The Acute Effect of Ethanol on Albumin, Fibrinogen and Transferrin Synthesis in the Rat

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Decrease of absolute synthesis of albumin and fractional synthesis of transferrin was observed within 3 h of orally administering ethanol (4 ml/kg) to rats maintained on a 40%-protein diet. In contrast, absolute synthesis of fibrinogen was unaffected. With this ethanol intake, the changes in protein synthesis occurred without significant ultrastructural change in the liver. When the ethanol intake was greater (8 ml/kg) ultrastructural disruption was observed. However, both the decrease of plasma protein synthesis and the ultrastructural alterations could be prevented by the simultaneous administration of a mixture of amino acids with the ethanol. The latter findings, not reported hitherto, suggest that ethanol may interfere with hepatic plasma protein synthesis and ultrastructure more through a disturbance of amino acid metabolism than through direct physical damage to the hepatocyte. An Appendix outlines the deconvolutional method used to correct for losses of labelled protein in the period during which measurements were made. The principle may also be applied to labelled plasma urea. The details of the calculations are given in a supplementary paper that has been deposited as Supplementary Publication 50007 at the National Lending Library for Science and Technology, Boston Spa, Yorks. LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1972) 126, 5.

Ethanol is known to influence the metabolism of the liver and when ingested chronically in excessive quantities results in histological changes such as fatty infiltration and cirrhosis (Lieber & Rubin, 1969). The synthesis of plasma proteins is an important liver function and an examination of the effect of acute ethanol ingestion on such synthesis might clarify the mechanism of the apparent hepatotoxicity of ethanol, uncomplicated by secondary nutritional effects that may occur with the chronic ingestion of ethanol.

The present study was designed to observe the acute effects of ethanol ingestion on plasma protein synthesis by using McFarlane's (1963*a*) approach. The results indicate that under our conditions, ethanol decreased absolute synthesis of albumin and fractional synthesis of transferrin without affecting absolute synthesis of fibrinogen, and caused ultrastructural changes in the hepatocyte. This decrease in albumin synthesis was not prevented by simultaneous injection of tryptophan. However, the changes caused by ethanol, both in synthesis and in ultrastructure, were prevented by injection of a mixture of amino acids.

Materials and Methods

Materials

Animals. Male Wistar rats (190–250 g) were used.

Ethanol. Ethanol was given to rats as 2 ml of a

47% (v/v) solution in 0.9% NaCl; i.e. the dose was about 4 ml/kg body wt.

Amino acid mixture. Aminosol 5% (Abbott Laboratories Ltd., Montreal 101, Quebec) was used. This is a modified hydrolysate of fibrin (approx. 2/3 free amino acids and 1/3 small peptides), 50 g/l.

Labelled compounds. Na₂¹⁴CO₃ (specific radioactivity 50 mCi/mmol) and [¹⁴C]urea, were supplied by Amersham/Searle, Don Mills, Ontario, Canada.

¹²⁵I-labelled albumin and fibrinogen. Rat albumin was prepared by DEAE-cellulose chromatography and labelled with ¹²⁵I by the method of McFarlane (1964). Fibrinogen was precipitated from rat plasma with (NH₄)₂SO₄, and then labelled with ¹²⁵I (McFarlane, 1963*b*).

¹²⁵I-labelled transferrin. Pooled rat plasma was diluted with an equal volume of 0.09 M-sodium citrate and centrifuged for 10 min at 10000 rev./min to sediment the platelets. The supernatant was mixed with sufficient 4 M-(NH₄)₂SO₄ to make the final volume twice that of the initial citrated plasma. The precipitate consisted largely of fibrinogen and γ -globulin, and was removed by centrifugation for 10 min at 5000 rev./min. The supernatant was mixed with 4 M-(NH₄)₂SO₄ in the ratio of 1:0.43 (v/v) to bring the solution to 2.6 M. The precipitate was rich in transferrin but also contained some albumin. It was separated by centrifugation for 10 min at 5000 rev./min. The precipitate was dissolved in 0.9% (w/v) NaCl, 'spiked' with ⁵⁹FeCl₃, and dia-

lysed to remove $(\text{NH}_4)_2\text{SO}_4$, then further dialysed against 0.01 M-sodium phosphate buffer (0.01 M- NaH_2PO_4 was added to 0.01 M- Na_2HPO_4 to give a pH of 7.5 at 25°C). The supernatant was applied to a column (30×1.5 cm) of DEAE-Sephadex A-50 equilibrated with the same buffer. Any remaining γ -globulin was eluted with 150 ml of 0.01 M-sodium phosphate buffer, pH 7.5, and transferrin was then eluted with 0.03 M-sodium phosphate buffer, pH 7.5. Fractions (6.5 ml) were collected, and those in which both the E_{280} and the radioactivity rose and fell simultaneously and proportionately were pooled and concentrated by using a Diaflo UM-10 membrane. This peak was found by starch-gel electrophoresis and immunoelectrophoresis to contain only those two rat transferrins described by Gordon & Louis (1963). This fraction was labelled with ^{125}I by McFarlane's ICI technique (McFarlane, 1964).

Experimental

Plan of experiment. A total of six batches of 10 to 12 Wistar rats was used for studies with ethanol, alone or in combination with amino acids. The results were compared with published (Jeejeebhoy *et al.*, 1970) control values on albumin and fibrinogen synthesis obtained from the same strain of animals fed in an identical manner. A further two control batches for fibrinogen synthesis were studied during the present experiment; the results were amalgamated with those obtained previously. Results from the present controls did not differ from those obtained previously. Animals were placed on a diet that contained 40% protein (as casein) and which provided $7100\text{ kJ}\cdot\text{m}^{-2}$ ($1700\text{ kcal}\cdot\text{m}^{-2}$) surface area for 4–6 days before the day of the experiment. Only animals that ate all the food and that gained weight steadily were used for the study. Water was given *ad libitum* and the last feed was given to the rats at about 1600 h of the day preceding injection. At about 0800 h each rat was placed under anaesthesia and then was given 2 ml of ethanol solution via an intra-gastric tube.

The animals were given $25\mu\text{Ci}$ of $\text{Na}_2^{14}\text{CO}_3$ intraperitoneally under anaesthesia 2 h later, followed by $5\mu\text{Ci}$ of either ^{125}I -labelled albumin, ^{125}I -labelled fibrinogen, or ^{125}I -labelled transferrin, via the dorsal vein of the penis.

After injection of the ^{14}C - and ^{125}I -labelled compounds, blood was withdrawn (under ether anaesthesia) from the retro-orbital plexus into heparin at 10 min after the injection and then hourly for 3 h after the administration of $\text{Na}_2^{14}\text{CO}_3$. At the fifth hour the rats were anaesthetized and bled to obtain a large sample of blood. Blood from two to three rats was pooled to obtain sufficient plasma for the determinations. The samples were centrifuged and the plasma was separated.

Separate batches of rats were given $5\mu\text{Ci}$ of

$[^{14}\text{C}]\text{jurea}$ via the dorsal vein of the penis to determine the distribution and turnover of $[^{14}\text{C}]\text{jurea}$ (McKinley *et al.*, 1970).

Experimental procedure and calculation of results. With two exceptions, the method used and the manner of calculation have been published in detail (Jeejeebhoy *et al.*, 1969, 1970). The equation used to calculate synthesis, unless otherwise stated, is that of Koj & McFarlane (1968) [i.e. equation (iii) noted subsequently]. The first exception concerns the separation of fibrinogen, transferrin and albumin from the same sample of plasma for the determination of arginine guanidine ^{14}C specific radioactivity. Details follow in the next section. The second exception concerns the manner of correcting for losses of albumin, fibrinogen and transferrin arginine guanidine ^{14}C during the period over which synthesis was measured. As before, the disappearance curves of ^{125}I -labelled albumin, fibrinogen and transferrin were used to modify the respective appearance curves of arginine guanidine ^{14}C . In our previous publication these necessary corrections were made by an analogue computer technique. In the present paper these corrections were made by a digital computer program in collaboration with Dr. K. H. Norwich. This method of correction employed analytical deconvolution of the integral of plasma arginine guanidine ^{14}C specific radioactivity and is described by Dr. Norwich in the Appendix.

Separation of fibrinogen, transferrin and albumin from a single sample of rat plasma for the determination of arginine guanidine ^{14}C specific radioactivity. This procedure is shown in Scheme 1. The plasma sample was diluted with an equal volume of 0.9% (w/v) NaCl, and a volume of 4 M- $(\text{NH}_4)_2\text{SO}_4$ nearly equal to that of the diluted plasma was added to give a final $(\text{NH}_4)_2\text{SO}_4$ molarity of 2 M to precipitate the fibrinogen and γ -globulin. The precipitate was removed by centrifugation for 10 min at 5000 rev./min and redissolved in a volume of 0.9% NaCl equal to that of the diluted plasma. To this solution, thrombin (64 National Institute of Health units of thrombin/ml of undiluted plasma) was added to clot the fibrinogen. The fibrin clot so formed was placed in a 50 ml tube on a slow shaker, and repeatedly squeezed and washed for 2 days in water, with frequent changes, to remove contaminating proteins. The clot was finally transferred to ampoules and hydrolysed for 18 h with 6 M-HCl at 110°C.

To the 2 M- $(\text{NH}_4)_2\text{SO}_4$ supernatant containing the transferrin and albumin, 0.43 vol. of 4 M- $(\text{NH}_4)_2\text{SO}_4$ was added to give a final molarity of 2.6, which precipitates a fraction rich in transferrin. The precipitate was separated by centrifugation at 5000 rev./min for 10 min and was dissolved in 1.5 ml of 0.9% NaCl. The solution was 'spiked' with $^{59}\text{FeCl}_3$ and was then dialysed first against running tap-water overnight and then against 0.01 M-sodium

Scheme 1. Flow sheet for separation of the proteins

For details see the text.

phosphate buffer, pH 7.5, before the supernatant was applied to a column (0.9 cm × 15 cm) of DEAE-Sephadex A-50 previously equilibrated with the same buffer. Residual γ -globulin was removed by elution with 30 ml of 0.01 M-sodium phosphate buffer and the transferrins were eluted (as noted under 'labelled compounds') by using 0.03 M-sodium phosphate buffer, pH 7.5. The fractions that contained the transferrins were pooled and concentrated as noted earlier, and were precipitated with perchloric acid at a final concentration of 0.2 M to remove traces of acid-soluble glycoproteins. The transferrin precipitate was washed once with 0.2 M-perchloric acid and then transferred to ampoules for hydrolysis by 6 M-HCl for 18 h at 110°C.

The 2.6 M-(NH₄)₂SO₄ supernatant, which contained mainly the albumin, was adjusted to pH 4.4–4.7 with 0.5 M-H₂SO₄ to precipitate albumin. The precipitate was removed by centrifugation for 10 min at 5000 rev./min and was then dissolved in 5 ml of 0.9% NaCl and dialysed overnight in running tap-water to remove all contaminating (NH₄)₂SO₄. To this dialysed albumin solution was added two volumes of 6.2% (w/v) of trichloroacetic acid in acetone to precipitate any remaining globulin. After centrifugation at 5000 rev./min for 10 min to remove this contaminant, diethylamine was carefully added dropwise to the supernatant that contained the albumin to neutralize the trichloroacetic acid. The albumin precipitate was washed twice with 66% (v/v) acetone and once with 100% acetone before being transferred to ampoules for hydrolysis by 6 M-HCl for 18 h at 110°C.

Protocol for studying the effect on albumin synthesis of the concurrent administration of ethanol with either

through a tube passed into the stomach. Concurrently either 6 ml of 5% Aminosol or 20 mg of tryptophan in 6 ml of 0.9% NaCl was given intraperitoneally. Then 2 h after the above procedure 25 μ Ci of Na₂¹⁴CO₃ was given intraperitoneally and 5 μ Ci of ¹²⁵I-labelled rat albumin was given intravenously via the dorsal vein of the penis. In one further batch of rats given Aminosol or saline 5 μ Ci of ¹²⁵I-labelled rat fibrinogen was injected intravenously instead of albumin. In separate experiments, the disappearance of exogenous [¹⁴C]urea was studied by injecting identically treated batches of rats with 5 μ Ci of [¹⁴C]urea intravenously instead of Na₂¹⁴CO₃ and ¹²⁵I-labelled albumin. Blood was withdrawn in the manner and at the intervals indicated earlier.

Calculation of albumin synthesis in experiments involving the injection of Aminosol (resulting in an unsteady state). In those determinations not involving the use of Aminosol there was no significant change, during the synthesis interval, in the concentration of plasma urea and albumin, and the animals appeared to be in a steady state. By contrast, in experiments involving the use of Aminosol there was a steady rise in the amount of urea in plasma suggesting that there was an expansion of the total body urea pool. In view of this probable alteration in urea pool size it is necessary to examine the question of whether the equation proposed by Koj & McFarlane (1968) [i.e. eqn. (iii) below], and which we have used in the studies of animals in a steady state (e.g. Tables 3, 4 and 5) applies under the present circumstances.

The basic relationship used for the measurement of protein synthesis was developed by McFarlane *et al.* (1965) as follows:

$$\frac{\text{Synthesis rate of protein guanidine C}}{\text{Synthesis rate of urea C}} = \frac{\text{Total radioactivity incorporated into protein guanidine C}(t_0, t_1)}{\text{Total radioactivity incorporated into urea C}(t_0, t_1)}$$

Dividing both sides of this equation by the size of the protein guanidine carbon pool and rearranging the equation we have:

$$\frac{\text{Fractional synthesis rate of protein}}{\text{rate of protein}} = \frac{\text{Hypothetical specific radioactivity (HSA) of protein guanidine C} \times \text{synthesis rate of urea C}}{\text{Total radioactivity incorporated into urea C}(t_0, t_1)} \quad (\text{i})$$

$$= \frac{\text{HSA of protein guanidine C} \times \text{urea pool} \times \text{fractional synthesis rate (FSR) of urea}}{\text{HSA of urea C} \times \text{urea pool}} \quad (\text{ii})$$

$$= \frac{\text{HSA of protein guanidine C} \times \text{FSR of urea}}{\text{HSA of urea C}} \quad (\text{iii})$$

a mixture of amino acids (Aminosol) or tryptophan alone. Batches of six rats were starved for 24 h before the study to ensure the absence of any dietary protein during the experiment. The rats of each batch were given by syringe either 2 ml of saline or 2 ml of 47% (v/v) ethanol in 0.9% NaCl (i.e. about 4 ml/kg)

where the hypothetical specific activity (HSA) is as defined by Koj & McFarlane (1968); i.e. 'the value that would have been obtained if all the ¹⁴C-labelled protein produced in the synthesis interval t had been retained in the plasma and all the [¹⁴C]urea in the body water'. From eqn. (ii) it is apparent that a

change in urea pool size will influence both numerator and denominator equally and will cancel out. Hence the eqn. (iii) proposed by Koj & McFarlane (1968) should be applicable in the present circumstances where the urea pool size increased during the synthesis interval.

To confirm the above theoretical conclusions it was decided to compute synthesis both by a modification of the more fundamental and apparently more widely applicable basic formula, eqn. (i), and by the method of Koj & McFarlane (1968) which we have used for animals in a steady state [eqn. (iii)]. The details of the application and calculation with the basic equation (i) are available as Supplementary Publication 50007 available from the National Lending Library for Science and Technology, Boston Spa, Yorkshire, LS23, 7BQ, U.K.

Electron-microscopic changes with acute ethanol ingestion. Liver biopsies were taken from control animals, and from those given ethanol at 4 and 8 ml/kg, and from those given ethanol at 8 ml/kg with Aminosol, intraperitoneally.

Immediately after killing after taking the 5 h blood sample, liver tissue was obtained for electron microscopic examination. Blocks of tissue were fixed for 1 h at 0°C in 1% OsO₄ that was buffered with 0.1 M-sodium phosphate buffer (pH 7.2). The blocks were dehydrated in a graded series of ethanol solutions, and transferred to propylene oxide and then to a mixture of propylene oxide and resin. The embedding medium was prepared essentially by the method of Mollenhauer (1964). Sections from the plastic-embedded tissue were cut with glass knives by using an LKB microtome. Sections 1.0 µm thick were

stained with Toluidine Blue (Trump *et al.*, 1961) and examined by light microscopy and those from the periportal and terminal hepatic vein regions were selected. Ultra-thin sections of grey interference colour were picked up on uncoated grids and were stained at room temperature with Reynold's solution of lead hydroxide for 15 min (Pease, 1964). The sections were examined in a Philips 200 electron microscope.

Results

Biosynthetic changes

Plasma volume, plasma albumin, plasma fibrinogen, total circulating albumin and total circulating fibrinogen. The results are given in detail in Table 1. The total amount of circulating albumin (plasma volume × plasma concentration of albumin) was not significantly different ($0.5 > P > 0.4$) in control and ethanol-fed animals, being 111.0 ± 12.5 and 114.6 ± 12.0 mg/100 g of rat weight respectively. Similarly, the total amount of circulating fibrinogen also was not significantly different in the two groups, being 10.21 ± 2.07 and 10.57 ± 2.00 mg/100 g ($0.3 > P > 0.2$) in control and ethanol-fed rats. Hence the pools of albumin and fibrinogen were of similar size in both sets of animals at the start of the study. On the other hand, urea synthesis was decreased in the ethanol-fed animals, the slope of the plasma [¹⁴C]urea specific radioactivity curve being less steep in these (mean 0.282/h) than in control animals (mean 0.372/h).

Arginine guanidine ¹⁴C specific radioactivity and

Table 1. *Comparison of plasma volume, plasma albumin and fibrinogen concentrations and the slope of [¹⁴C]urea specific radioactivity in rat plasma, in control and ethanol-treated rats*

Values are the mean ± s.d. Numbers in parentheses refer to the number of rats for the measurement concerned, except for the mean slope of [¹⁴C]urea specific radioactivity, where the numbers refer to the number of batches (of about six rats each).

	Control	Ethanol-treated (4 ml/kg)
Plasma volume (ml/100 g body wt.)	3.88 ± 0.370 (26)	4.67 ± 0.399 (13)
Plasma albumin concentration (g/100 ml)	2.90 ± 0.30 (21)	2.30 ± 0.17 (15)
Total circulating albumin (mg/100 g body wt.)	111.0 ± 12.5 (19)	114.6 ± 12.0 (10)
Plasma fibrinogen concentration (mg/100 ml)	247 ± 43.8 (28)	238 ± 24.4 (11)
Total circulating fibrinogen (mg/100 g body wt.)	10.21 ± 2.07 (26)	10.57 ± 2.00 (11)
Mean slope of [¹⁴ C]urea plasma specific radioactivity ($0.693/t_{\frac{1}{2}}$ in h)		
After injection of Na ₂ ¹⁴ CO ₃	0.393 (7)	0.263 (3)
After injection of [¹⁴ C]urea	0.372 (6)	0.282 (3)

hypothetical specific radioactivity of the arginine guanidine carbon. The mean relative specific radioactivities of plasma albumin, fibrinogen and transferrin are pictured in Fig. 1. The administration of ethanol resulted in a delayed appearance of the peak of albumin and fibrinogen arginine guanidine C specific radioactivity. In controls the peak value was reached at 2h, whereas in ethanol-treated rats the peak was reached at 3h after injection of $\text{Na}_2^{14}\text{CO}_3$. The time of peak specific radioactivities (3h) of transferrin was not changed by feeding ethanol. Table 2 shows that the total arginine guanidine C specific radioactivity remained constant for any time interval from 4h onward after injection of $\text{Na}_2^{14}\text{CO}_3$, showing that no labelled protein significantly affecting the calculation is delivered after 4h. In consequence the arginine guanidine C specific radioactivities from the single large specimen of blood drawn at the end of the experiment (5h) was used to calculated synthesis in the ethanol-treated rats.

Comparison of ^{14}C urea specific radioactivity obtained after injecting ^{14}C carbonate with that after injecting ^{14}C urea. In ethanol-treated animals, the mean 'k' factor ($0.693/t_{1/2}$ in h) for ^{14}C urea specific radioactivity after injection of $\text{Na}_2^{14}\text{CO}_3$ varied between 0.239 and 0.283 with a mean of 0.263, compared with a range and mean of 0.256 to 0.296 and 0.282 respectively after injecting ^{14}C urea. Thus there was no significant difference between the 'k' values for the ^{14}C urea curves observed after injection of $\text{Na}_2^{14}\text{CO}_3$, and for those seen after injection of ^{14}C urea. This confirms the results obtained by Jeejeebhoy *et al.* (1970). Hence the 'k' values used for the calculation of synthesis were obtained from ^{14}C urea curves generated endogenously by the injected $\text{Na}_2^{14}\text{CO}_3$.

Albumin, fibrinogen and transferrin synthesis after ethanol administration. The results are shown in Tables 3, 4 and 5. The fractional albumin synthesis in ethanol-treated animals was $58.88 \pm 7.35\%$ (mean $\pm$ s.d.) of the intravascular pool in 20 animals studied (batches I and III, Table 3) and $25.28 \pm 1.86\%$ of the intravascular pool in one batch (II, Table 3) of 12 animals. Even the higher of these values was significantly lower than in the control group where synthesis amounted to $77.18 \pm 10.02\%$ of the intravascular pool ($P < 0.01$). Since the circulating albumin pools in ethanol-treated rats and controls were similar it

Fig. 1. Mean relative specific radioactivities of the arginine guanidine carbon of plasma albumin, fibrinogen and transferrin in control and ethanol-treated rats

The maximum value reached in plasma is taken as 100%. o, Control rats; ●, ethanol-treated rats; (a), albumin; (b) fibrinogen; (c), transferrin.

Table 2. *Losses of radioactivity from the plasma compartment [digital computer estimates as percentage of maximum value in plasma (pool 1)] in ethanol-treated rats*

Synthesis interval (h)	Pool 1 (plasma)	Pools (3+4) (catabolism+ extravascular)	Pools 1+3+4 (total radioactivity)
Albumin			
1	74.0	12.0	86.0
2	92.0	40.2	132.4
3	100.0	42.9	142.9
4	90.0	58.8	148.8
5	72.0	68.4	140.4
Fibrinogen			
1	52.7	2.9	55.6
2	88.6	12.2	100.8
3	100.0	27.1	127.1
4	94.6	43.6	138.2
5	87.7	49.2	136.9
Transferrin			
1	55.0	9.4	64.4
2	88.0	24.7	112.7
3	100.0	43.8	143.8
4	93.0	61.2	154.2
5	76.0	79.0	155.0

may be inferred that for albumin both fractional and absolute (= fractional synthesis rate $\times$ total circulating albumin) synthesis rates were decreased by feeding ethanol. Similarly fractional synthesis of transferrin (Table 5) in ethanol-treated rats was significantly lower than in controls, being 58.10 ± 22.9 and $111.3 \pm 44.4\%$ of the intravascular pool respectively, ($P < 0.01$).

In contrast, fractional synthesis of fibrinogen in ethanol-treated rats was not significantly different from that in controls, amounting to 65.0 ± 12.1 and $66.2 \pm 13.5\%$ of the intravascular pool respectively (Table 4). Neither was the absolute amount of synthesis different in the two groups, the size of the circulating fibrinogen pools being very similar (Table 1). Fibrinogen synthesis was similar to control values even in the 12 rats in batch II (Tables 3 and 4), where albumin synthesis was markedly decreased to $25.28 \pm 1.86\%$ of the intravascular pool.

The effect of the simultaneous administration of Aminosol with the ethanol, on albumin and fibrinogen synthesis. The results are shown in Tables 6 and 7. Albumin synthesis was 75.14 ± 14.36 and $76.54 \pm 8.38\%$ of the intravascular pool in animals given Aminosol-ethanol and Aminosol-saline respectively when calculated by the method of Koj & McFarlane (1968). The difference was not significant. When the results were recalculated by the basic eqn. (i), albumin synthesis in these Aminosol-treated rats was 87.07 ± 19.06 and $95.73 \pm 18.39\%$ of the intravascular pool in animals given ethanol or saline respectively. The

difference here also was not significant ($0.7 > P > 0.6$). These values for synthesis, calculated by the basic equation, were 16–25% higher than those calculated by the Koj & McFarlane (1968) equation ($P < 0.05$), but a comparison of synthesis in the two states, with only one or other equation, indicated that the simultaneous administration of Aminosol with ethanol prevented the decreased albumin synthesis seen after giving ethanol alone.

Fibrinogen synthesis was definitely increased in the presence of Aminosol ($P < 0.001$) (Table 7, cf. Table 4, col. 7) but was not significantly different with the further addition of ethanol ($0.6 > P > 0.5$).

The effect of the simultaneous administration of tryptophan on the response of albumin synthesis to ethanol administration. The results are noted in Table 8. The fractional synthesis rate of albumin with tryptophan and ethanol was significantly lower than with tryptophan alone, being respectively 50.41 ± 6.90 and $68.09 \pm 7.58\%$ of the intravascular pool/day ($0.02 > P > 0.01$). The difference in absolute synthesis rates was even more marked (total circulating albumin values have not been given here).

Electron microscopic changes

The hepatocellular ultrastructure of the non-ethanol treated animals was identical with that observed by other investigators (Bruni & Porter, 1965; Herdson *et al.*, 1964) in fed and starved control rats. In fed rats given 4 ml/kg of ethanol, there was

Table 3. *Rat albumin synthesis*

The hypothetical specific radioactivity (HSRA) of plasma protein arginine guanidine ^{14}C (col. 4) is the measured arginine guanidine ^{14}C specific radioactivity (col. 3) corrected for losses of label into the extravascular pool and by catabolism during the period over which synthesis is measured. The method of correction (by digital computer) is given in the text and Appendix. The means are given $\pm$ s.d. with the numbers of determinations in parentheses. Synthesis (col. 7) (as % of intravascular pool/day) = $[\text{col. (4)}]/[\text{col. (5)}] \times \text{col. (6)} \times 24\text{h} \times 100$ [calculated after Koj & McFarlane (1968); i.e. eqn. (iii)].

	Batch no. (1)	Rat no. (2)	Specific radioactivity of plasma albumin arginine guanidine ^{14}C (d.p.m./mg of C)		Plasma [^{14}C]urea specific radioactivity		Fractional synthesis (% of intravascular pool/day) (7)
			Measured (3)	Hypothetical (4)	At time t_0 (d.p.m./mg of C) (5)	Slope ' k ' ($0.693/t_4$ in h) (6)	
Ethanol-treated (4 ml/kg)	I	1+8	4966	11536	138211	0.283	56.69
		4+11	11620	26994	286530	0.283	63.98
		5+12	5202	12126	133607	0.283	61.64
		7+14	5937	13792	145082	0.283	64.57
		6+13	6259	14540	226071	0.283	43.68
	III	9+10	10289	19629	192031	0.239	58.63
		11+12	9784	18666	184697	0.239	57.97
		15+16	8722	16640	169649	0.239	56.26
		17+18	8034	15327	167927	0.239	52.35
		19+20	11191	21350	166463	0.239	73.57
Control	II	1+7	3780	6183	182861	0.265	21.50
		2+8	6033	9868	244473	0.265	25.67
		3+9	4592	7511	191606	0.265	24.93
		4+10	5006	8189	199452	0.265	26.11
		5+11	5746	9399	227304	0.265	26.30
		6+12	5517	9025	208631	0.265	27.51
			Mean $\pm$ s.d.				46.34 $\pm$ 17.93 (16)
			Mean $\pm$ s.d.				77.18 $\pm$ 10.02 (13)

Table 4. *Rat fibrinogen synthesis*
For further details see Table 3 and the text. Batches II and III refer to same animals as in other tables.

	Batch no. (1)	Rat no. (2)	Specific radioactivity of plasma fibrinogen arginine guanidine ¹⁴ C (d.p.m./mg of C)		Plasma [¹⁴ C]urea specific radioactivity		Fractional synthesis (% of intravascular pool/day) (7)
			Measured (3)	Hypothetical (4)	At time t ₀ (d.p.m./mg of C) (5)	Slope 'k' (0.693/t _{1/2} in h) (6)	
Ethanol-treated (4 ml/kg)	II	1 + 7	11398	17894	182861	0.265	62.24
		2 + 8	14601	22923	244473	0.265	59.63
		3 + 9	8996	14124	191606	0.265	46.88
		4 + 10	14307	22461	199452	0.265	71.59
		5 + 11	11209	17598	227304	0.265	49.24
		6 + 10	11422	17932	208631	0.265	54.66
	III	9 + 10	10717	22572	192031	0.239	67.42
		11 + 12	11600	24432	184697	0.239	75.88
		13 + 14	11770	24642	160813	0.239	87.89
		17 + 18	9617	20255	167927	0.239	69.29
Control		19 + 20	9685	20398	166463	0.239	70.29
							64.99 ± 12.07 (11)
							66.2 ± 13.5 (14)

Table 5. *Rat transferrin synthesis*
For further details see Table 3 and the text. Batches I and III refer to the same animals as in other tables.

Ethanol-treated (4 ml/kg)	Batch no. (1)	Rat no. (2)	Specific radioactivity of plasma transferrin arginine guanidine ¹⁴ C (d.p.m./mg of C)		Plasma [¹⁴ C]urea specific radioactivity		Fractional synthesis (% of intravascular pool/day) (7)
			Measured (3)	Hypothetical (4)	At time <i>t</i> ₀ (d.p.m./mg of C) (5)	Slope 'k' (0.693/ <i>t</i> _{1/2} in h) (6)	
	I	2+ 9	3572	7200	203000	0.283	24.10
		4+11	9500	19000	286530	0.283	45.04
		3+10	6546	13100	239000	0.283	37.23
		5+12	4881	9760	133603	0.283	49.62
	III	9+10	7777	15715	192031	0.239	46.94
		11+12	12852	25971	184697	0.239	80.66
		15+16	10000	20208	169649	0.239	68.33
		17+18	11240	22713	167927	0.239	77.58
		19+20	13415	27109	166463	0.239	93.41
							58.10 ± 22.91 (9)
Control	A	2+ 3	5212	12200	168000	0.288	50.19
		6+ 7	8086	18600	133000	0.288	96.66
	B	1+2+ 6	11148	26400	138000	0.260	119.37
		4+ 5	5948	13700	113500	0.260	75.32
		9+10	7633	17600	143500	0.260	76.53
	C	3+ 5	10623	21900	112500	0.264	123.34
		4+ 6	21526	44400	143900	0.264	195.50
		8+ 9	15261	31400	127400	0.264	156.16
		1+2+ 7	12512	25800	150500	0.264	108.62
							113.3 ± 44.40 (9)

Mean ± s.d.

Mean ± s.d.

Table 6. *Effect of Aminosol with ethanol on rat albumin synthesis*

Fractional synthesis (col. 8) was calculated by basic eqn. (i) (details are in Table 1 of material submitted for deposition). For further details see Table 3 and the text.

	Batch no. (1)	Rat no. (2)	Specific radioactivity of plasma albumin arginine guanidine ¹⁴ C (d.p.m./mg of C)		Plasma [¹⁴ C]urea specific radioactivity		Fractional synthesis (% of intravascular pool/day)	
			Measured (3)	Hypothetical (4)	At time t ₀ (d.p.m./mg of C) (5)	Slope 'k' (0.693/t ₄ in h) (6)	By (Koj & McFarlane) eqn. (iii) (7)	By eqn. (i) (8)
Aminosol + ethanol (4ml/kg)	IVA	1	12833	24600	136162	0.154	66.77	89.99
		2	14335	27500	149155	0.154	68.14	55.17
		3	14624	28000	120859	0.154	85.63	114.55
		4	14279	27500	143160	0.154	71.00	97.35
		5	11082	21300	147782	0.154	53.27	66.42
		6	13222	25300	143008	0.154	65.39	94.62
	V	1	13899	30300	124907	0.154	89.42	72.40
		2	10780	23500	113338	0.154	76.48	88.17
		3	10633	23180	85362	0.154	100.15	104.98
Mean ± s.d.							75.14 ± 14.36 (9)	87.07 ± 19.06 (9)
Aminosol + saline	IVA	8	9981	18500	121080	0.169	61.97	73.89
		9	12971	23900	143793	0.169	67.42	87.42
		10	11826	21800	112032	0.169	78.92	89.72
		12	11239	20800	105328	0.169	80.10	131.66
	V	5	9220	21390	104024	0.169	83.13	85.66
		6	9147	21220	105583	0.169	81.12	101.61
Mean ± s.d.							76.54 ± 8.38 (7)	95.73 ± 18.39 (7)

Table 7. *Effect of Aminosol with ethanol on rat fibrinogen synthesis*

For further details see Table 3 and the text.

Batch no. (1)	Rat no. (2)	Specific radioactivity of plasma fibrinogen arginine guanidine ^{14}C (d.p.m./mg of C)		Plasma [^{14}C]urea specific radioactivity		Fractional synthesis (% of intravascular pool/day) (7)
		Measured (3)	Hypothetical (4)	At time t_0 (d.p.m./mg of C) (5)	Slope 'k' (0.693/ $t_{1/2}$ in h) (6)	
Aminosol+ethanol (4ml/kg)	XI	7+ 8+ 9 13+14+15	12381 17901	26950 38990	0.124 0.124	74.6 114.8
	Mean $\pm$ S.D.					94.7 ± 28.4 (2)
Aminosol+saline	XI	4+11	11336	30000	0.182	113.5
		5+ 6	11177	29600	0.182	94.8
		10+12	20564	54400	0.182	127.8
		16+17	13085	34600	0.182	112.0
Mean $\pm$ S.D.						112.0 ± 13.5 (4)

Table 8. *Effect of tryptophan with ethanol on rat albumin synthesis*

For further details see Table 3 and the text.

Batch no. (1)	Rat no. (2)	Specific radioactivity of plasma albumin arginine guanidine ^{14}C (d.p.m./mg of C)		Plasma [^{14}C]urea specific radioactivity		Fractional synthesis (% of intravascular pool/day) (7)
		Measured (3)	Hypothetical (4)	At time t_0 (d.p.m./mg of C) (5)	Slope 'k' (0.693/ $t_{1/2}$ in h) (6)	
Tryptophan + ethanol (4ml/kg)	IVB	1 2 3 4	8895 8068 7630 10733	18860 17100 16180 22750	0.238 0.238 0.238 0.238	43.40 46.14 53.64 58.47
	Mean $\pm$ S.D.					50.41 ± 6.899 (4)
Tryptophan + saline	IVB	5 6 7 8 9	8831 9024 9378 10477 7898	18020 18410 19130 21370 16110	0.277 0.277 0.277 0.277 0.277	60.90 73.85 71.29 75.35 59.05
	Mean $\pm$ S.D.					68.09 ± 7.575 (5)

EXPLANATION OF PLATE I

(a) Liver from starved control rat. Note that the rough endoplasmic reticulum is predominantly in the form of free ribosomes (fr) and ribosomal aggregates. The mitochondria (m) and other organelles are compactly disposed. Glycogen (gly) is present in small amounts (p, peroxisome). Lead hydroxide stain; $\times 25650$. (b) Liver from fed rat given ethanol (8 ml/kg). Note the marked similarity with the appearance in the starved state. Cellular organelles are compact. The rough endoplasmic reticulum is predominantly in the form of free ribosomes (fr) and ribosomal aggregates. Mitochondria (m) tend to be round rather than ovoid, and to show slight enlargement (lys, lysosome; p, peroxisome). Lead hydroxide stain; $\times 25650$.

EXPLANATION OF PLATE 2

Liver from Aminosol-treated rat given ethanol (8 ml/kg). The fine structure depicted is normal. The rough endoplasmic reticulum (rer) is predominantly in the form of elongated cisternae with smaller numbers of free ribosomes as compared with the starved state (compare with Plate 1*a*). Cellular organelles are more widely spaced. Glycogen particles (gly) and smooth endoplasmic reticulum are more abundant (m, mitochondrion; p, peroxisome). Lead hydroxide stain; $\times 28\,500$.

only minimal increase in fat as assessed by ultrastructural observation, despite the above-noted demonstration of changes in protein synthesis.

In fed rats that were given more ethanol (8ml/kg) a number of fine structural alterations were noted (Plate 1b). There was almost complete absence of cytoplasmic glycogen. The cytoplasmic volume was reduced and the mitochondria, rough endoplasmic reticulum and other organelles appeared crowded compared with the fed controls. The rough endoplasmic reticulum was dispersed, and was usually vesiculated and reduced in amount. The numbers of free ribosomes and ribosomal aggregates were greater. The mitochondria were enlarged and tended to be spherical in outline. These changes were found throughout the entire liver lobule, and were found in all animals of this group. These changes were comparable with those seen in starved rats (Plate 1a).

In contrast, rats given Aminosol with ethanol (8ml/kg) showed a normal ultrastructure with the exception of some glycogen depletion in the periportal zone of the hepatocytes (Plate 2). It appears that the administration of an amino acid mixture with the ethanol protects the liver from the ultrastructural changes caused by ethanol alone.

Discussion

The results show that acute ethanol ingestion in well-nourished rats decreases the fractional and absolute synthesis of albumin and the fractional synthesis of transferrin with no net change in the fractional and absolute synthesis of fibrinogen. [Even in one batch (II, Tables 3 and 4) where albumin synthesis was markedly decreased, there was still no change in fibrinogen synthesis.]

The effect of ethanol in decreasing the synthesis of albumin and transferrin may be considered as due either to gross, direct actions of ethanol on the structure of endoplasmic reticulum, or to a more subtle metabolic inhibition. Because a dose of ethanol of 4ml/kg was followed by a significant biochemical effect (but little structural change), and double this dose (8ml/kg) was required to produce a definitive ultrastructural change, we consider that ethanol may decrease plasma protein synthesis by interfering with the availability or utilization of amino acids rather than by acting directly on ultrastructure (e.g. by disintegrating membrane-bound ribosomes). Consistent with this hypothesis is the observed decrease in the rate of synthesis of urea (Table 1, and A. Bruce-Robertson, K. N. Jeejeebhoy, J. Ho, M. Foley, U. Sodtke & R. Lui, unpublished work with man).

Giving 8ml/kg of ethanol to the fed rat resulted in changes in hepatic cellular organelles, particularly the endoplasmic reticulum and mitochondria, which

were similar to ethanol-induced changes reported by others (Rubin & Lieber, 1967; Porta *et al.*, 1970). The present study shows that the acute ultrastructural changes in the hepatocytes of the fed ethanol-treated rats are almost indistinguishable from those seen during starvation. In particular, the rough endoplasmic reticulum has a dispersed, vesicular and fragmented appearance, and there is a concomitant increase in the number of free ribosomes and ribosomal aggregates. Again, the ultrastructural alterations, which indicate damage to the rough endoplasmic reticulum, could be due to a direct effect of ethanol or could be secondary to the influence of ethanol on amino acid metabolism [the polyribosome profile being sensitive to the supply of amino acids (Munro, 1970)]. To assist in distinguishing between these two possibilities some rats were treated with Aminosol. Giving an excess of amino acids (as Aminosol) not only greatly inhibited the ultrastructural changes seen with a high dose of ethanol (8ml/kg) alone (in fact, centrilobular hepatocytes in the Aminosol-ethanol-treated animals had a normal ultrastructure), but also prevented the decreased synthesis of plasma proteins induced by a low dose of ethanol (4ml/kg), suggesting to us that both the disruptions (structural) and the decreases (biochemical) may be the result of ethanol action, primarily on amino acid utilization.

In control rats given Aminosol + saline the fractional synthesis of albumin [76.54 ± 8.38 (7)] was not increased over that of control rats not given Aminosol [77.18 ± 10.02 (13)] but which like their counterparts, ate a 40% protein diet until a few hours before the study. (This comparison is also true for the respective absolute synthesis rates although the total circulating albumin values are not given here.) This suggests that albumin synthesis cannot be stimulated by increasing the supply of amino acids above that present when basic nutrition is adequate. In other studies (K. N. Jeejeebhoy, M. J. Phillips, A. Bruce-Robertson, J. Ho & U. Sodtke, unpublished work) we have not been able, by the administration of additional amino acids, to increase albumin synthesis acutely (within 2–3 h) above control values in nutritionally replete animals.

To obtain additional information about the protective effect of amino acids on the ethanol-induced decrease of plasma protein synthesis, experiments were performed to see if tryptophan alone would reverse this decrease. Tryptophan was selected because of its known effect on the polyribosome profile and liver protein synthesis (Munro, 1970). However, tryptophan alone had no appreciable effect on the ethanol-induced decrease of albumin synthesis.

The finding that fibrinogen synthesis is normal during ethanol treatment indicates either that ethanol does not influence the synthesis of this protein or that

two opposing factors are acting, with ethanol not only decreasing synthesis of all proteins through its influence on liver, but simultaneously and perhaps non-specifically, stimulating fibrinogen synthesis (which is generally considered to be easily enhanced). Although the latter view might seem to be supported by the observation that fibrinogen synthesis is increased by treatment with Aminosol (and especially when there is no increase in synthesis of albumin) it is not supported by the fact that ethanol has no significant effect on the synthesis of fibrinogen in the presence of Aminosol. Nor is there support for this view by our results for batch II (Table 3) in which, as previously remarked, a very substantial decrease in albumin synthesis is accompanied by normal fibrinogen synthesis that is not significantly different from that in batch III, in which albumin synthesis was only moderately decreased.

When the pattern of change in these syntheses is compared with the alterations in plasma protein concentrations seen in experimental and clinical protein malnutrition there appears to be a similarity, in that malnutrition also causes a decrease of albumin and transferrin concentrations (Platt *et al.*, 1964; Peetom, 1962). Preliminary studies show that short-term protein deprivation in the rat has relatively little effect on fibrinogen synthesis (K. N. Jeejeebhoy, A. Bruce-Robertson, J. Ho & U. Sodtke, unpublished work).

In summary, our results suggest that ethanol inhibits plasma protein synthesis in a selective way, and that this change, and the ultrastructural ones seen at higher dosage, can be prevented by giving amino acids. The exact mechanism by which ethanol operates needs elucidation.

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APPENDIX

Rates of Plasma Protein Synthesis by Deconvolution

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The plasma specific radioactivity of arginine guanidine ^{14}C -labelled protein has been measured at various times after the injection of labelled protein precursor ($\text{Na}_2^{14}\text{CO}_3$). The resulting 'appearance' curve is a balance between the rate of synthesis and delivery of new protein and the rate of destruction and distribution of new plus existing protein.