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EFFECT OF CHRONIC ETHANOL TREATMENT ON TRYPTOPHAN OXYGENASE, TYROSINE AMINOTRANSFERASE AND GENERAL PROTEIN METABOLISM IN THE INTACT AND PERFUSED RAT LIVER

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Abstract—Male Wistar rats (275–300 g body wt) were given ethanol to provide 31 per cent of the total calories, while sucrose replaced ethanol isocalorically in controls. Thirty-six per cent of the calories were provided by fat in both groups.

Tryptophan oxygenase (TO) activity was reduced to about 60 per cent of control activity in livers from rats treated with ethanol for 38 days. This effect was not a consequence of a reduction of *in vivo* corticosterone levels in the treated animals. Tyrosine aminotransferase (TAT) activity was unchanged in these livers. When the livers were perfused with dexamethasone (20 μg/ml), the induction of both enzymes was significantly lower in ethanol-treated rats. The incorporation of labelled amino acids into protein 30 and 45 min after the addition of label was also reduced in livers from treated rats. The degradation of TO, TAT and general proteins, measured in the perfused liver after cycloheximide (50 μg/ml) was not significantly affected by previous ethanol consumption. No obvious leakage of liver proteins to the perfusate could be registered after ethanol treatment. Ethanol treatment did not significantly influence plasma bilirubin, liver bile production, liver lipids or gross histological appearance of the liver. It was concluded that liver TO, TAT and general protein synthesis was reduced as a consequence of the previous chronic intake of ethanol independent of changes in the other liver parameters measured.

STUDIES on the effects of ethanol on the liver have been concerned mostly with lipid and carbohydrate metabolism and less with protein metabolism. Some authors failed to observe an effect of acute ethanol treatment on general liver protein synthesis,[1–3] although reduction of albumin synthesis[4,5] and *in vitro* protein synthesis[6,7] has been reported recently. Chronic ethanol treatment has been found by some authors to reduce general liver protein synthesis[8,9] and reduce mitochondrial protein synthesis,[7] while others have observed no effect of such treatment on general protein[10] or albumin synthesis.[4] Increased protein synthesis *in vitro* has been reported after prolonged ethanol intake.[6]

This report deals with the effect of chronic ethanol consumption on the metabolism of total liver protein and that of two liver enzymes, tryptophan oxygenase (TO) and tyrosine aminotransferase (TAT) in isolated perfused rat livers. Both enzymes have a rapid turnover[11,12] and their activities are thus easily influenced by changes in enzyme synthesis or breakdown. Both enzymes are induced by corticosteroids[12,13] and may also serve as markers of corticosteroid action on the liver.

Intake of ethanol has been shown to cause fatty liver[14] and more pronounced signs

of liver damage such as increased enzyme leakage and histological changes.[15,16] The exact mechanisms underlying these changes are still discussed. The route of administration, amount and concentration of alcohol[17] as well as the composition of the diet[18] may influence the response of the liver to ethanol. In the experiments reported here the state of the liver has been characterized by determination of lipid content, enzyme leakage, histological changes and bile production.

MATERIAL AND METHODS

Animals. Male Wistar rats (275–300 g body wt) were divided into two groups; the rats from each group were housed in separate plastic boxes (two animals per box) at 21°, 60 per cent humidity with 12-hr periods of darkness from 7 p.m. to 7 a.m.

Ethanol treatment. The animals were given the following diets for 38 days. One group (ethanol group) received a liquid diet containing 32% ethanol (v/v) and 25% sucrose (w/w) *ad libitum*.[18] The mean consumption per rat per day of this solution was 9 ml when it was the sole drinking fluid or when an equal volume of tap water was given in addition. Forty per cent sucrose (w/w) replaced ethanol isocalorically in the second group (control group). The amount of tap water given to the first group was adjusted so that the rats in both groups received the same amount (approximately 18 ml) of liquid per day. A solid food mixture of fat (soybean oil/refined lard, 1:1), protein (casein), and carbohydrate (sucrose/starch, 1:1) was given to both groups *ad libitum* to provide approximately 52 per cent of the total calories. The rats derived 36 and 13 per cent respectively of their total calories from fat and protein. The ethanol group obtained 20 per cent of the calories from carbohydrate and 31 per cent from ethanol, while carbohydrates provided 51 per cent of the total calories to the control group. In addition to the main nutrients the solid food contained per 10 kg: 900 g U.S.P. XIV salt mixture (Nutritional Biochemical Cooperation), 0·9 g Rovimix AD_3, 500/72 (Hoffmann–La Roche & Co.), 6 g Rovimix E (absorbate 25%, 250 I.E. per g, Hoffmann–La Roche & Co.), 150 mg 2-methyl-1,4-naphthohydroquinone (3·7 g Synkavit, Hoffmann—La Roche & Co.), 20 g choline chloride (Sigma), 30 g D,L-methionine (Sigma), 40 g L-cystine (Sigma), 3·7 g inositol (Sigma), 2·4 g *p*-aminobenzoic acid (Grade II-F, Sigma), 120 mg thiamine-HCl (Nutritional Biochemical Cooperation), 120 mg riboflavine (Nutritional Biochemical Cooperation), 1·5 g niacin amide (Nutritional Biochemical Cooperation), 300 mg D-Ca-pantothenate (Nutritional Biochemical Cooperation), 120 mg pyridoxine-HCl (Nutritional Biochemical Cooperation), 45 mg folic acid (crystalline, Nutritional Biochemical Cooperation), 5 mg D-biotin (crystalline, Nutritional Biochemical Cooperation), and 15 mg vitamin-B_{12} (crystalline, Sigma). The lipotropic index (mg choline + 1/3 mg methionine per 100 cal[19]) of the final diet consumed by the rats was 25. The liquid and solid diets were either given up to the time of decapitation, see below (fed rats) or they were replaced with water only for the last 24–30 hr before killing (fasted rats).

Preparation of samples for histologic examination. Fed rats from both groups were killed by decapitation and the livers were excised quickly. Small samples of liver (100 mg) were fixed in 4% formaldehyde, stored in 60% ethanol (v/v), embedded in paraffin, sliced, stained and examined under the light microscope.

Lipid determinations. A liver sample (approximately 3 g) obtained from fed rats killed by decapitation (see above) was frozen in liquid nitrogen. The lipids were ex-

tracted by Folch's procedure[20] and triglycerides and phospholipids were separated by thin layer chromatography.[21] Triglycerides were then determined by the method of Carlson and Wadstrøm,[22] the phospholipids were determined by phosphate analysis[23] after complete hydrolysis. Tripalmitate and KH_2PO_4 were used as the respective standards.

Analysis of rat plasma. Blood was withdrawn under ether anaesthesia from the abdominal aorta of fasted rats in order to determine plasma corticosterone concentration.[24,25] Plasma bilirubin concentration was determined with an autoanalyser (Technicon method AA II-18 based upon the method of Jendrassik and Grof[26]). Plasma protein was determined by a modification (Technicon method N-14b) of the biuret[27] method. Activities of the following enzymes were measured in plasma; β-glucuronidase (β-D-glucuronide glucuronohydrolase, EC 3.2.1.31),[28] alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1), aspartate aminotransferase, GOT (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1), and alanine aminotransferase, GPT (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2). β-glucuronidase activity was expressed as μg reaction product (phenolphthalein) formed per hr and 5 ml plasma, the other enzyme activities as international units. All enzyme activities except that of β-glucuronidase were measured automatically (LKB, kinetic test recording initial reaction velocity) based upon original methods.[29–31]

Liver analysis. A minor hepatic lobe of fasted rats was ligated under anaesthesia (see above), cut off and frozen in liquid nitrogen. Duplicate specimens were analysed for tryptophan oxygenase activity (L-tryptophan: oxygen oxidoreductase, EC 1.13.1.12),[32] and tyrosine aminotransferase activity (L-tyrosine-2-oxoglutarate aminotransferase, EC 2.6.1.5).[33] Enzyme units were equal to the number of μmoles of reaction product formed per hr at 37°. pH[34] and protein content[35] were measured in duplicate specimens of liver homogenates. Cyclic AMP levels were determined according to Gilman.[36]

Liver perfusion. The livers from which a lobe had been taken (see above) were perfused as described elsewhere.[34] Male Wistar rats (400 g) given ordinary laboratory rat chow were fasted for 24 hr and used as blood donors. The perfusate consisted of blood diluted with buffer[34,37] to give a final hematocrit of 20%. Forty ml perfusate was recirculated through the liver at 37°. The pH of the perfusate was maintained at 7·4 by means of 0·5 N $NaHCO_3$. The mean perfusate flow rate was 2 ml/min/g liver for both ethanol-treated and control rats. The bile production was the same (approximately 50 μl/hr/g) in both groups, and no abnormalities in the general appearance of all livers used were recorded. The relative protein content increased slightly in livers from both ethanol-treated and control animals, from 20·1 and 20·4 per cent of wet weight liver initially to 24·2 and 23·0 per cent respectively measured after 3 hr perfusion. After 5 hr of perfusion the relative protein contents were 23·2 (ethanol-treated) and 23·5 (control) per cent. All perfusions started about noon, two ethanol-treated and two control livers were always perfused in parallel. In some experiments dexamethasone (Decadron, a gift from Merck-Sharp & Dohme) was added to the perfusate after 10 min (final concentration 20 μg/ml), and cycloheximide (Sigma) after 3 hr (final concentration 50 μg/ml). Liver specimens were taken in duplicate for TO and TAT analysis at the times indicated.

Measurement of incorporation of labelled amino acids into protein. Three μCi (200 μl)

of (U-^{14}C) protein hydrolysate (CFB. 25, 54 mCi/m Atom carbon, The Radiochemical Centre, Amersham) was added to the perfusate after 15 min. Liver samples were taken at the times indicated, frozen in liquid nitrogen and analysed for labelled protein in the following way: The samples were homogenized, and protein was precipitated with 20 vol. ice-cold 10% trichloroacetic acid (TCA). One aliquot of the supernatant was taken for determination of TCA-soluble counts, and another aliquot for the determination of ninhydrin positive substances[38] after removal of TCA by one ether extraction. The precipitate was washed three times in ice-cold 5% TCA, and nucleic acids were solubilized by incubation in 5% TCA at 80° for 20 min. After two additional washes at 0° in 5% TCA, the precipitate was washed once in 70% ethanol at 0°, twice in 96% ethanol at room temperature and finally twice in ether at room temperature. Proteins from the perfusate were prepared in the same way.

One mg liver protein or 5 mg plasma protein was dissolved in glass counting vials using 0·2 ml Soluene 100 (Packard) at 50° for 30 min. Ten ml of scintillation fluid (PPO 4 g, dimethyl POPOP 0·05 g, toluene 1000 ml, glacial acetic acid 1 ml)[39] was added, and the radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer, model 3003, operated at 0°–5°.

RESULTS

Effect of ethanol treatment on tryptophan oxygenase activity

Tryptophan oxygenase activity was reduced in rats which had received ethanol for 38 days. This reduction was to about 60 per cent of control activity when related to either 100 g rat weight, wet weight of liver or g liver protein. The reduction was not due to changes in postmortal TO-activation[34] or to changes in kynurenine metabolism in the assay mixture[34] (Table 1).

TABLE 1. EFFECT OF CHRONIC ETHANOL TREATMENT ON RAT WEIGHT, LIVER WEIGHT, PROTEIN CONTENT, TRYPTOPHAN OXYGENASE ACTIVITY, HOMOGENATE pH, TYROSINE AMINOTRANSFERASE ACTIVITY AND CYCLIC AMP CONCENTRATION IN FASTED RATS*

	Ethanol-treated rats	Control rats	Significance
No. of rats	10	10	—
Initial body weight (g)	286·5 ± 5·8	295·5 ± 3·8	N.S.
Final body weight (g)	297·5 ± 9·3	302·2 ± 8·6	N.S.
Liver weight (g)	7·44 ± 0·23	8·12 ± 0·26	0·031
Liver protein (mg/g wet wt)	201·0 ± 5·0	204·1 ± 5·3	N.S.
Tryptophan oxygenase activity:			
(units/100 g rat wt)	5·9 ± 0·8	10·6 ± 1·1	0·008
(units/g liver wet wt)	2·36 ± 0·33	3·93 ± 0·41	0·008
(units/g liver protein)	11·7 ± 1·5	19·3 ± 2·0	0·008
Liver homogenate pH	6·83 ± 0·02	6·85 ± 0·01	N.S.
Tyrosine aminotransferase activity:			
(units/100 g rat wt)	225 ± 13	220 ± 24	N.S.
(units/g liver wet wt)	90 ± 5	82 ± 9	N.S.
(units/g liver protein)	448 ± 24	404 ± 47	N.S.
Cyclic AMP (pmoles/mg liver wet wt)	0·81 ± 0·07	0·92 ± 0·11	N.S.

* The results are given ± standard error, S.E.M. The significance levels according to Wilcoxon's test are given, considering levels above 0·05 as insignificant, N.S.

Intracellular acidity was recorded (Table 1) since an increase in acidity has been shown to be followed by reduced TO-activity.[34] Table 1 shows that previous ethanol treatment did not influence the pH of the liver homogenate. TO-activity has been found to increase after increasing plasma concentration of corticosterone,[40,41] the main adrenal steroid hormone in rats. The plasma corticosterone level (Table 3) in control rats was 68·5 μg/100 ml vs. 95·8 μg/100 ml in rats subjected to ethanol-treatment.

Both groups of rats showed reduced weight gain compared to rats on ordinary laboratory chow diet. The liver weights were somewhat reduced after fasting in the ethanol-treated rats (Table 1).

Biochemical mechanism responsible for reduction of tryptophan oxygenase activity

The biochemical mechanism underlying the reduced TO-activity was investigated by the following experiments.

Mixed homogenate experiments. A liver homogenate from an ethanol-treated rat was mixed with a homogenate from a control rat. The activity of the mixture was measured and compared to the calculated activity. In four such experiments the mean activity measured was exactly equal to the activity calculated.

Liver perfusion experiments. Livers from which a specimen had been taken for TO-analysis were perfused (Fig. 1, dotted lines). The initial difference in TO-activity was almost unchanged after 3 hr perfusion. When dexamethasone was added to the perfusates, TO-activity increased both in control and ethanol pretreated livers (Fig. 1, solid lines). The increase in enzyme activity after 3 hr was 36·7 units/g liver protein i.e. 190 per cent of the original value (control group) and 17·1 units/g liver protein i.e.

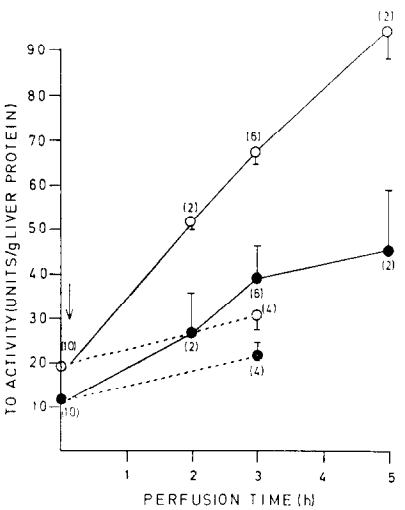

FIG. 1. Tryptophan oxygenase activity in isolated perfused livers from fasted rats. The initial values represent the enzyme activities while the livers were still *in situ*. Nothing was added to four livers from control animals (○ – – – ○), and to four livers from ethanol pretreated animals (● – – – ●). Dexamethasone (20 μg/ml was added after 10 min (↓) to six control livers (○——○), and to six livers from rats pretreated with ethanol (●——●). The number of livers analysed for each point is shown in parentheses. Vertical lines indicate the standard error, S.E.M. The significance level of the difference between control and ethanol-pretreated livers after 3 hr dexamethasone induction was 0·016 according to Wilcoxon's paired-comparison test.

145 per cent of the original value (ethanol group) respectively, compared to perfusions without hormone. The induction recorded in ethanol-pretreated livers was thus only about 50 per cent of that in control livers. The TO-activities were also higher in control rats than in treated rats 2 and 5 hr after dexamethasone induction.

When, after perfusion for 3 hr, TO-synthesis was stopped by the inhibitor of protein synthesis cycloheximide,[42] the enzyme activity declined during the following 2 hr (Fig. 2a). The relative reduction (slope of lines) was apparently independent of the enzyme activity measured at 3 hr. The mean relative reduction of TO-activity (Fig. 2b) was slightly although not significantly greater in control rats (64·1 per cent/2 hr) than in ethanol-treated rats (57·4 per cent/2 hr).

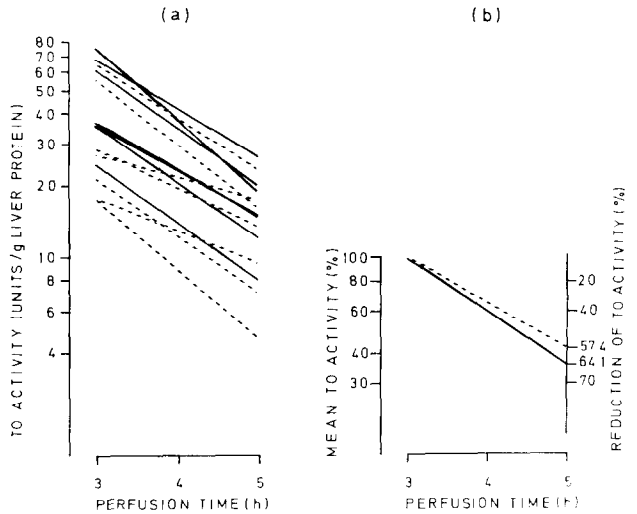

FIG. 2. Reduction of induced and basal tryptophan oxygenase activity in perfused rat livers after the administration of cycloheximide (50 μg/ml) at 3 hr. The enzyme activities were measured at 3 and 5 hr. The results of the individual experiments are shown as a semilogarithmic plot in (a) for seven control livers (——) and seven livers from animals pretreated with ethanol (- - -). The mean per cent reduction is plotted in (b). The difference between control and ethanol-treated animals (64·1–57·4) was not statistically significant according to the Wilcoxon test.

Effect of chronic ethanol consumption on tyrosine aminotransferase activity

Chronic ethanol treatment did not influence the hepatic TAT-activity (Table 1). The level of cyclic AMP, one of the factors controlling TAT-activity,[43] was not significantly changed (Table 1).

After perfusion for 3 hr the activity of TAT was almost unchanged in livers from both ethanol-treated and control animals (Fig. 3). When TAT was induced by dexamethasone during perfusion, a much larger increase of enzyme activity was recorded in control livers than in livers from ethanol-treated rats (Fig. 3). The respective increases in enzyme activities were 548 units/g liver protein i.e. 255 per cent of original value (control) and 204 units/g liver protein i.e. 175 per cent of original value (ethanol) compared to perfusions without hormone; i.e. ethanol consumption reduced the induction to about 40 per cent of that found in controls.

After perfusion for 3 hr TAT-synthesis was stopped by the addition of cycloheximide. During the following 2 hr the enzyme activity declined as shown in Fig. 4.

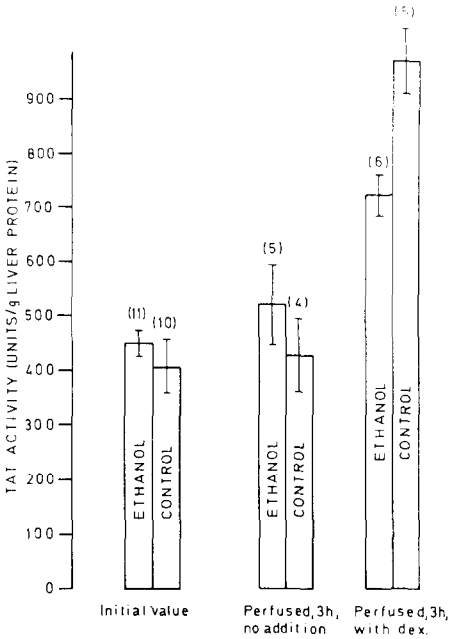

FIG. 3. Tyrosine aminotransferase activity in isolated perfused livers from fasted rats. The initial values represent the enzyme activities while the livers were still *in situ*. Dexamethasone (dex), 20 μg/ml, was added after 10 min. The number of livers analysed for each column is shown in parentheses. Vertical lines indicate $\pm$ the standard error, S.E.M. The significance level of the difference between induced control and ethanol-pretreated livers was 0·002 according to the Wilcoxon test.

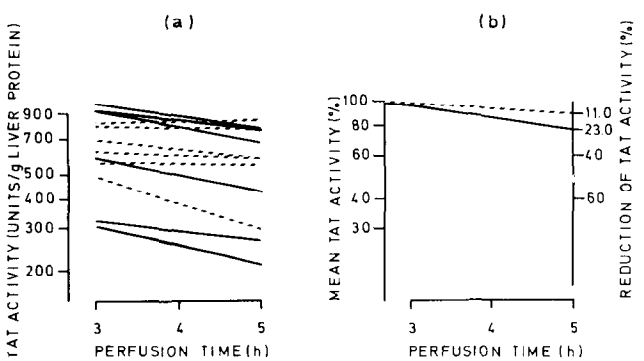

FIG. 4. Reduction of induced and basal tyrosine aminotransferase activity in perfused rat livers after the administration of cycloheximide (50 μg/ml) at 3 hr. The enzyme activities were measured at 3 and 5 hr. The results of the individual experiments are shown as a semilogarithmic plot in (a) for six control livers (——) and for six livers from ethanol-treated rats (- - -). The mean per cent reduction is shown in (b). The difference between control and ethanol-treated animals (23·0–11·0) was not statistically significant according to the Wilcoxon test.

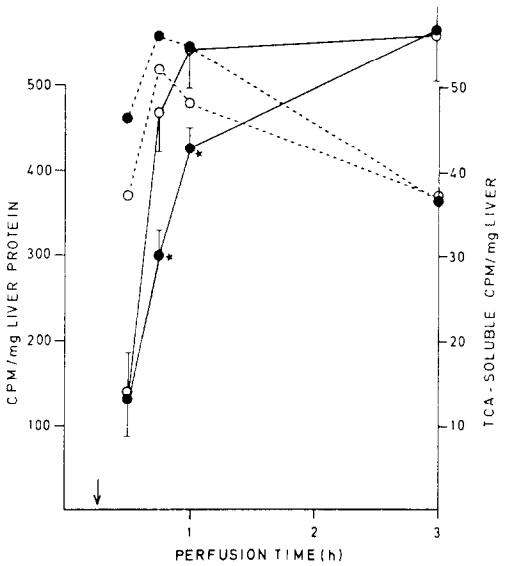

FIG. 5. Incorporation of labelled amino acids into protein in livers perfused with dexamethasone. Dexamethasone (20 μg/ml) was added to the perfusate after 10 min perfusion, protein hydrolysate (3 μCi, U-^{14}C) was added after 15 min (↓) to six control livers (○) and six ethanol-treated livers (●). Counts incorporated into protein (——) and TCA-soluble counts (- - - -) were registered. The corresponding points on the TCA-soluble count-curves were not statistically significantly different, while points marked with (*) were significantly (Wilcoxon, $\alpha < 0{\cdot}05$) lower than the corresponding control values. Vertical lines indicate the standard error, S.E.M.

The mean relative reduction of TAT-activity (Fig. 4b) was slightly but not significantly greater in control livers than in livers from ethanol-treated rats.

Influence of ethanol treatment on general liver protein synthesis

The amount of protein per liver was slightly reduced after ethanol-treatment (Table 1). Ethanol-treatment reduced the amount of TCA-soluble ninhydrin-positive material per g liver to about 80 per cent both before and during liver perfusions (six experiments).

The incorporation of labelled amino acids into protein was followed in perfused livers (Figs. 5 and 6). During perfusion for 3 hr, the amount of label incorporated per unit time gradually declined. This might be a consequence of a gradual reduction of protein synthesis during perfusion, a relative lack of one or more of the labelled amino acids or both. Such a decline in incorporation also takes place as the specific activity of the protein synthesized approaches that of the amino acid pool.

When dexamethasone was added to the perfusates, ethanol-treated livers incorporated significantly less amino acids into protein 30 and 45 min after the addition of label (Fig. 5, solid lines). At these times, livers from ethanol-treated rats had incorporated 64 and 78 per cent of the corresponding control values. This phenomenon was not seen in perfusions without addition of hormone (Fig. 6, solid lines). The difference in incorporation was not due to a decreased uptake of label by treated livers (Fig. 5 and 6, dotted lines), as TCA-soluble counts were slightly increased in ethanol-treated livers.

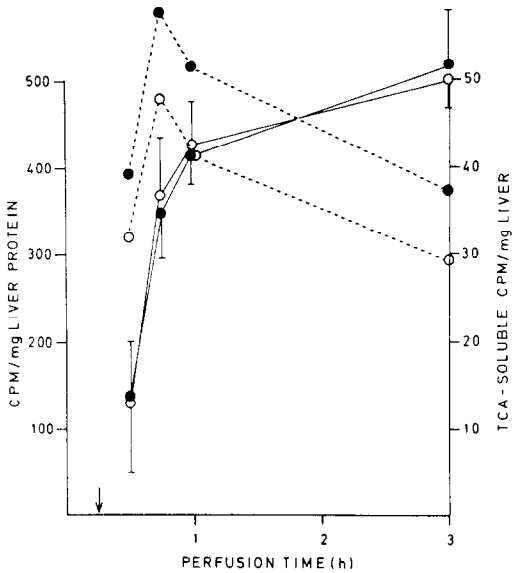

FIG. 6. Incorporation of labelled amino acids into protein in livers perfused without dexamethasone. Protein hydrolysate (3 μCi, U-^{14}C) was added after 15 min (↓) to four control livers (○) and four ethanol-treated livers (●). Counts incorporated into protein (——) and TCA-soluble counts (- - - -) were registered. The corresponding points on either pair of curves were not statistically significantly different. Vertical lines indicate the standard error, S.E.M.

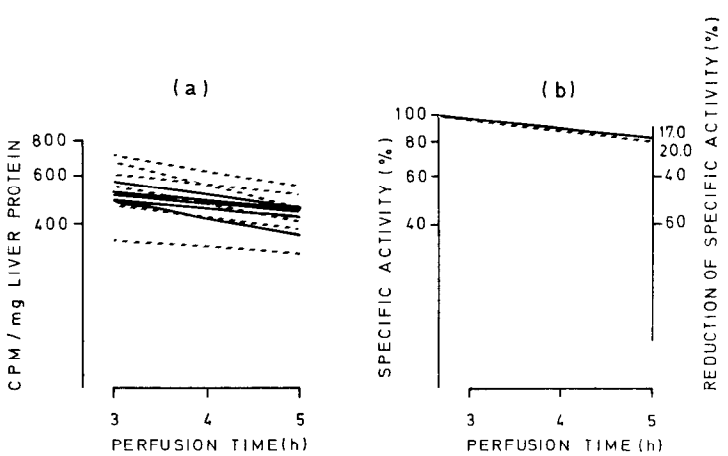

FIG. 7. Reduction of labelled proteins in perfused rat livers after the administration of cycloheximide (50 μg/ml) at 3 hr. Labelled proteins were counted at 3 and 5 hr. The results of the individual experiments are shown in (a) for five control livers (——) and for six livers from ethanol-treated rats (- - -) in a semilogarithmic plot. The mean per cent reduction is shown in (b).

The ratios TCA-precipitable to TCA-soluble counts (both referred to mg liver protein) were for control livers with dexamethasone: 1·83 and 2·32, and for those without dexamethasone: 1·56 and 2·08, 30 and 45 min after addition of the label respectively. The corresponding figures after ethanol consumption were: 1·07 and 1·66 (with dexamethasone) and 1·22 and 1·68 (without dexamethasone).

Figure 7 shows the reduction of liver protein specific activity after a dose of cycloheximide, which in prior experiments had been shown to inhibit protein synthesis more than 95 per cent. There was no difference in protein degradation between the experimental and control livers during 2 hr of perfusion.

Total radioactivity of proteins in the perfusate was significantly lower in ethanol pretreated rats, only after dexamethasone treatment (Table 2). Total protein content of the perfusate was not significantly influenced by either treatment (Table 2).

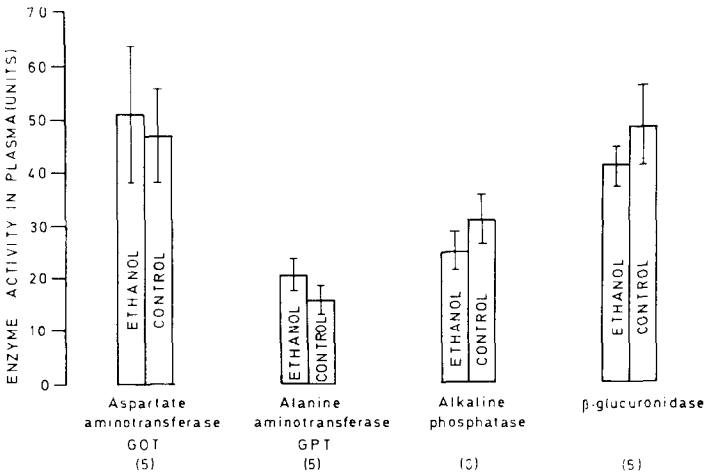

FIG. 8. Effect of chronic ethanol treatment on plasma levels of hepatic enzymes. The enzyme activity was measured 24–30 hr after the last intake of ethanol. Vertical lines indicate ± the standard error, S.E.M. All differences between ethanol treated and controls were statistically insignificant according to the Wilcoxon test.

Effect of ethanol treatment on enzymes and bilirubin in plasma and on bile production

Ethanol treatment for 38 days produced slight but insignificant changes in the plasma levels of GOT, GPT, alkaline phosphatase or β-glucuronidase (Fig. 8), and an insignificant reduction in the amount of circulating plasma protein (Table 3). Plasma bilirubin concentration in ethanol-treated rats also did not differ significantly from control levels (Table 3). Approximately 50 μl of bile per hour and g liver was produced during perfusion of ethanol-treated and control livers during the first 2 hr.

Effect of ethanol treatment on hepatic structure and lipid content

No significant changes were noticed in five livers from each group examined under the light microscope.

Hepatic triglycerides and phospholipids were not changed significantly by the ethanol treatment used in these experiments (Table 4).

DISCUSSION

Ethanol treatment for 38 days was not accompanied by an increase in liver triglycerides and phospholipids, in plasma GOT, GPT or bilirubin or by major structural changes. The plasma concentrations of two other liver enzymes, β-glucuronidase and alkaline phosphatase were not significantly changed. Bile production of *in vitro* per-

TABLE 2. PROTEIN CONTENT OF THE PERFUSATE AFTER 5 hr PERFUSION*

	Ethanol-treated rats + dexamethasone	Control rats + dexamethasone	Significance	Ethanol-treated rats	Control rats	Significance
No. of rats	5	6	—	4	4	—
Protein content (g/total perfusate)	1·48 ± 0·08	1·58 ± 0·05	N.S.	1·66 ± 0·06	1·56 ± 0·06	N.S.
Labelled protein (CPM/total perfusate)	$3{\cdot}5 \pm 0{\cdot}4 \times 10^5$	$4{\cdot}3 \pm 0{\cdot}2 \times 10^5$	0·041	$4{\cdot}0 \pm 0{\cdot}5 \times 10^5$	$4{\cdot}5 \pm 0{\cdot}3 \times 10^5$	N.S.

* 3 μCi of (U-^{14}C) protein hydrolysate was added to the perfusates after 15 min. Cycloheximide was added at 3 hr perfusion in some experiments. This addition did not significantly influence the amount of labelled protein registered at the end of perfusion 2 hr later. The results are given ± standard error, S.E.M. The significance levels according to Wilcoxon's test are given, considering levels above 0·05 as insignificant, N.S.

TABLE 3. EFFECT OF CHRONIC ETHANOL TREATMENT ON PLASMA CONCENTRATION OF TOTAL PROTEIN, BILIRUBIN AND CORTICOSTERONE IN FASTED RATS*

	Ethanol-treated rats	Control rats	Significance
No. of rats	6	6	—
Total protein (g/100 ml)	5·4 ± 0·2	5·7 ± 0·1	N.S.
Bilirubin (mg/100 ml)	0·08 ± 0·02	0·07 ± 0·01	N.S.
Corticosterone (μg/100 ml)	95·8 ± 10·0	68·5 ± 3·5	0·008

* The results are given ± standard error, S.E.M. The significance levels according to Wilcoxon's test are given, considering levels above 0·05 as insignificant, N.S.

fused livers was unaffected by previous ethanol treatment. In other liver perfusion experiments (to be published) we have found both normal bromosulfalein disappearance from the perfusate, and normal excretion in bile after previous ethanol treatment under the conditions described in this report.

Most investigations on changes in liver composition due to chronic ethanol treatment have utilized alcohol in amounts to provide 35 per cent or more of the calories

TABLE 4. EFFECT OF CHRONIC ETHANOL TREATMENT ON LIVER WEIGHT, TRIGLYCERIDES, AND PHOSPHOLIPIDS IN FED RATS*

	Ethanol-treated rats	Control rats	Significance
No. of rats	5	5	—
Liver wt (g)	11·59 ± 0·63	10·92 ± 0·82	N.S.
Triglycerides (mg/g wet wt)	5·41 ± 1·60	7·34 ± 2·23	N.S.
Phospholipids (mg/g wet wt)	27·48 ± 2·20	28·47 ± 2·87	N.S.

* The results are given ± standard error, S.E.M. A significance level of the difference between treated and controls above 0·05 according to Wilcoxon's test, was considered as statistically insignificant, N.S.

consumed, in order to mimic the consumption of human drinkers.[14] The feeding schedule of ethanol used in my experiments provides about 30 per cent of the total calories. The small reduction in daily ethanol intake (from 35 to 30 per cent) may in part explain the lack of lipid accumulation reported here, since consumption of a diet providing 25 per cent of the calories as ethanol had been found not to cause fatty liver at all.[14]

Tryptophan oxygenase activity was, however, reduced in the present experiments. The reduction of this enzyme is of some interest *per se* in view of its role in serotonin metabolism[44] and NAD synthesis.[45] It also provides a model for protein metabolism and the discussion will be restricted to this latter aspect. The experiments with mixed liver homogenates excluded lack of enzyme activators or presence of inhibitors as a cause for the reduced enzyme activity.[34] Thus, either reduced enzyme synthesis or increased degradation might explain the diminished enzyme activity seen after ethanol treatment. The mean per cent turnover was approximately the same in both groups of liver (Fig. 2b) when TO-synthesis in perfused livers was blocked by cycloheximide,

which does not interfere with TO degradation.[46] Moreover (Fig. 2a) the relative rate of degradation was independent of the initial (3 hr) enzyme activity in livers otherwise treated the same way, an observation also reported under other circumstances by others.[47] Experiments with dexamethasone induction showed that livers pretreated with ethanol were unable to produce the same amount of new TO molecules as control livers (Fig. 1). Reduced enzyme synthesis, and not enzyme inhibition or enzyme degradation must therefore be the cause of the diminished TO-activity after ethanol treatment for 38 days. Experiments with TAT and incorporation of labelled amino acids were performed in order to test whether synthesis of other proteins was also inhibited. The induction of TAT due to dexamethasone was reduced (Fig. 3) whereas the degradation of the enzyme measured after the administration of cycloheximide (Fig. 4) was not altered after ethanol treatment. These results suggest that ethanol treatment affects also synthesis of TAT, although the effects of steroids and cycloheximide on TAT are less clearcut than those on TO.[46,48,49] The basal initial activity of this enzyme was not affected by chronic ethanol consumption.

Incorporation of labelled amino acids into protein was significantly reduced by ethanol treatment, only after dexamethasone administration (Fig. 5). Differences in liver protein pool size between experimental and control animals could be ruled out on the basis of protein content (Table 1). An increase in size of the liver amino acid pool after ethanol or a lowered uptake of plasma amino acids by the liver could also explain the reduced incorporation. TCA-soluble, ninhydrin-positive material was, however, reduced after ethanol treatment, in agreement with observations of others[9] and uptake of amino acids seemed slightly increased (Figs. 5 and 6) after prolonged ethanol treatment. The difference in protein synthesis between ethanol-treated and control animals could thus be even greater than suggested by Figs. 5 and 6.

Increased leakage of newly synthesized proteins could in part explain the results obtained with TO, TAT and labelled proteins. This increase would have to be extreme and probably not compatible with normal liver function (bile production, liver appearance, protein content) in order to solely explain the reductions in enzyme activity and incorporation of radioactivity into protein. Increased leakage is unlikely due to the fact that the amount of radioactive proteins appearing in the perfusate is reduced after ethanol treatment (Table 2). Reduced synthesis of plasma proteins could explain this difference between experimental and control livers.

The low basal, initial TO-activity, as well as the reduction of enzyme and protein synthesis seen in the perfused liver after ethanol treatment did not depend on the presence of ethanol since ethanol had not been given for the last 24–30 hr before TO-determination and perfusion. This observation allows this effect of ethanol to be distinguished from the direct one on albumin synthesis,[4,5] and from the acute pH-dependent reduction in TO-synthesis.[34] Reduced plasma corticosterone could not be responsible for the effect of ethanol treatment on TO, since the concentration of this hormone was increased after ethanol treatment (Table 3) in accordance with data of Kuriyama *et al.*[6] The corticosterone levels found in the experiments reported here were comparatively high probably reflecting effects of fasting and ether anaesthesia. Ethanol-treated animals must therefore be capable of producing steroid hormones normally and might, in addition, exhibit reduced corticosterone degradation. The reduced enzyme and general protein synthesis were recorded in isolated perfused livers, and were therefore not dependent on blood-borne factors changed by ethanol

treatment. The effects were significant after dexamethasone addition to the perfusate, which may indicate defective steroid receptors or a deficient machinery for protein synthesis which becomes more apparent when the system is stressed by a steroid load. The acute administration of ethanol may be followed by increased corticosterone levels in the rat.[40,50,51] The induction of TO and TAT due to this steroid would thus probably decline after a period of chronic ethanol consumption.

Banks *et al.*[9] found a reduction of general liver protein synthesis *in vivo* in rats treated with ethanol (40 per cent of calories) for 28 days. These rats had developed a moderate fatty liver. In the experiments reported here the effect of ethanol on liver TO-activity *in vivo* seemed to occur without accompanying changes in the other liver parameters studied. It is, therefore, tempting to assume that changes in liver protein synthesis during ethanol treatment precede the more commonly studied changes in fat metabolism. The protein content of the diet in the present experiment was low, and this could perhaps make changes in protein synthesis due to ethanol more readily detectable. Generally, changes in enzyme synthesis could help to explain changes in enzyme activities often recorded during chronic ethanol consumption.[52] Furthermore, altered protein synthesis in the liver could be of central importance during the development of other changes in liver metabolism and morphology seen after chronic ethanol treatment.

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