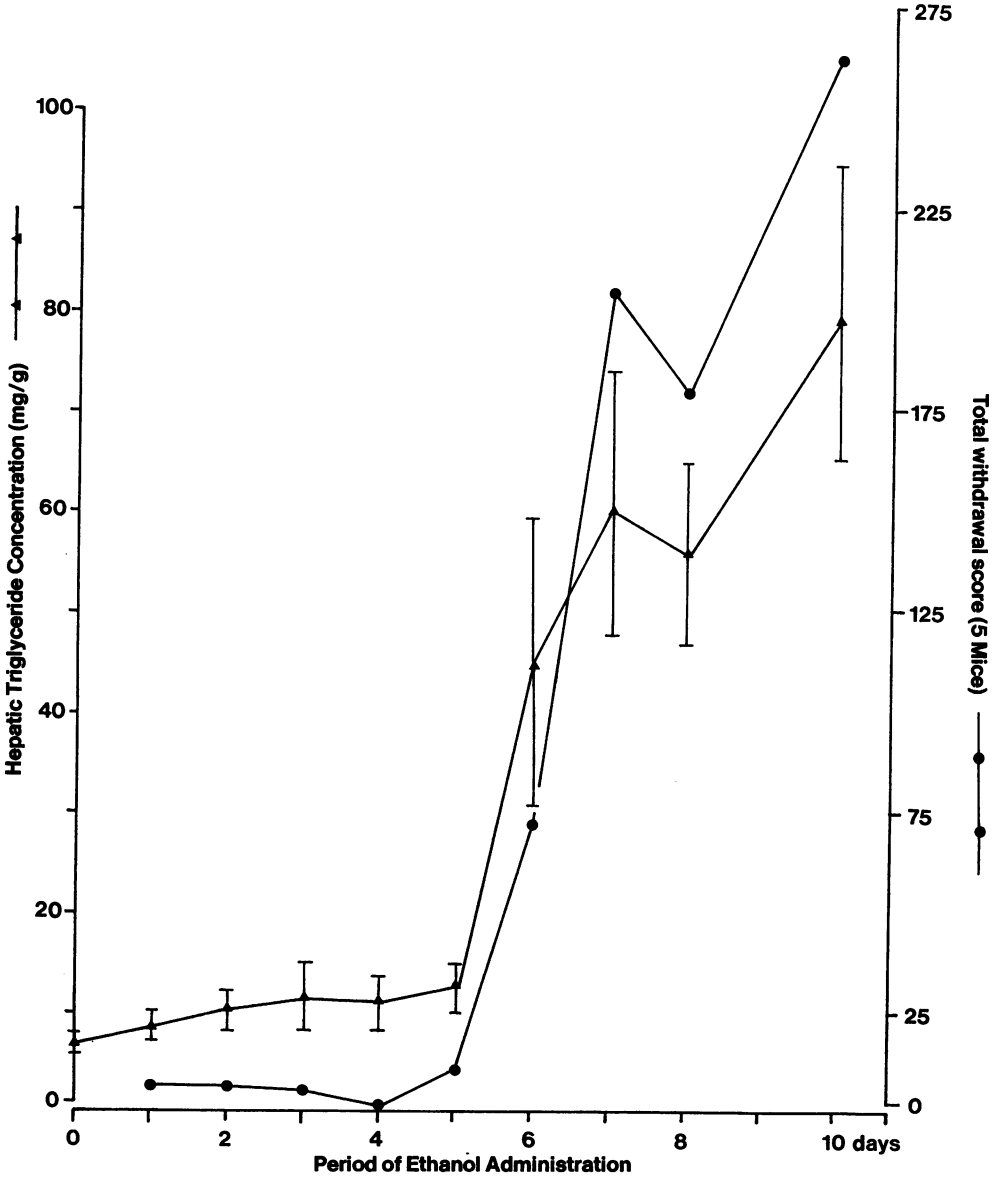

FIG. 1(a). See page 610 for legend.

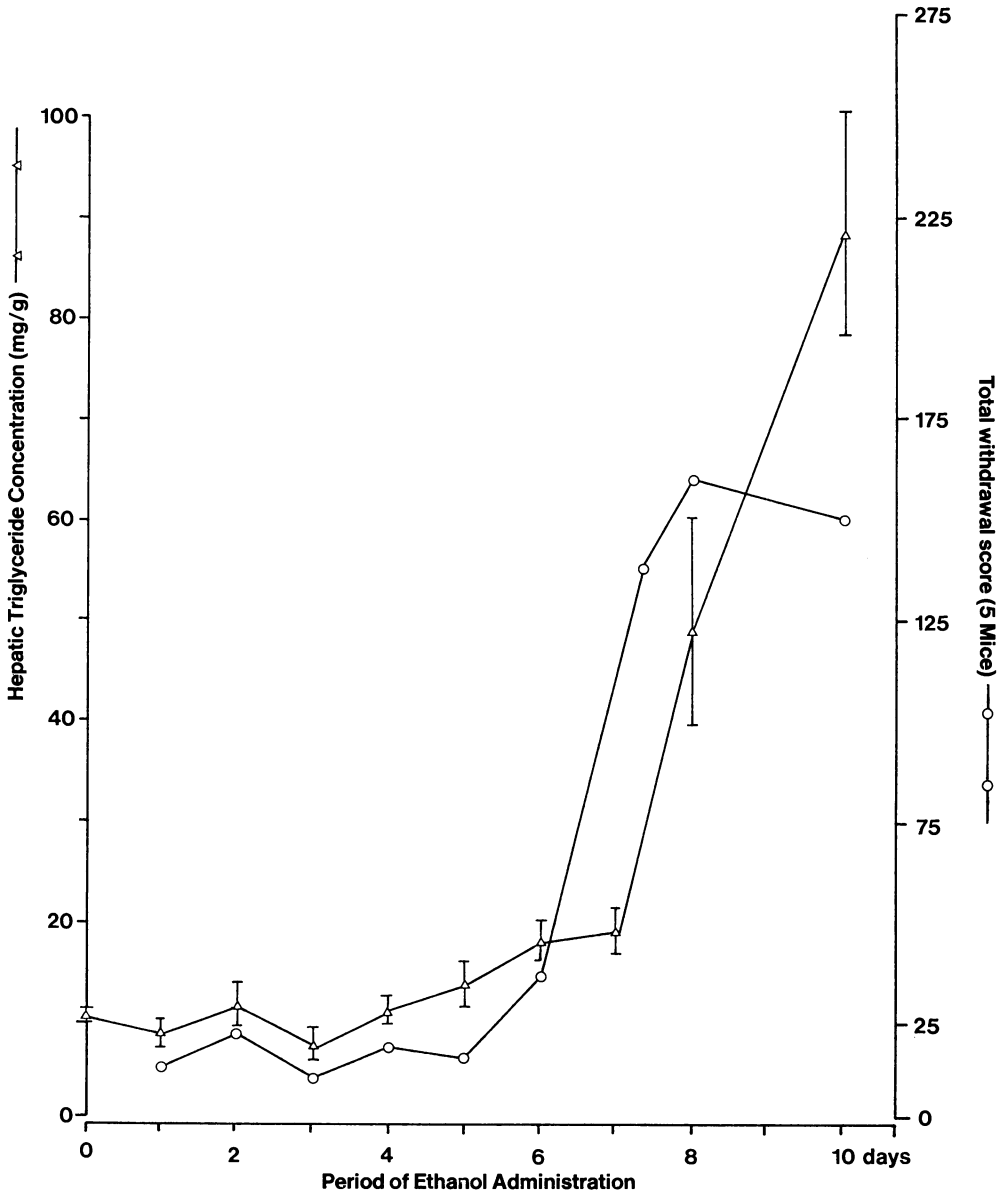

FIG 1(b)

FIG. 1.—Male and female mice were exposed to the same concentration of ethanol vapour for 10 days. On each day 10 animals of each sex were removed from the inhalation chamber, 5 killed for hepatic triglyceride accumulation and 5 tested for the ethanol withdrawal syndrome. Fig. 1a shows the results obtained for male mice; Fig. 1b the results for female mice. Hepatic triglyceride accumulation is expressed as the concentration of triglycerides in mg/g wet weight of liver. Bars represent the s.e. mean of at least 5 observations. The withdrawal score is expressed as the total score obtained for 5 animals tested hourly over a 10-h period. Further details, see text.

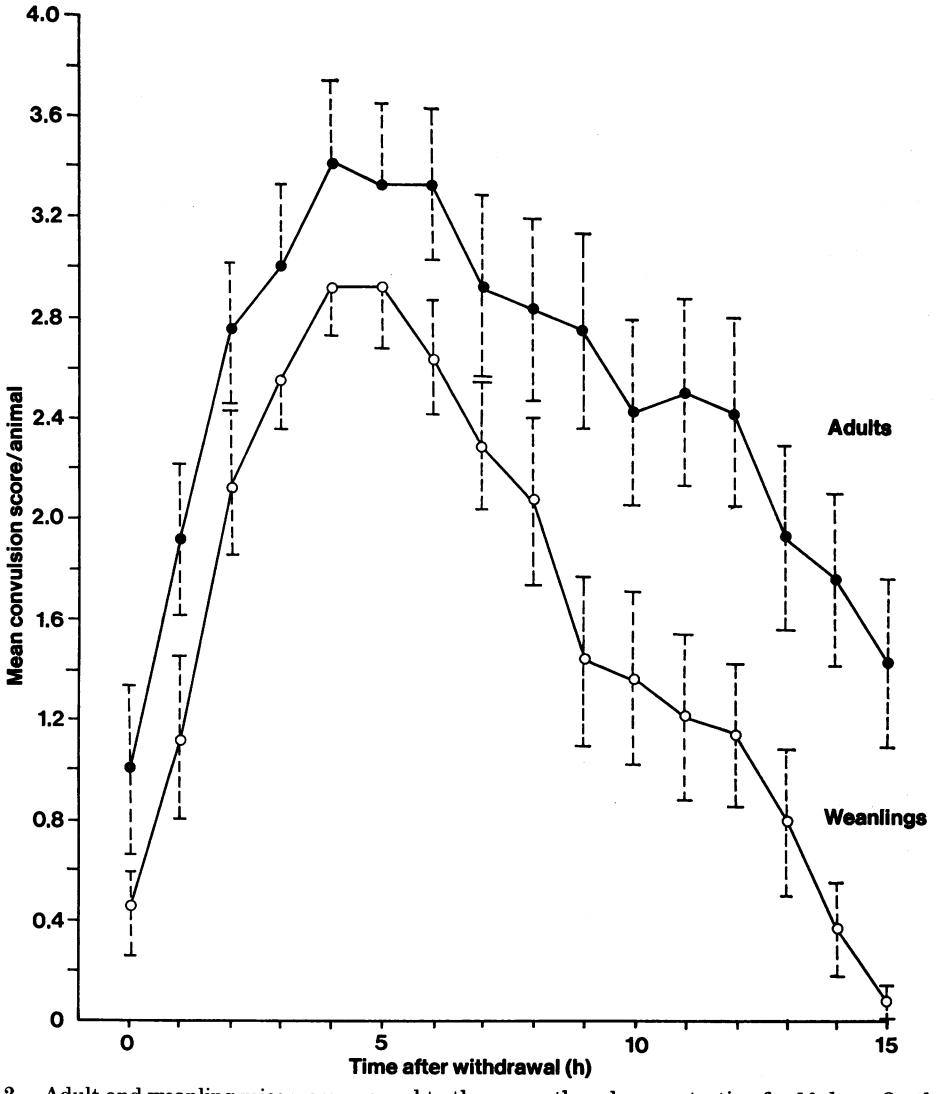

FIG. 2.—Adult and weanling mice were exposed to the same ethanol concentration for 10 days. On the tenth day ethanol was removed from the inspired air and the mice tested for the ethanol withdrawal syndrome. Fig. 2 shows a comparison between the convulsion scores for adult and weanling mice over a period of 15 h after withdrawal. Bars represent the s.e. mean of at least 15 observations at each point. Further details are given in the text.

(Fig. 2). After this time significant differences in the severity of withdrawal signs were observed, weanling animals achieving significantly ($P<0{\cdot}05$) lower withdrawal scores (Fig. 2). Comparison of hepatic triglyceride concentrations in adults and weanlings after withdrawal showed that hepatic triglyceride concentrations had fallen much more rapidly in weanlings (Table I).

Effect of carnitine on hepatic triglyceride accumulation and on the ethanol withdrawal syndrome

The addition of 7% w/w DL-carnitine to the normal laboratory diet appeared

TABLE I: *Fall in Hepatic Triglyceride Concentrations during Withdrawal of Ethanol from Dependent Mice*

Treatment	Blood ethanol on withdrawal mg/ml ±s.e.m.	n	Hepatic triglyceride concentration: At withdrawal mg/g ±s.e.m.	n	4 h after withdrawal mg/g ±s.e.m.	n	8 h after withdrawal mg/g ±s.e. m.	n	12 h after withdrawal mg/g ±s.e.m.	n
Control adult	—		9·79±1·13	5	—		—		—	
Control weanling	—		7·03±0·35	5	—		—		—	
Adults. Ethanol vapour 10 days	2·21±0·65	5	28·20±2·93	5	23·97±2·11	5	21·10±0·92	5	18·82±0·77	4
Weanlings. Ethanol vapour 10 days	2·57±0·41	5	53·39±4·32	5	39·09±2·62	5	23·87±1·72	5	10·97±0·19	5

Adult and weanling mice were exposed to the same concentration of ethanol vapour for 10 days. At no time during this period was a significant difference observed between blood ethanol concentrations in the two groups. On withdrawal of ethanol after 10 days animals of each group were killed for hepatic triglyceride estimation (expressed as triglyceride concentration/g wet weight of liver) and the remainder used to assess the ethanol withdrawal syndrome. After 4, 8, and 12 h, mice were taken at random from the group undergoing withdrawal and killed for hepatic triglyceride estimation. For further details see text.

TABLE II. *Effect of Carnitine on Hepatic Triglyceride Accumulation and Ethanol Withdrawal Severity in Young Adult Mice*

Treatment	Blood ethanol on withdrawal mg/ml ±s.e.m.	n	Mortality %	Hepatic triglyceride on withdrawal mg/g ±s.e.m.	n	Mean withdrawal score/animal Mean score ±s.e.m.	n
Control (normal diet)	—		—	7·4±0·65	6	3·1±0·8	7
Control (Carnitine supplement)	—		—	7·02±0·84	6	2·7±0·7	7
Ethanol vapour (normal diet)	1·72±0·07	7	30	62·8±4·32*	5	26·6±0·9*	9
Ethanol vapour (carnitine supplement)	2·00±0·21	7	10	36·66±5·78*	6	11·2±2·4*	10

* Represents a significant ($P < 0·05$ in a Student's one-tailed t test) difference between the two experiments groups.

Groups of young adult male mice receiving either a normal powdered laboratory diet, or a diet to which 7% w/w DL-carnitine had been added, were exposed to the same concentration of ethanol vapour for 10 days. At no time during this period were significant differences observed between blood ethanol concentrations in these groups. After 10 days some mice were killed for estimation of hepatic triglycerides, and the remainder were assessed for the severity of the ethanol withdrawal syndrome. For further details see text.

not to affect ethanol metabolism in experimental animals, since similar blood ethanol concentrations were obtained whether animals received this addition or not (Table II). Administration of carnitine in this way largely prevented the accumulation of triglycerides in livers of ethanol-treated mice (Table II) and also markedly reduced the mean total withdrawal score (Table II). An additional observation was that DL-carnitine seemed to reduce the mortality associated with ethanol administration (this was noticed in three successive experiments). The addition of carnitine had no effect on the triglyceride concentration in livers from control animals maintained on this diet (Table II).

Effect of carbon tetrachloride administration on hepatic triglyceride accumulation and on the ethanol withdrawal syndrome

The administration of carbon tetrachloride (1·3 ml/kg i.p.) on the first day of ethanol administration produced a small but not significant increase in the severity of the withdrawal syndrome when ethanol was withdrawn 9 days later (Table III).

TABLE III.—*Effect of Carbon Tetrachloride Administration on the First Day of Ethanol Exposure on Subsequent Hepatic Triglyceride Accumulation and Ethanol Withdrawal Severity in Young Adult Mice*

Treatment	Blood ethanol on withdrawal mg/ml ±s.e.m.	n	Mortality %	Hepatic triglyceride on withdrawal mg/g ±s.e.m.	n	Mean withdrawal score/animal Mean score ±s.e.m.	n
Untreated control	—		—	7·08±0·95	6	—	
Control+arachis oil BP	—		—	13·53±1·39	8	—	
Control+CCL_4 i.p.	—		—	15·97±2·37	6	—	
Ethanol vapour+arachis oil BP	1·48±0·14	7	25	36·66±3·88	5	27·0±2·3	9
Ethanol vapour+CCl_4 i.p.	1·61±0·14	7	50	37·90±4·56	5	33·9±1·3	9

Groups of young adult male mice were injected with carbon tetrachloride (1·3 ml/kg in arachis oil 0·2 ml i.p.) or with the vehicle alone on the first day of exposure to the same concentration of ethanol vapour. After 10 days of exposure to ethanol (no significant difference in blood ethanol concentrations between groups) mice were removed from the inhalation chambers. Some were killed for hepatic triglyceride estimation, and the remainder were used to assess the severity of ethanol withdrawal. For further details, see text.

TABLE IV.—*Effect of Carbon Tetrachloride Administration on the Penultimate Day of Ethanol Exposure on Hepatic Triglyceride Accumulation and Ethanol Withdrawal Severity in Young Adult Mice*

Treatment	Blood ethanol on withdrawal mg/ml ±s.e.m.	n	Mortality %	Hepatic triglyceride on withdrawal mg/g ±s.e.m.	n	Mean withdrawal score/animal Mean score ±s.e.m.	n
Untreated control	—		—	7·08±0·95	6	—	
Control+arachis oil BP	—		—	59·02±8·82	6	—	
Control+CCl_4 i.p.	—		—	69·00±3·48	7	—	
Ethanol vapour+arachis oil BP	1·91±0·08	7	20	72·95±6·07*	6	26·5±2·5	9
Ethanol vapour+CCl_4 i.p.	2·19±0·1	7	25	114·44±4·5*	5	30·9±2·3	9

Experimental conditions were the same as those described under Table III, except that in these experiments additional drug treatments were made on the ninth day of ethanol exposure. For further details see text.

* Indicates a significant difference ($P<0·05$ in Student's one-tailed t test) between the experimental groups.

There was no significant alteration of ethanol-induced triglyceride accumulation when this was measured immediately before ethanol withdrawal (Table III). Blood ethanol concentrations were similar in the two groups. An increase in mortality was observed in carbon tetrachloride-treated mice. Control animals showed little evidence of triglyceride accumulation 9 days after carbon tetrachloride administration (Table III). When tested for the ethanol withdrawal syndrome for a short period 9 days after carbon tetrachloride administration, control animals showed no evidence of a "withdrawal syndrome".

The administration of carbon tetrachloride on the penultimate day of inhalation of ethanol produced a marked increase in hepatic triglyceride accumulation measured before ethanol withdrawal (Table IV). Despite this increase in triglyceride concentration there was no significant change in the severity of the ethanol withdrawal syndrome (Table IV). The small increase in severity observed was due entirely to an increase in piloerection scores for carbon tetrachloride-treated animals. Since piloerection was observed to be marked even while these animals were still receiving ethanol, this change cannot be considered to indicate potentiation of a *withdrawal* sign. There was little difference in mortality between groups and blood ethanol concentrations were similar.

The administration of carbon tetrachloride, or indeed of arachis oil alone, produced significant increases in hepatic triglyceride concentrations in control animals after 24 h (Table IV). However, there was no evidence of any of the behavioural changes associated with ethanol withdrawal in these animals other than some piloerection.

DISCUSSION

The complexity of changes in central neurotransmitter metabolism observed in ethanol-dependent animals (see Littleton, 1975) has encouraged us to seek a more fundamental alteration of brain metabolism in dependence. As a first hypothesis we have considered whether ethanol-induced hepatic dysfunction may contribute to changes associated with ethanol dependence.

When adult male mice are exposed to ethanol vapour the onset and development of physical dependence runs a characteristic time course. We now report that accumulation of triglycerides in liver runs a similar time course. Male and female mice of the same strain differ in the onset of signs of physical dependence, in that these are delayed in female mice by 1 or 2 days. A similar delay occurs in the accumulation of triglycerides in livers of female mice. These results suggest that a relationship exists between accumulation of triglycerides in the liver and the onset of signs of physical dependence on ethanol. Results obtained by comparing weanling and adult mice suggest a further relationship between hepatic triglyceride concentration and the ethanol physical withdrawal syndrome. Weanling mice exhibit a withdrawal syndrome which is of shorter duration than that of adults. Weanling mice also show a more rapid reversal of hepatic triglyceride accumulation after withdrawal of ethanol. Additional evidence for a relationship between triglyceride accumulation and dependence is provided by the observation (Griffiths, Abu Murad and Littleton, 1977) that mice of different strains show hepatic triglyceride accumulation during ethanol administration in proportion to the severity of the subsequent withdrawal syndrome.

These results raise the question of how these changes in liver could be related to the changes in brain which produce the signs of physical dependence. One possibility is that triglyceride accumulation in liver produces or reflects a dysfunction of the liver which is related to these central changes. The alternative is that accumulation of triglycerides in liver is part of a more general alteration of lipid metabolism which is itself related to dependence. These alternatives were investigated by the use of compounds which affect the hepatotoxicity of ethanol.

Carbon tetrachloride potentiates ethanol hepatotoxicity (Strubelt, Buettner and Siegers, 1975) and produces hepatic triglyceride accumulation primarily by an effect on the liver (Recknagel, 1967; Plaa and Witschi, 1976). Therefore, if ethanol-induced liver damage causes the changes in brain associated with dependence, then carbon tetrachloride administration should potentiate the induction of ethanol dependence. This was not observed to be the case. Although carbon tetrachloride administration increased hepatic triglyceride accumulation, it did not increase the severity of the ethanol withdrawal syndrome. These results do not prove that liver function plays no role in ethanol dependence, but they favour the alternative explanation, that ethanol-induced triglyceride accumulation in the liver is part of a wider disturbance of lipid metabolism involved in dependence.

DL-carnitine reduced the accumulation of triglycerides in livers of mice exposed to ethanol vapour, and also reduced the severity of the ethanol withdrawal syndrome. DL-carnitine has been reported to reduce both ethanol hepatotoxicity and hepatic triglyceride accumulation, but by different mechanisms (Hosein and Bexton, 1975). Carnitine has a general role in fatty acid transport across mitochondrial membranes, and probably reduces triglyceride accumulation by stimulating fatty acid

utilisation (Hosein and Bexton, 1975). Thus carnitine could be affecting ethanol dependence either by a protective action on the liver, by its widespread effects on lipid metabolism, or by some other mechanism. The results discussed previously suggest that the second mechanism is the most likely.

The implications of these findings is that ethanol produces an alteration in lipid metabolism which may be causally related to the induction of physical dependence. That massive dietary supplementation with carnitine can inhibit this change argues that carnitine-dependent transfer of fatty acids across mitochondrial membranes may be deficient. Katsumata (1970) has demonstrated that mitochondria from animals treated chronically with ethanol are relatively resistant to carnitine, suggesting that the fundamental defect may lie in the mitochondrial membrane.

REFERENCES

ABU MURAD, C., GRIFFITHS, P. J. & LITTLETON, J. M. (1976) Catecholamine Metabolism and the Role of Liver Dysfunction in the Induction of Ethanol Dependence. *Br. J. Pharmac. Chemother.*, **56**, 377.

BERGHAU, G., KURZ, A. & DOTZAUER, G. (1975) Fatty Acid Pattern of Human Fat and Liver Tissue in Alcoholics and Non-alcoholics: Fatty Acid Pattern as a Measure of Alcoholism. *Dt. med. Wschr.*, **100**, 1233.

FOLCH, J., LEES, M. & SLOANE STANLEY, G. H. (1957) A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues. *J. Biol. Chem.*, **226**, 497.

GOLDSTEIN, D. B. (1973) Inherited Differences in Intensity of Alcohol Withdrawal Reactions in Mice. *Nature*, **245**, 154.

GRIFFITHS, P. J., ABU MURAD, C. & LITTLETON, J. M. (1977) Ethanol-induced Hepatic Triglyceride Accumulation in Mice and Genetic Differences in the Ethanol Physical Withdrawal Syndrome. *Br. J. Addict. Alcohol.* (in press).

GRIFFITHS, P. J., LITTLETON, J. M. & ORTIZ, A. (1973) A Method for the Induction of Dependence on Ethanol to Mice. *Br. J. Pharmac. Chemother.*, **47**, 669.

GRIFFITHS, P. J., LITTLETON, J. M. & ORTIZ, A. (1974) Changes in Monoamine Concentration in Mouse Brain Associated with Ethanol Dependence and Withdrawal. *Br. J. Pharmac. Chemother.*, **50**, 489.

HOSEIN, E. A. & BEXTON, B. (1975) Protective Action of Carnitine on Liver Lipid Metabolism after Ethanol Administration to Rats. *Biochem. Pharmac.*, **24**, 1859.

HUNT, W. A. & MAJCHROWICZ, E. (1974) Turnover Rates and Steady State Levels of Brain Serotonin in Alcohol Dependent Rats. *Brain Res.*, **72**, 181.

INGRAM, L. O. (1976) Adaptation of Membrane Lipids to Alcohols. *J. Bact.*, **125**, 670.

KALANT, H., KHANNA, J. M., SEYMOUR, F. & LOTH, J. (1975) Acute Alcoholic Fatty Liver—Metabolism or Stress. *Biochem. Pharmac.*, **24**, 431.

KATSUMATA, K. (1970) Effect of Carnitine on the Oxidation of Palmitate in Alcohol-fed Liver Mitochondria. *J. Vitam.*, **16**, 249.

LESCH, P., SCHMIDT, E. & SCHMIDT, F. W. (1973) Effects of Chronic Alcohol Abuse on the Fatty Acid Composition of the Major Lipids in the Human Brain. *Z. klin. Chem.*, **11**, 159.

LIEBER, C. S. & RUBIN, E. (1968) Alcoholic Fatty Liver in Man on a High Protein and Low Fat Diet. *Am. J. Med.*, **44**, 200.

LITTLETON, J. M. (1975) The Experimental Approach to Alcoholism. *Br. J. Addict Alcohol*, **70**, 99.

PLAA, G. L. & WITSCHI, H. (1976) Chemicals, Drugs and Lipid Peroxidation. *Ann. Rev. Pharm. & Tox.*, **16**, 125.

POHORECKY, L. A., JAFFE, L. S. & BERKELEY, H. A. (1974) Effects of Ethanol on Serotonergic Neurons in the Rat Brain. *Res. Commun. Chem. Pathol. Pharmac.*, **8**, 1.

RECKNAGEL, R. O. (1967) Carbon Tetrachloride Hepatoxicity. *Pharm. Revs.* **19**, 145.

STRUBELT, O., BUETTNER, F. & SIEGERS, C. P. (1975) The Hepatotoxic Effect of Ethanol after Pretreatment with Carbon Tetrachloride, Allyl Alcohol or D-galactosamine. *Naunyn Schmiedeberg's Arch. exp. Path. Pharmac.*, **287**, suppl. R102.

VAN HANDEL, E. & ZILVERSMIT, D. B. (1957) Micromethod for the Direct Determination of Serum Triglycerides. *J. Lab. clin. Med.*, **50**, 152.