Alcohol-Induced Depression of Albumin Synthesis: Reversal by Tryptophan

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ABSTRACT The influence of alcohol on albumin synthesis was studied in the isolated perfused rabbit liver. Carbonate-^{14}C was used to label the intracellular arginine pool which serves as the precursor of both the carbon of urea and the guanido carbon of arginine in albumin. The control group synthesized albumin at a rate of 33 mg/100 g of wet liver weight during 2.5 hr of perfusion. When alcohol, 220 mg/100 ml, was added to the perfusate, albumin synthesis decreased to between 7 and 11 mg, less than one-third the control rate. The addition of 10 mM tryptophan to perfusates containing alcohol prevented most of the inhibitory effects and albumin synthesis increased to average 24 mg. Further, the addition of alcohol to the perfusate decreased the hepatic protein/DNA ratio from 70 to 54 and the RNA/DNA ratio from 2.3 to 1.8, changes equivalent to those seen after a 24 hr fast. The addition of tryptophan to the perfusate prevented these findings in both instances.

Endoplasmic membrane-bound polysomes were examined for aggregation. Alcohol decreased the quantity of heavier aggregates. Reaggregation occurred when tryptophan was added but quantitative changes in albumin synthesis could not be related to the degree of reaggregation.

INTRODUCTION

The acute effect of alcohol on the liver's ability to synthesize albumin has not been clarified though alteration of the subcellular mechanisms responsible for albumin synthesis occurs rapidly (1). Further, the alcohol-induced changes may be only transient since albumin synthesis frequently is found to be normal once the intake of alcohol and poor nutrition are removed (2). The effect of alcohol per se on albumin synthesis was examined in the isolated perfused rabbit liver. The results indicated that the acute exposure to alcohol in the perfusate rapidly shuts off albumin synthesis and the addition of tryptophan to the same perfusate counteracts most of the inhibiting effects of alcohol on albumin synthesis.

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PROTOCOL

Four experimental groups of young adult male rabbits (weight 1.3–1.5 kg) were used as donors for the perfused livers and were compared with perfused livers from rabbits fed ad lib., group 1 (controls). Group 2 consisted of seven rabbits also fed ad lib. for the same period of time but whose livers were subsequently perfused with a perfusate containing 220 mg/100 ml alcohol initially. The half-time of alcohol removal from the perfusates was about 70 min and the final concentrations at the end of the perfusions averaged 60–80 mg/100 ml. Group 3 consisted of six rabbits maintained on alcohol and a restricted caloric intake for 8 wk. The diet was adjusted daily so that the alcohol consumed constituted 40–50% of the calories ingested. An average of 45 ml of 20% (w/w) alcohol were consumed daily with a food intake of 20–25 g. The combined caloric intake was less than 50% of the average intake of the control rabbits (group 1) whose food intake was not limited. Group 4, consisted of four rabbits, treated in the same fashion as group 3 except that the alcohol was replaced by an equivalent amount of sucrose in terms of calories.

Since the addition of tryptophan to the perfusate results in a marked increase in albumin synthesis in rabbit livers obtained from fasted rabbits, studies were done in which tryptophan (10 mM) was added to the perfusate to see if the acute inhibition of albumin synthesis by alcohol could be prevented. Group 5 consisted of eight donors fed on a control diet as in group 2 but, in which the standard perfusate contained 10 mM tryptophan and 220 mg/100 ml alcohol.

Perfusion. The techniques for removal of the liver and its perfusion have been previously described in detail. Briefly, under light ether anesthesia the livers were exposed, the portal vein cannulated proximally while the liver was *in situ*, and perfusion immediately started. The inferior vena cava was cannulated below and ligated above the dia-

TABLE I

Albumin Synthesis in the Perfused Rabbit Liver

A	B	C	D	E	F	G	H	I
Group	Studies	Diet	Perfusate alcohol	Liver weight	Synthesized urea carbon spec. act.	Perfusate circulating albumin	Albumin guanido c spec. act.	Albumin synthesis
	n			*g*	*cpm/ml* $\times 10^{-5}$	*mg*	*cpm/ml*	*mg/100 g in 2.5 hr*
1	17	Control	0	68 ±7	4.4 ±0.3	3900 ±300	2560 ±270	33 ±1
2	7	Control	+	58 ±3	5.4 ±0.6	3800 ±50	845 ±125*	11 ±2*
3a	4	Low calorie alcohol	0	51 ±8	4.0 ±0.1	3860 ±165	1430 ±62	27 ±4
3b	2	Low calorie alcohol	+	51 ±7	5.3 ±0.5	3450 ±150	640 ±200*	7,9
4	4	Low calorie sucrose	0	46 ±5	5.3 ±0.3	3910 ±270	1530 ±215	25 ±3
5	8	Control	+ and 10 mM tryptophan	62 ±6	4.5 ±0.4	4000 ±155	1740 ±215	24 ±2

* Significantly less than groups 1 and 5 (P less than 0.01).
Values presented as the Mean ±SEM.

Columns E through H are the determinants of albumin synthesis, $I = \left(G \times \frac{H}{F}\right) \times \frac{100}{E}$. While the specific activity of the synthesized urea C, the circulating albumin pools and the liver weights were not different, the values for albumin guanido C specific activity were depressed in the alcohol perfused groups 2 and 3b but not in group 5 which had both tryptophan and alcohol in the perfusate.

phragm and the liver removed and reoriented on a platform in a heated, humidified box and the portal vein inflow cannula transferred to a pump system (3).

Perfusion was directed into the portal vein at a rate of 1.0–1.4 ml/g of liver per min. The perfusion volume of 140–170 ml was recirculated and oxygenated by a disk oxygenator that received the output from the inferior vena cava. Bile was collected from the cannulated biliary duct (3).

The perfusate consisted of two parts fresh whole rabbit blood diluted with one part amino acid fortified Krebs-Henseleit buffer containing 40-50 mg/100 ml glucose (3). In addition, the perfusate was adjusted to contain 0.69 μmoles of L-glutamine and 0.24 μmoles of L-arginine/ml. The amino acid level in the standard perfusate averaged 200–300 μmoles/100 ml and only trace levels of tryptophan were present. The albumin level of the perfusate was adjusted to 2.9–3.3 g/100 ml with rabbit albumin and the pH maintained at 7.32–7.40. All livers were preperfused for 20–25 min with a separate but identical perfusate before the addition of the CO_2-^{14}C label. Then the perfusion was continued for 2.5 hr, a time period adequate to permit release of all newly labeled albumin (3). The viability of the liver preparation has been determined (3). Lactate did not accumulate, O_2 extraction was steady, glucose levels rose (though in fed livers this represents in part, release from glycogen stores). Glycogen levels averaged 76 ±7 mg/g wet liver weight before perfusion and 66 ±5 mg/g after perfusion, urea production proceeded at a steady rate during the perfusion with alcohol, and bile production continued during the course of perfusion at 2–3 ml/hr.

Albumin synthesis. The carbonate-^{14}C technique was used to label the hepatic-arginine intracellular pool (4–6). Since arginine is the immediate precursor not only of the arginine residue in albumin but also of urea, a direct product precursor relationship exists as has been described by Swick (4), Reeve, Pearson, and Martz (5), and McFarlane (6). This technique has been examined in detail in numerous publications (3–8). After 30 min of perfusion (control and experimental) 100 μCi of carbonate-^{14}C (specific activity 5 mCi/mmole) was injected directly into the inflow tube to the portal vein and the perfusion continued for 2.5 hr.

Albumin synthesis was determined by the following formula:

$$\text{Albumin synthesis} = \left[\frac{\text{Albumin guanido C specific activity}}{\text{Synthesized urea C specific activity}}\right] \times \text{circulating albumin (8).}$$

Synthesized urea carbon–specific activity is presumed to equal the precursor arginine guanido carbon–specific activity.

The representative data for the components of this formula are given in Table I columns E–I. The specific activity of newly synthesized urea was not significantly different in the different groups while that of the albumin guanido carbon was depressed when derived from the alcohol perfusate. The specific activity of the newly synthesized urea carbon represents the specific activity of the immediate precursor arginine guanido carbon–specific activity. The fact that the urea carbon–specific activity was not lower in the alcohol perfused group when compared with the control group is related to the observation that CO_2 production in the liver perfused with alcohol is depressed below the control value (9). Thus the intrahepatic CO_2 pool is small and the precursor radioactive CO_2-^{14}C specific activity reaching the arginine pool is higher than it would be if CO_2 production were normal: and similarly the albumin guanido carbon-specific activity should be lower than the already depressed values seen for it comes from the same CO_2-^{14}C and arginine pool.

In order for the carbonate-^{14}C method to be valid, the rates of synthesis of albumin and urea should remain constant during the experimental period. Otherwise situations may arise wherein the major portion of urea is synthesized when the specific activity of the arginine precursor pool is high while the major portion of albumin is synthesized when the specific activity of this precursor pool is low. This would lead to a calculated albumin synthesis that is falsely low. The reverse situation would result in falsely high calculated values for albumin production. In the isolater perfused liver system, after the introduction of the CO_2-^{14}C, peak total urea radioactivity occurs by 15 min of perfusion or earlier. Thereafter there is no increase in the count rate due to urea-^{14}C. Thus the formation of radioactive albumin (from the same arginine-^{14}C from which the urea was derived) by the incorporation of radioactive arginine into albumin, occurs in that 15 min period. It is this event that determines the specific activity of the guanido carbon of the isolated albumin. Any subsequent alteration in the synthesis of albumin would have an undetectable effect on the albumin specific activity because of the large mass of perfusate albumin (4000 mg). On the other hand, any alteration in urea synthesis would alter the specific activity of the synthesized urea resulting in false values for albumin synthesis. In these studies however, urea production was steady. Urea synthesis was 0.46 ± 0.14 mg/min per 100 g liver weight during the first 5, 10, and 60–90 min of perfusion and 0.39 ± 0.10 mg during the last 60–90 min. These values are not statistically different. In perfusions containing alcohol, or alcohol and tryptophan, the rates of urea synthesis were slowed but were still constant over these same time periods. Urea synthesis was 0.27 ± 0.02 mg at the early times and 0.30 ± 0.06 mg at the later times. Thus in our experiments, the urea synthesis rates were constant and so any change in the calculated amount of albumin synthesized must have been due to an altered albumin synthetic rate during that 15 min interval of radioactive incorporation. While the minute by minute values for albumin synthesis cannot be determined by any method, this average rate of incorporation of the label into albumin is a measure of albumin synthesis as long as the urea synthetic rate remains constant.

Since alcohol might inhibit the release of newly synthesized albumin, the rate of release of labeled albumin was studied in both alcohol and control perfusates. Labeled albumin was detected in the alcohol and control perfusates by at least 35 min after the carbonate-^{14}C injection. The peak specific activity occurred by 100–120 min as it did in the nonalcohol-perfused control studies indicating essentially the same release of the labeled albumin in control and alcohol studies. If release were delayed by alcohol, the peak specific activity of the released albumin would have occurred later.

ANALYTICAL METHODS

The total protein in the perfusate was measured by a biuret method (10) and albumin partition by a Kern microelectrophoresis unit (11). Perfusate albumin was isolated by preparative acrylamide gel electrophoresis. 5 ml of red cell-free perfusate was dialyzed overnight in the cold against 500 ml of 0.128 M glycine-Tris (tris(hydroxymethyl) aminomethane) buffer, pH 8.3. After the addition of 50 mg sucrose to the dialyzed perfusate, 3.5 ml were layered over a 13×90 mm (diameter $\times$ height) 7.5% polyacrylamide column at pH 8.9 (1.5 M Tris $-$ HCl) in a Fractophorator (Buchler Instruments, Inc, Fort Lee, N. J.). A constant current of 5 ma was applied for 30 min to migrate and stack the proteins into the gel. The current was increased to 10 ma and 4 ml fractions collected every 5 min with a fraction collector. Tubes were read at 278 mμ. The fraction of the first peak, from the midpoint of the ascending slope to the peak of this slope were pooled and portions examined by qualitative polyacrylamide electrophoresis (8) and immunoelectrophoresis (12) at a 6% protein level.

The perfusate albumin from the pooled fractions was hydrolyzed with 6 N HCl, the hydrolyzate neutralized and passed through a resin column according to the method of McFarlane (6). After reacting the effluent with arginase, a portion was incubated with urease according to the method of Conway and Byrne (13), the ammonia released by 45% K_2CO_3 was trapped in 2 N H_2SO_4 and assayed with Nessler's reagent. An identical portion was incubated with urease, treated with H_3PO_4, the CO_2 released was trapped in phenethylamine which was then dissolved in 15 ml of a scintillator; the ^{14}C was assayed in an ambient-temperature liquid scintillation counter. The urea carbon-specific activity of the perfusate was determined in the same way using the supernatant from heated, tungstic acid precipitated samples to drive off any trapped CO_2-^{14}C (6).

SGOT (glutamic oxaloacetate transaminase) levels were measured in control and experimental perfusions before and after the perfusion (14).

After perfusion, the liver was chilled by the gentle injection of 25 ml of ice-cold 0.25 M sucrose in TKM buffer (50 mM Tris $-$ HCl (pH 7.5)–25 mM KCl $-$ 5 mM $MgCl_2$). The liver was weighed, minced, and homogenized in 2 volumes of the same buffer in a glass homogenizer with a loose-fitting teflon pestle. DNA was determined by the indole method of Ceriotti as modified by Keck (15) as follows: 0.1 ml of liver homogenate was added to 2 ml of ice-cold 0.3 M $HClO_4$. After 10 min in the cold the suspension was spun at 5000 *g* for 5 min. The precipitate was washed twice with 2 ml of ice-cold 0.2 M $HClO_4$. After the final centrifugation the excess acid was drained by inverting the tubes. The precipitate was suspended in 0.7 ml H_2O and dissolved upon the addition of 0.7 ml 0.6 N KOH. Occasionally solution was enhanced upon incubation at 37°C for 5–10 min. After solution, 2.6 ml of H_2O was added and mixed. To 2 ml of the final solution were added 1 ml of 0.06% indole and 1 ml 2.5 N HCl and heated in a boiling water bath for 20 min together with a reagent blank and calf thymus DNA standards. They were then cooled and extracted three times with 4 ml of $CHCl_3$. After the last extraction the tubes were centrifuged in order to clarify the upper aqueous phase. The absorbance of the aqueous phase was measured at 490 mμ.

The determination of RNA was essentially that of Fleck and Begg (17): 0.1 ml of liver homogenate was added to 2 ml of ice-cold 0.3 M $HClO_4$ and mixed. After 10 min in the cold, the mixture was spun at 5000 *g* for 5 min. The precipitate was washed twice with 2 ml of 0.2 M $HClO_4$ and after the final centrifugation the excess acid was drained off by inverting the tubes. The precipitate was suspended in 0.5 ml H_2O and then dissolved by the addition of 0.5 ml 0.6 N KOH. The RNA was hydrolyzed by incubation at 37°C for 1 hr. The solution was cooled on ice and 2 ml of ice-cold 0.6 M $HClO_4$ was added. After 10 min in the cold, the suspension was spun at 5000 *g* for 5 min and the supernate diluted 1:3 with water. RNA was determined from the absorbance at 260 mμ determined with $E_{1cm}^{1\%} = 312$. Total protein was determined by the method of Lowry (16). Lactate and pyruvate levels were obtained in control, alcohol

perfused- and alcohol tryptophan-perfused livers employing Sigma Chemical Co. kits 826B and 726. Samples of perfusate blood were obtained at 30 min intervals and the concentrations of lactate and pyruvate determined on each sample.

Polysomes were isolated from the whole liver, after 30 min of perfusion and after the standard 2.5 hr perfusion, employing the techniques described by Blobel and Potter (18) as modified below. Nuclei, debris, and mitochondria were separated by a 10 min spin at 15,000 *g* and the supernate layered over at 1.38–2 M sucrose discontinuous gradient in TKM containing cell sap as an RNAse inhibitor as suggested by Blobel and Potter (18). After a 20 hr spin at 105,000 *g* (No. 40 rotor, Spinco model L ultracentrifuge) the bound polysomes sedimenting into the 1.38 M sucrose layer were removed, treated with ¼ vol of 20% Triton–5% sodium deoxycholate solution and recentrifuged through 2 M sucrose in TKM-cell sap for 20 hr as above. The pellet obtained was considered to represent the polysomes which had been bound to the endoplasmic reticulum.

Polysome analysis. The polysomes obtained from the endoplasmic membranes were suspended in 1 ml of cold distilled water and 3–5 absorbance units (260 mμ) was layered over a 4.5 ml linear sucrose gradient (0.3–1.1 M in TKM over a 0.2 ml cushion of 60% sucrose). These gradients were spun at 40,000 rpm in a SW 50L at 4°C for 35 min and the resultant gradient was analyzed in a ISCO ultraviolet analyzer (Instrumentation Specialties Co.) at 254 mμ (model UA-2).

The average concentration of alcohol in the perfusate at the onset of perfusion was 220 mg/100 ml and the half-time of disappearance was 70 min. In the rabbits given the alcohol diet, the highest blood alcohol level observed was 57 mg/100 ml.

RESULTS

The animals on restricted diets containing either alcohol or sucrose (100 cal/day) maintained their weights, while the control rabbits whose caloric intake was about twice as great gained an average of 200–300 g over the 8 wk dietary period.

The results of these studies are presentd in Table II. The five groups of rabbits were as described in Methods.

Group 1. control diet—control perfusate
2. control diet—alcohol perfusate
3a. alcohol diet—control perfusate
3b. alcohol diet—alcohol perfusate
4. sucrose diet—control perfusate
5. control diet—alcohol and tryptophan perfusate

In the control group 1, albumin synthesis was 33 mg/100 g wet liver weight in the 2½ hr of perfusion. With the addition of alcohol to the perfusate albumin synthesis fell 67% (group 2) ($P < 0.01$). The feeding of alcohol for 8 wk failed to lower the albumin synthetic rate, which equalled that found when sucrose replaced the alcohol in the diet, (group 4). However when two animals were fed alcohol and alcohol was present in the perfusate albumin synthesis was again markedly depressed by 79 and 73% from the control rate.

TABLE II
Hepatic Composition after Perfusion

Donor rabbit	Perfusate	Studies	Protein/DNA	RNA/DNA
		n		
Fed	Control	11	70 ±1	2.3 ±0.1
Fasted*	Control	5	53 ±2‡	1.8 ±0.2‡
Fed	Alcohol	7	54 ±5‡	1.8 ±0.1‡
Fed	Alcohol (30 min)	4	80 ±7	2.4 ±0.2
Fed	Alcohol tryptophan	8	70 ±3	2.4 ±0.1
Fasted*	Tryptophan	6	65 ±7	2.0 ±0.2

Values presented as ±SEM.
* Previously reported (8).
‡ *P* value less than 0.01 compared with the fed control and fed alcohol tryptophan studies.
Alcohol (30 min) signifies after 30 min of perfusion with alcohol the time that the CO_2-^{14}C was added. The liver or samples of liver were removed at that time and the ratios determined.
The sucrose and the alcohol-fed rabbits had ratios which were identical with the control values.

With the addition of tryptophan to the alcohol perfusates (group 5) albumin synthesis rose significantly from the low levels seen in groups 2 and 3b without tryptophan ($P < 0.01$) to a value only 27% below that found in the control group.

Free amino acid levels were measured in the alcohol-containing perfusates before and after perfusion and no significant changes were noted from previously reported pre- and postperfusion levels for control perfusions (8).

SGOT values ranged between 5 and 25 sigma units before perfusion and did not change significantly in the control perfusions. In the perfusions in which alcohol was added the SGOT values rose to average 122 ±34 units (SE). Hemolysis was minimal in these perfusions as well as in the control studies.

Both protein/DNA and RNA/DNA ratios decreased after the 2.5 hr perfusion in the presence of alcohol and in livers from fasted donors. These ratios were maintained at control values upon the addition of 10 mM tryptophan (Table II). With only 30 min of alcohol perfusion, no change in these ratios were noted.

Lactate/pyruvate ratios were measured in most studies. The control range was 11–20 at the initiation of perfusion and decreased to 5–10 after 2.5 hr of perfusion. In control, alcohol, and alcohol tryptophan studies the lactate levels did not change greatly during the study, ranging from 3–6 μmoles/ml in any one perfusion. However in the alcohol-containing perfusates the pyruvate levels fell rapidly from the control values of 0.5–0.7 μmoles/ml to nearly undetectable levels during the per-

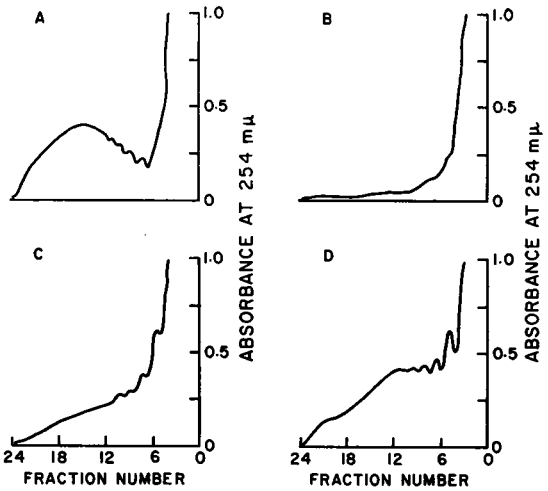

FIGURE 1 Polysomal profiles. Endoplasmic membrane-bound polysomal profiles from control (A) alcohol perfused (B) alcohol tryptophan-perfused (C and D) livers. The profiles were examined on linear sucrose gradients 0.3–1.1 TKM buffer over a 0.2 ml cushion of 60% sucrose. The gradients were spun at 40,000 rpm in the SW 50 L at 4°C for 35 min. See text. Albumin synthesis (A) 40 mg, (B) 6 mg, (C) 24 mg, (D) 16 mg. Albumin synthesis was markedly depressed in the alcohol study and disaggregation was complete. However, the degree of reaggregation induced by tryptophan was not related to the degree of stimulation of albumin synthesis. (See text.)

fusion resulting in very high lactate/pyruvate ratios. The pyruvate levels remained depressed until the perfusion had continued for 90–120 min. Tryptophan did not affect these results. At this time in the perfusion, the alcohol levels were below 100 mg/100 ml and pyruvate levels increased to 0.2–0.3 μmoles/ml and remained at these levels until the end of the perfusion. Lactate/pyruvate ratios ranged from 12–35 at the end of perfusion in the alcohol or alcohol tryptophan studies.

The sedimentation profiles of the isolated-bound polysomes are shown in Figs. 1 and 2. When the polysomes were examined after the 2.5 hr of perfusion the presence of alcohol resulted in disaggregation of the bound polysome (1b). However, the degree of reaggregation of the bound polysome upon the addition of tryptophan bore no relationship to the absolute rate of albumin synthesis, and a minimal degree of reaggregation (1c) was found to be associated with a twofold increase in albumin synthesis.

The polysome profiles of four alcohol and alcohol tryptophan studies were also examined at the time when the carbonate-^{14}C would be added to the liver. This time represents the beginning of incorporation of labeled arginine into albumin (Fig. 2). While the degree of aggregation was not as marked as in the control group (Fig. 1a) disaggregation had not occurred and both groups revealed similar polysomal profiles, yet the alcohol-perfused liver did not synthesize albumin normally.

DISCUSSION

The isolated rabbit liver perfused with alcohol, fails to synthesize albumin at a normal rate while the addition of tryptophan to a final concentration of 10 mM reverses this alcohol-induced inhibition. The feeding of alcohol for 8 wk equivalent to 50% the caloric intake does not alter albumin synthesis as long as alcohol is not present in the perfusion system. There are few published data on the acute effects of alcohol on hepatic protein synthesis, but a decrease in the rate of release of labeled lipoproteins has been noted in rat liver perfusions when alcohol was added (19). However, the rate of incorporation of the label into total protein and into albumin was not delayed in this study.

The acute effects of alcohol on subcellular elements in the hepatocyte have been observed repeatedly with disorganization of ribosome attachment to the endoplasmic reticulum a prominent feature (1). Since albumin is synthesized on a polysome bound to the endoplasmic reticulum, the rapid loss of some of the albumin-synthesizing capacity in the present studies was not unexpected (20, 21). The addition of tryptophan to the alcohol-loaded perfusates resulted in a reversal of most of the acute effects of alcohol with respect to albumin

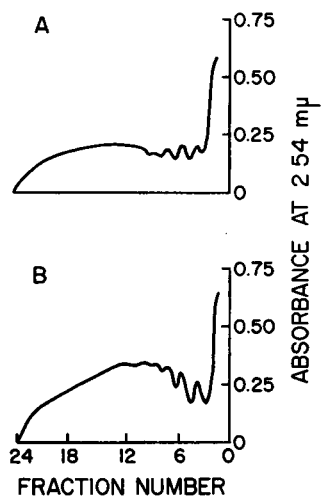

FIGURE 2 Polysomal profiles. Endoplasmic membrane-bound polysomal profiles from alcohol perfused (A) and alcohol tryptophan-perfused livers (B). The livers were homogenized after 30 min of perfusion at the time when the carbonate-^{14}C would be added. Thus these profiles represent the profiles at the time that the livers are synthesizing albumin-^{14}C. Aggregation is present in both profiles. 3.1 OD units were layered over the sucrose gradient in (A) and 4.1 OD units in gradient (B).

synthesis in the perfused liver. The specific mechanism by which tryptophan reactivates albumin synthesis is not known. Studies in vivo and in vitro have shown tryptophan to cause reaggregation of polysomes and these changes have been related to increases in labeled amino acid incorporation into protein (*22*, *23*). Similarly, when tryptophan was added to the perfusate employing livers from fasted animals, the results indicated parallelism between increased albumin synthesis and bound polysome reaggregation (8). Further, if the action of tryptophan is to cause polysome reaggregation, then no effect of tryptophan would be expected on a fully aggregrated system. In nine studies previously reported (8), tryptophan failed to stimulate albumin synthesis when the liver was derived from a fed donor. That such correlations are hard to interpret is shown in Figs. 1 and 2. 30 min after alcohol perfusion a reasonably aggregated-bound polysome pattern was found in these livers, yet albumin synthesis was essentially zero. On the other hand, polysome patterns from alcohol-tryptophan perfused livers were not remarkable in the degree of aggregation and albumin synthesis was near normal. Unless a particular region of the polysome profile can be ascribed to a specific protein then polysome profiles, per se, do not reflect quantitative changes in protein synthesis.

Another acute effect of alcohol in the 2.5 hr of perfusion was a loss in hepatic RNA and protein (Table II). The ratios of protein and RNA to DNA resembled those found in livers from fasted animals. The presence of tryptophan in the alcohol-containing perfusates and in perfusates from the fasted livers prevented this decrease in RNA and protein. With only 30 min of alcohol perfusion, loss of RNA and protein was not observed and the decrease in the incorporation of the ^{14}C-label into albumin, at this time could not be ascribed to a loss of RNA.

The metabolism of alcohol results in an alteration in the oxidative state within the liver, resulting in reduced gluconeogenesis and lowered urea production (9, 19, 23–25) and in the present studies, urea synthesis though steady was depressed by about 35%. Tryptophan however did not alter the alcohol-induced changes of the oxidative state within the hepatic cell as evidenced by the lack of reversal of the high lactate/pyruvate ratios. Recently, excess tryptophan has been suggested as playing a major or key role in shifting metabolism between gluconeogenesis and protein synthesis by inhibiting phosphoenolpyruvate carboxykinase (*26*) and this metabolic action must also be considered.

While the mechanism underlying the acute effects of both alcohol and tryptophan on albumin production remain to be answered, the present studies do show that the acute administration of alcohol results in rapid decreases in albumin production with a loss of cellular RNA and protein. The acute effects of alcohol on albumin synthesis may be moderated if excess tryptophan is present in the perfusate at the same time.

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