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EFFECT OF CHRONIC ETHANOL AND SUCROSE INGESTION ON LIVER POLYSOMAL POLY(A)mRNA CONTENT AND INCORPORATION OF [5-^{3}H]URIDINE INTO mRNA*

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Abstract—Mice fed a combination of 10 per cent (v/v) ethanol and 5 per cent (w/v) sucrose for 5 months showed a 2-fold increase in the amount of [5-^{3}H]uridine label that was incorporated into putative mRNA in the polysomes of the liver. Part of the increase was due to a change in the total amount of UTP present in the cell, although the uptake and processing of [5-^{3}H]UTP were not altered. When corrected for UTP pool size variation, ethanol–sucrose-treated mice still incorporated 1.5-fold more [5-^{3}H]uridine-labeled RNA into polysomes than controls. Poly(A$^+$)mRNA content was 1.2 times greater in ethanol–sucrose-treated mice, and [5-^{3}H]poly(U) hybridization to poly(A)mRNA suggested that individual poly(A) tracts may be 1.5 times longer in ethanol-sucrose-treated mice than in controls. [Poly(A)mRNA is the same as poly(A$^+$)mRNA]. The proportion of newly labeled poly(A$^-$)mRNA declined in ethanol–sucrose-treated mice compared to controls. The results indicate that ethanol–sucrose treatment may cause an increase in the amount of poly(A$^+$)mRNA that has longer poly(A) tracts and a concomitant decline in the amount of [5-^{3}H]uridine label incorporated into poly(A$^-$)mRNA. A decline in protein synthesis could not be explained by a lack of mRNA in ethanol–sucrose fed mice.

Chronic alcohol ingestion in conjunction with a nutritionally adequate diet affects protein and RNA synthesis.* Because of the use of different diets, different strains of animals, and animals of different ages, some of the results from studies on alcohol-induced changes in protein synthesis have been conflicting. For example, Kuriyama *et al.* [1] and Jarlstedt [2] found that chronic alcohol treatment causes a rise in the rate of liver protein synthesis *in vivo*, whereas others have found a consistent decrease *in vivo* [3–6] and *in vitro* [7, 8] that is apparently not due to an unavailability of amino acids [8]. The decrease of protein synthesizing capability may be due to a decrease in tRNA acceptor activity [9] and changes in the ability of ribosomes to bind to mRNA [10]. Porta *et al.* [11] found that a chronic alcohol–sucrose diet did not affect the RNA content per milligram protein content in rats, and Banks *et al.* [6] determined that RNA content per gram rat liver did not change during chronic alcohol treatment. At the same time, the syntheses of mitochondrial RNA, nRNA, tRNA and rRNA in mouse brain appears to be inhibited by chronic alcohol administration [10, 12–14]. The content of poly(A)mRNA in mouse brain polysomes, however, is not altered as a consequence of alcoholism [15]. The present study was designed to test the hypothesis that alcohol–sucrose-induced changes in liver protein synthesis may be due, at least in part, to a change in the number of polysomes present in the mouse liver. The effect of alcohol–sucrose treatment on the incorporation of newly made mRNA into mouse liver polysomes and the amount of polysome associated poly(A)mRNA was also studied.

MATERIALS AND METHODS

Animals. Female Swiss Webster ICR strain mice (22 g), obtained from Timco (Houston, TX), were given a 10 per cent (v/v) ethanol–5 per cent (w/v) sucrose solution for 20–22 weeks as the sole source of liquid, while the control animals were offered water. Both groups were fed laboratory chow (Purina) *ad lib.* and kept in the same 12-hr alternating light and dark cycle. All experiments were initiated between hr 1 and hr 3 of the light cycle because polysome abundance undergoes a diurnal rhythm [16]; the animals were not withdrawn from alcohol prior to experimentation.

Labeling of polysomes. Liver polysomes were labeled by intraperitoneal injection of each mouse with 500 μCi of [5-^{3}H]uridine, 29 Ci/mmole (Research Products International, Elk Grove Village, IL). After 60 min of incorporation, mice were killed by cervical dislocation, and the liver polysomes were extracted.

Extraction of polysomes. All glassware used in this study was washed in triple distilled water and heat treated for 3 hr at 350° to destroy RNAse activity.

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* The terms alcohol and ethanol are used interchangeably. Poly(A)mRNA is the same as poly(A$^+$)mRNA. The A : C ratio is the ratio found by dividing the alcohol sucrose data by the appropriate control data.

The general procedure of Sala-Trepat *et al.* [17] was used in preparing polysomes. Livers, after weighing, were homogenized in 5 ml of 0.025 M Tris–HCl (pH 7.5), 0.1 M KCl, 0.04 M NaCl, 0.075 M $MgCl_2$, 0.05 M NH_4Cl, 0.5 mg/ml heparin, 1.5 mg/ml yeast tRNA and 0.006 M 2-mercaptoethanol, using a Dounce homogenizer (A) pestle. The homogenates were centrifuged at 15,000 *g* (r_{av} 9.5 cm) for 10 min at 4°. The resulting postmitochondrial supernatant fractions from alcohol–sucrose and control livers were then equalized on the basis of protein concentration by adding buffer to the more concentrated sample. The Bio-Rad assay [18] was used to determine protein concentration. Heparin was then added to a final concentration of 3 mg/ml, and 1/9th volume of a 10 per cent (v/v) Triton X-100–10 per cent (w/v) sodium deoxycholate solution was added with continuous stirring.

Polysome gradients. Equal volumes of the postmitochondrial supernatant fractions from alcoholic and control mice were layered onto 10–35 per cent (w/v) sucrose gradients in homogenization buffer lacking Triton X-100 and sodium deoxycholate. As a control for nuclear RNA contamination, EDTA (0.1 M) was added to equal volumes of control and experimental postmitochondrial supernatant fractions, which were layered onto 10–35 per cent (w/v) sucrose gradients as above, except that 0.025 M EDTA was substituted in the gradient for 0.01 M $MgCl_2$. The gradients were centrifuged in a Beckman SW 41 rotor at 205,600 *g* (r_{av} 10.93 cm) for 70 min at 4°. A Beckman L5-50 ultracentrifuge was used in all gradient experiments. The gradients were then fractionated with a density gradient fractionator (ISCO, Lincoln, NE). The relative amount of polysomes in each gradient was determined by planimetry of the absorbance scans. In order to measure incorporated [5-^{3}H]uridine, the fractions from the polysome gradients were precipitated by the addition of 2.5 vol. of ethanol (carrier RNA or DNA was added to a final concentration of 40 μg/ml). (With an ISCO density gradient fractionator, unincorporated [5-^{3}H]uridine is introduced down through the gradient by wall friction, making the precipitation step necessary.) After sitting overnight at −20°, the precipitates were centrifuged at 10,000 *g* (Sorvall HB-4 rotor, r_{av} 9.15 cm) for 10 min at 4° and resuspended in 1 ml of water. Four milliliters of Biocount (Research Products International) was added to each sample in a minivial prior to counting. Samples were counted for 50 min at a counting efficiency of 38 per cent.

Extraction of polysomal RNA. Polysomes from postmitochondrial supernatant fractions (see "Extraction of polysomes") were centrifuged through 3 ml of 1 M sucrose in 0.1 M NaCl, 0.001 M EDTA, and 0.05 M Tris–HCl (pH 7.5) (NET buffer). The RNA was extracted from polysomes by the method of Perry *et al.* [19], (phenol-nucleic acid grade, Bethesda Research Laboratories, Rockville, MD; chloroform, Mallinckrodt Chemical Works, St. Louis, MO). The RNA was precipitated overnight with 2.5 vol of ethanol at −20° and then centrifuged. The pellet was resuspended in NET buffer. Diethylpyrocarbonate (Sigma Chemical Co., St. Louis, MO) was added to a concentration of 3 μl/ml and the sample was layered over a 1.5 ml pad of 20 per cent (w/v) sucrose in NET buffer. The sample was centrifuged in an SW 41 rotor at 205,600 *g* (r_{av} 10.93 cm) for 1 hr at 4°. Insoluble materials present in the preparation were pelleted; the purified RNA remained in the supernatant fraction.

RNA gradients. Polysomal RNA (45 μg) from both alcoholic and control mouse polysomes was centrifuged in denaturing sucrose gradients as described previously [20]. The RNA gradients were fractionated and processed as described previously for polysome gradients.

[5-^{3}H]*Poly(U) hybridization with poly(A) tracts of mRNA.* Synthetic poly(A) (0.1 μg, Sigma Chemical Co.) was used as a standard and processed the same as polysomal RNA fractions. Separate or pooled fractions from each RNA gradient were precipitated in ethanol, centrifuged and resuspended in NET buffer. Each RNA sample was incubated with [5-^{3}H]poly(U) (Miles Laboratories, Elkhart, IN, 35 μCi/μmole phosphate) at 40° for 15 hr and processed as described previously [21]. Samples were counted at 18 per cent efficiency in a nonaqueous mixture (toluene containing 4 g/l PPO and 0.1 g/l POPOP).*

Oligo(dT)-cellulose chromatography. Poly(A) mRNA was purified free of rRNA and tRNA using oligo(dT)-cellulose chromatography as described previously [21]. Individual fractions were quantified as to volume, and the amount of nucleic acid present was determined spectrophotometrically. The 260/280 ratios were routinely between 2.15 and 2.17, indicating highly purified RNA.

Measurement of uridine nucleotide availability in alcoholic and control mouse livers. Alcoholic and control mice were killed 1 hr after intraperitoneal injection of [5-^{3}H]uridine (500 μCi/mouse). After removal of the gall bladders, the livers were excised, sliced and frozen in liquid nitrogen within 20 sec of death. The frozen liver tissue was weighed and transferred rapidly to a tube containing 6 ml of 0.6 N perchloric acid and homogenized thoroughly with a Sorvall (Newton, CT) Omnimix. The homogenates were centrifuged at 14,000 *g* (Sorvall HB-4 rotor, r_{av} 9.5 cm) for 10 min at 4°, and 0.5 M Tris–HCl (pH 6.5) was then added. The pH of the supernatant fractions was adjusted to pH 6–7 by dropwise addition of 5 M KOH. The supernatant fractions were then chilled rapidly to −20°, and the precipitated potassium perchlorate was removed by centrifugation at 14,000 *g* (Sorvall HB-4 rotor, r_{av} 9.5 cm) for 5 min at 4°. The resulting supernatant fraction was stored at −20°.

Two dimensional ion-exchange chromatography on poly-(ethyleneimine)-cellulose (PEI-cellulose) thin-layer plates (Brinkmann Instruments, Houston, TX) was used to separate nucleotides [22]. Samples (50 μl) were first concentrated using activated charcoal (Sigma Chemical Co.) [23]. A UTP standard bound with 80 per cent efficiency and was undegraded by this treatment. ATP, GTP, UTP and CTP were used as standards (10^{-2} M solutions in water)

* PPO = 2,5-diphenyloxazole; POPOP = 1-4-bis[2-(5-Phenoxazolyl)benzene; Phenyloxazolylphenyl-oxazolyl-phenyl)

Table 1. Effect of chronic ethanol–sucrose treatment on mouse body and organ weight, food and liquid consumption, [5-^{3}H]uridine incorporation into liver mRNA, poly(A)mRNA content, and poly(A) tract length*

	Number of replicate animals/treatment	Number of experiments	Ethanol–sucrose treated	Control	Ratio A:C	Significance
Initial body weight (g)	10	1	22.3 ± 1.3	22.3 ± 2.0	1.0	NS
Final body weight (g)	5	1	31.0 ± 0.9	31.0 ± 2.3	0.97	NS
Average food consumption (g/day per mouse)	8	1	3.7 ± 2.4	3.94 ± 1.44	0.94	NS
Average liquid consumption (ml/day per mouse)	8	1	5.76 ± 0.456	5.52 ± 0.432	1.04	NS
Liver weight (g)	8	1	1.21 ± 0.225	1.24 ± 0.168	0.98	NS
Liver supernatant protein (mg/g body wt)	1	7	140.4 ± 37.9	127.1 ± 19.4	1.1	NS
Labeled mRNA in polysomes (net dpm/mg supernatant protein $\times 10^{-3}$)	1	4	3.0 ± 0.45	1.5 ± 0.08	2.04	0.003
Labeled mRNA in polysomal RNA (net dpm/mg polysomal RNA $\times 10^{-5}$)	1	3	3.1 ± 0.54	1.5 ± 0.11	2.09	0.013
Labeled poly(A)mRNA bound to oligo(dT)-cellulose (net dpm/mg polysomal RNA $\times 10^{-5}$)	1	2	1.7 ± 0.22	0.59 ± 0.07	2.93	0.029
Labeled poly(A$^-$)mRNA not bound to oligo(dT)-cellulose (net dpm/mg polysomal RNA $\times 10^{-5}$)	1	2	1.0 ± 0.032	0.83 ± 0.059	1.25	0.029
Total poly(A)mRNA bound to oligo(dT)-cellulose (μg/mg polysomal RNA)	1	2	24.05 ± 1.06	19.6 ± 0.57	1.23	0.025
Specific activity of poly(A)mRNA (net dpm/μg poly(A)mRNA $\times 10^{-3}$)	1	2	7.2 ± 0.61	3.0 ± 0.27	2.39	0.018
[5-^{3}H]Poly(U) hybridized to polysomal RNA (net dpm/mg polysomal RNA $\times 10^{-5}$)	1	3	4.1 ± 1.1	2.1 ± 0.31	1.91	0.026
Estimated poly(A) tract length NA of poly(A)mRNA (nucleotides)	NA	NA	116 (8.7%)†	75 (5.6%)†	1.54	NA

* Values are means ± S.D. Student's *t*-test (two-tailed) for paired observations was used in determining the level of significance. Levels above 0.05 are considered insignificant (NS); not applicable (NA).

† Estimated percentage of poly(A) in total poly(A)mRNA.

on a separate plate. The fractionated nucleotides were identified by their respective R_F values and by comparison to standards run on separate plates using u.v. light to visualize the nucleotides. Sections of the plates were cut out using a cork borer, and the nucleotides were eluted using 0.07 M $MgCl_2$–0.2 M Tris–HCl (pH 7.4). Nucleotides were quantified spectrophotometrically, and the A_{max} of each nucleotide was determined as a further means of identification. Samples were counted at 38 per cent efficiency for 50 min using Biocount.

RESULTS

Animals and ethanol–sucrose treatment. Mice fed alcohol–sucrose for 5 months had the same mean weight as controls, and no significant difference in mean liver weight was observed (Table 1). There was no difference in either food or liquid consumption (Table 1), although there was a slower growth rate in the alcohol–sucrose group during the first month of the experiment (this slower average growth rate was due to four mice in the alcohol–sucrose group that died during this time period). Following these deaths, the growth rates of both alcohol–sucrose and control groups were the same. Sucrose was administered with the alcohol to encourage the mice to drink more alcohol. Mice that drank 10 per cent (v/v) ethanol alone consumed 4.1 ml/day per mouse, while mice that drank 10 per cent (v/v) ethanol–5 per cent (w/v) sucrose consumed 5.76 ml/day per mouse (difference significant at 0.01 level).

Effect of alcohol–sucrose treatment on morphology. Routine histological sections were prepared with haematoxylin and eosin stain for the general architecture of liver, Mallory's triple stain for connective tissue and oil red 0 for lipids. The alcohol–sucrose treatment did not cause any distortion of the normal liver architecture, and no cell, or nuclear, enlargement was observed. There was no increase in the amount of connective tissue seen due to the alcohol–sucrose treatment. However, the treatment did cause a 2- to 3-fold increase in the amount of lipid deposited in periportal hepatocytes, a result similar to that obtained using rats [11, 24, 25]. Concentrations of alcohol higher than 10 per cent (v/v) and sucrose 5 per cent (v/v) fed to rats can cause significant architectural distortions, connective tissue invasion, and fat accumulation [24].

Effect of alcohol–sucrose treatment on [5-^{3}H]uridine incorporation into polysomes. Preliminary experiments using the Bio-Rad (Richmond, CA) protein assay [18] on crude liver homogenates showed that protein content did not differ between the two groups. Results were analyzed using Student's *t*-test; the confidence level was 0.01. The amount of postmitochondrial supernatant protein (PMSP) was chosen as a standard upon which to base the results of the experiment on [5-^{3}H]uridine incorporation into polysomes. This standard was chosen since protein content did not vary, and the standard accounted for vagaries in homogenization and centrifugation. Although the standard deviation of the PMSP data was somewhat high, there was no significant difference in the amount of PMSP recovered in alcohol–sucrose or control mice (Table 1).

One hour after injection of [5-^{3}H]uridine, polysomes were extracted from the livers of alcoholic and control mice and centrifuged in sucrose gradients, as described in 'Materials and Methods' (Fig. 1). In four experiments, there was no significant difference in the total amount of polysomes obtained per milligram PMSP, and no consistent difference in absorbance profiles of the polysome gradients was found. EDTA disrupted the polysomes, as indicated by the little absorbance in the polysome (> 80S) portion of the gradient (Fig. 1). EDTA does not disrupt the [5-^{3}H]uridine labeled ribonucleoprotein that is released from the nucleus during homogenization and contaminates the polysome portion of the gradient [15, 26]. The cpm in each fraction of the polysome portion of the EDTA-treated alcohol–sucrose gradient was subtracted from the corresponding fraction of the alcohol–sucrose polysome gradient. Similarly, the cpm in each fraction of the polysome portion of the EDTA-treated con-

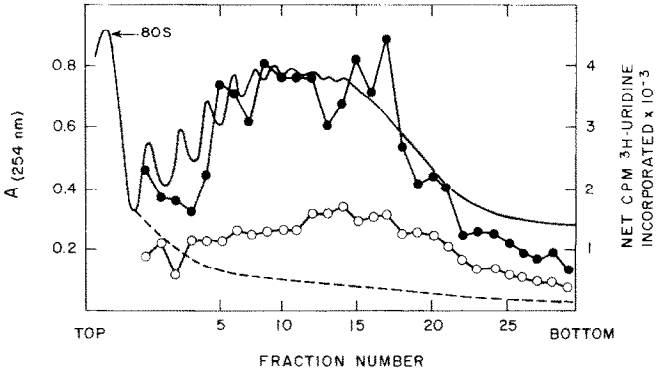

Fig. 1. Effect of alcohol–sucrose treatment on the incorporation of 60-min pulse labeled [5-^{3}H]uridine into mouse liver polysomes. Key: (——) absorbance profile typical of polysomes isolated from both alcohol–sucrose-treated and control mice; (-----) effect of EDTA treatment on absorbance profile; (●) net incorporation of [5-^{3}H]uridine into mRNA of alcohol–sucrose polysomes–EDTA control; and (○) net incorporation of [5-^{3}H]uridine into mRNA of control polysome-EDTA control. One alcohol-sucrose-treated and one control mouse were used.

trol gradient was subtracted from the corresponding control polysome gradient. In four experiments, an average of 60 mg of PMSP was layered on each gradient, and about 25 per cent of the cpm recovered was due to nuclear contamination. The net cpm due to putative mRNA in both experimental and controls of a single experiment are graphed in Fig. 1. In each fraction of the gradient, the amount of labeled precursor incorporated into alcohol–sucrose mRNA was generally twice that incorporated into control mRNA. Since polysome size is dependent on mRNA length [17, 27], it is most probable that large numbers of different mRNAs were affected by the alcohol–sucrose treatment.

In four separate experiments, the amount of [5-^{3}]uridine incorporated into liver polysomes of alcohol–sucrose-treated mice was greater than controls by a ratio of 2.04 (Table 1). This difference was significant at the 0.003 confidence level as determined by Student's *t*-test. For all four experiments, the average cpm/60 mg PMSP recovered in the alcohol–sucrose polysome region was 93,841. After deducting 23,453 cpm for nuclear contamination, a net of 70,361 cpm was realized. For the control group, an average of 46,117 cpm was recovered, and after deducting 11,529 cpm for nuclear contamination, a net of 34,588 cpm was obtained. These results were converted to dpm/mg PMSP and listed in Table 1.

Effect of alcohol–sucrose treatment on [5-^{3}H] *uridine incorporation into polysomal RNA.* When 45 μg of extracted polysomal RNA from experimental or control polysomes was centrifuged in sucrose gradients, the resultant absorbance profiles could be superimposed (Fig. 2). Since the amount of polysomes did not vary between groups, it was unlikely that the amount of RNA composing these polysomes varied. Therefore, the incorporation of [5-^{3}H]uridine into mRNA was based on equal amounts of polysomal RNA. The shape of the profiles indicated that the purified RNA was undegraded [23]. The pattern of [5-^{3}H]uridine incorporation into alcohol–sucrose and control polysomal RNA was heterogeneous and did not rise significantly where the 18S and 28S absorbance peaks were. This indicated that the majority of incorporated label in both polysome and RNA gradients was in mRNA and not rRNA. The RNA profiles also indicated that many different mRNAs were affected by the alcohol–sucrose treatment, there being a 2- to 3-fold increase in the amount of [5-^{3}H]uridine incorporated by all fractions of alcohol–sucrose versus control RNA (Fig. 2). The exact number of mRNAs affected cannot be determined unequivocally using sucrose gradient centrifugation because this technique cannot sufficiently separate the estimated 34,000 different mRNAs that may be present in rodent liver [28].

Isolated poly(A)mRNA from alcohol–sucrose and control polysomes exhibited the same density gradient profiles when equal quantities were applied to the gradients (Fig. 3). Sala-Trepat *et al.* [17] estimated the number average nucleotide size of rat liver poly(A)mRNA to be approximately 1475 nucleotides, while we obtain a value of 1400 nucleotides for mouse using the data from Fig. 3 and the formula:

$$\text{Number average nucleotide length} = \frac{\sum_i (A)\, L_i}{\sum_i (A)_i},$$

where A_i is the absorbance in fraction i and L_i = the nucleotide length of RNA sedimenting in fraction i.

The [5-^{3}H]uridine patterns were the same as those for polysome (Fig. 1) and polysomal RNA (Fig. 2) scans with the alcohol–sucrose poly(A)mRNA incorporating two to three times more isotope in each

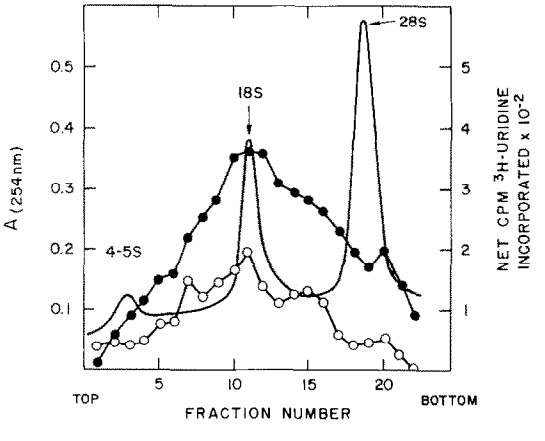

Fig. 2. Effect of alcohol–sucrose treatment on the incorporation of 60-min pulse labeled [5-^{3}H]uridine into mouse liver polysomal RNA. Key: (——) absorbance profile typical of 45 μg polysomal RNA isolated from both alcohol–sucrose-treated and control mice; (●) net incorporation of [5-^{3}H]uridine into mRNA of alcohol–sucrose polysomal RNA–EDTA control; and (○) net incorporation of [5-^{3}H]uridine into mRNA of control polysomal RNA–EDTA control. One alcohol–sucrose-treated and one control mouse were used.

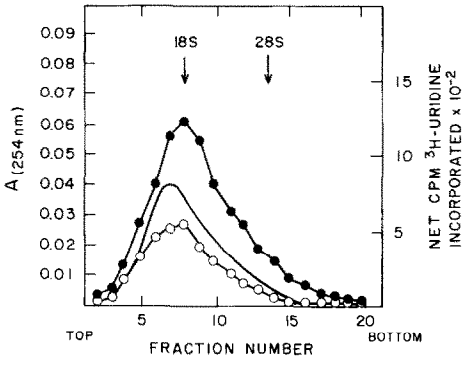

Fig. 3. Effect of alcohol–sucrose treatment on the incorporation of 60-min pulse labeled [5-^{3}H]uridine into mouse liver poly(A)mRNA. Key; (——) absorbance profile typical of 22 μg of poly(A)mRNA from both alcohol–treated and control mice;(●) net incorporation of [5-^{3}H]uridine into poly(A)mRNA of alcohol–sucrose mice; and (○) net incorporation of [5-^{3}H]uridine into poly(A)mRNA of control mice. One alcohol–sucrose-treated and one control mouse were used.

Table 2. Effect of alcohol–sucrose treatment on poly(A)mRNA content and incorporation of [5-^{3}H]uridine into poly(A)mRNA and poly(A$^-$)mRNA*

	Experiment 1		Experiment 2	
	Alcohol–sucrose	Control	Alcohol–sucrose	Control
Polysomal RNA applied (μg)	406	499	965	575
Poly(A)mRNA bound (μg)	10.1	10	22.5	11.1
Percent based on μg of poly(A)mRNA	2.5	2.0	2.3	1.9
[5-^{3}H]Polysomal RNA applied (cpm)	45,641	28,766	99,721	29,200
[5-^{3}H]Poly(A)mRNA bound (cpm)	29,200	12,156	60,573	11,855
Per cent of cpm bound as poly(A)mRNA	64	42	60.7	40.6

* Polysomal RNA labeled for 60 min with [5-^{3}H]uridine was chromatographed on oligo(dT)-cellulose. One alcohol–sucrose mouse and one control mouse were used in each experiment.

gradient fraction than controls. Both rRNA and tRNA peaks were absent in both absorbance and labeling profiles, indicating a very pure preparation of poly(A)mRNA.

Except for the above experiment, measurement of the amount of [5-^{3}H]uridine incorporated into polysomal RNA was done with an average of 100 μg of polysomal RNA/experiment. In the alcohol–sucrose gradients, an average of 15,786 cpm was recovered, of which 4093 cpm (26 per cent) was nuclear contamination, leaving a net recovery of 11,694 cpm. For controls, gross recovery was 7514 cpm, of which 1948 cpm (26 per cent) was contamination, leaving a net of 5566 cpm. Results are summarized in Table 1 based on the number of dpm/mg polysomal RNA.

*Effect of alcohol–sucrose treatment on poly(A)-mRNA and poly(A$^-$)*mRNA. [5-^{3}H]Uridine-labeled polysomal RNA was applied to oligo(dT)-cellulose in order to measure the total amount of poly(A)mRNA present and the proportion of newly made labeled poly(A)mRNA. In the first experiment, 2.5 per cent of the mass of the alcohol–sucrose polysomal RNA bound to oligo(dT)-cellulose, compared to 2 per cent for controls (Table 2). However, 64 per cent of the labeled newly made alcohol–sucrose polysomal RNA bound, compared to 42 per cent for controls. In the second experiment, 2.3 per cent of the mass of alcohol–sucrose polysomal RNA bound, compared to 1.9 per cent for controls, while 60.7 per cent of the newly made alcohol–sucrose polysomal RNA bound, compared to 40.6 per cent in controls.

The results of the number of micrograms of polysomal RNA bound to oligo(dT)-cellulose were averaged and are listed in Table 1 as the total poly(A)mRNA bound/mg of polysomal RNA. The average A:C ratio was 1.23. The data obtained on the amount of [5-^{3}H]uridine incorporated into polysomal RNA were averaged with the results of a third experiment (alcohol–sucrose 366,600 dpm/mg RNA, control 154,263 dpm/mg RNA) and expressed in Table 1 as the number of dpm of labeled mRNA/mg polysomal RNA. The average A:C ratio was 2.09. The oligo(dT)-cellulose binding data were averaged and are listed in Table 1 as the net dpm of poly(A)mRNA/mg polysomal RNA. The average A:C ratio was 2.93. Finally, data for the amount of labeled poly(A$^-$)mRNA not bound to oligo(dT)-cellulose were averaged and are listed in Table 1 as the net dpm of poly(A$^-$)mRNA/mg polysomal RNA. The average A:C ratio was 1.25.

Effect of UTP pool sizes on the incorporation results. The results from the [5-^{3}H]uridine incorporation experiments could have been influenced by alcohol-induced changes in uridine metabolism in the liver. To check this possibility, the distribution of isotope among UTP, UDP and UMP was compared in alcohol–sucrose and control mouse livers 1 hr post-injection with [5-^{3}H]uridine. Chronic alcohol–sucrose ingestion had no effect on the uptake and processing of [5-^{3}H]uridine in mouse liver (Table 3). After 60 min, there was more than twelve times as much radioactivity in [5-^{3}H]UTP as in [5-^{3}H]UDP or [5-^{3}H]UMP. This result is similar to those of other pool size studies of rat liver [29]. The amount of

Table 3. Effect of ethanol–sucrose on free hepatic [5-^{3}H]UMP, [5-^{3}H]UDP, [5-^{3}H]UTP and the specific activity of UTP*

	Ethanol–sucrose	Control	A:C ratio
[5-^{3}H]UMP (dpm/g protein)	9.45×10^4	8.33×10^4	1.14
[5-^{3}H]UDP (dpm/g protein)	8.34×10^4	8.22×10^4	1.01
[5-^{3}H]UTP (dpm/g protein)	1.21×10^6	1.22×10^6	0.99
UTP (μmole/g protein)	0.692	0.966	0.72
Specific activity (μCi/μmole)	0.785	0.572	1.37

* Values are based on one control mouse and one ethanol–sucrose-treated mouse.

UTP eluted from PEI-cellulose plates was determined spectrophotometrically and corrected for inefficiences in binding and elution from charcoal (20 per cent) and in elution from PEI-cellulose (5 per cent). The amount of UTP differed between alcohol–sucrose fed mice and controls by a ratio of 0.72 (Table 3), and therefore the specific activity of UTP in alcohol–sucrose livers was 1.37 times that of control livers.

The labeling experiments must therefore be corrected for the variation of pool specific activity by dividing the A:C ratio for each labeling experiment by 1.37. When the RNA labeling data are thus corrected, the labeling differences reflect differing amounts of newly made (60 min) RNA. The procedure assumed that the differences in the specific activity of the precursor pools remained the same throughout the entire 60-min labeling period. Since the pool size difference was due to the alcohol–sucrose UTP pool being reduced compared to controls, this assumption should be valid as it was unlikely that the cold pool would be changed dramatically during the 60-min labeling period.

When the data in Table 1 were corrected for pool size variation, the A:C ratio for labeled polysomes/mg PMSP was 1.47, for labeled polysomal RNA/mg RNA 1.51, for labeled poly(A)mRNA/mg polysomal RNA 2.11, and for poly(A$^-$)mRNA/mg polysomal RNA was 0.9.

[5-^{3}H]*Poly(U) hybridization with poly(A)mRNA.* [5-^{3}H]Poly(U) hybridized with the poly(A) tracts of the poly(A)mRNA showed that there was 1.91 times more poly(A) in alcohol–sucrose polysomal RNA than in controls (Table 1). The specific activity of the [5-^{3}H]poly(U) was 0.11 μCi/μg polymer and the counting efficiency was 18 per cent. From the data in Table 1, it was determined that there was 2.08 μg of poly(A)/mg of alcohol–sucrose polysomal RNA compared to 1.09 μg in controls. Based on the recovery of poly(A)mRNA/mg of alcohol–sucrose polysomal RNA (Table 1), it was estimated that the amount of the alcohol–sucrose poly(A) was 8.65 per cent of the total poly(A)mRNA, while for controls it was 5.56 per cent. Using a number (average nucleotide size) of 1400 for the poly(A)mRNA, the average poly(A) tract length would then be 116 and 75 nucleotides, respectively.

DISCUSSION

Porta and Gomex-Dumm [24] observed that mice drank more alcohol when sucrose was present in the solution than when alcohol was used alone, and our results support this conclusion. Although the combination of alcohol and sucrose in the drinking water may lead to dietary inadequacy, the growth rates, final body weights, and liver weights suggested this did not occur in this study. In previous studies [11, 24, 25], far higher concentrations of alcohol–sucrose were used than in this study. The 5-month exposure period was chosen to equal or exceed exposure periods used in protein synthesis studies [1–6]. At the end of 5 months, the only consistent morphological change observed was the accumulation of lipid by periportal hepatocytes. This suggested that alcohol–sucrose and control livers contain essentially the same cell popula ons, making biochemical comparisons valid.

The amount of PMSP was selected as a standard upon which to base results because total liver protein (unpublished observation) and liver weight were shown not to vary between alcohol–sucrose-treated and control mice. Although some variation occurred, the amount of PMSP recovered from alcohol–sucrose and control livers after homogenization was not significantly different. Therefore, we concluded that the alcohol–sucrose and control livers were equally homogenized and that the use of PMSP as a basis of standardization accounted for variation in homogenization. The amount of polysomes, as determined from absorbance scans of gradients per milligram PMSP, was the same in both experimental and control groups. This suggests that the amount of polysomal RNA contained in the polysomes should also be the same. Thus, the results of this study are the same as those reported by Porta *et al.* [11], who observed no difference in the recoverable RNA per gram of defatted dry weight of liver between alcohol-treated and controls. For these reasons, results obtained using phenol extracted RNA were expressed per milligram of polysomal RNA. This corrected for variations in the phenol-chloroform extraction of polysomal RNA from polysomes.

The preliminary pool size measurements reported here show no difference between experimental and control mice in the uptake and processing of [5-^{3}H]uridine into [5-^{3}H]UTP. The treatment did affect the total UTP pool, reducing it compared to controls. A similar reduction in the UTP pool had been reported for regenerating rat liver [29]. In both cases, a higher rate of RNA synthesis compared to controls could be expected to draw heavily on intracellular pools, thereby reducing them unless there was a compensatory increse in uridine phosphorylation. In this study, similar reductions were observed for ATP, CTP and GTP pools (data not shown).

After correction for pool size variation, the 60-min incorporation of [5-^{3}H]uridine into mRNA reflected the present rate of transcription, RNA processing, and cytoplasmic transport. The results of this study show that mice fed alcohol and sucrose incorporated 1.5 times more [5-^{3}H]uridine-labeled RNA into polysomes and polysomal RNA than controls. The result is based on seven separate labeling experiments, using two different methods. The sucrose gradient labeling profiles confirmed that the RNA extracted was undergraded and that mRNAs of all size classes showed the same difference in incorporation. We have observed similar results in mice fed alcohol alone (data not shown), suggesting an independence of sucrose in the diet.

The alcohol–sucrose-induced changes in [5-^{3}H]uridine incorporation may be due to increased transcriptional activity and RNA processing or to faster mRNA transport to the cytoplasm, although it has been found that RNA transport in mouse brain is inhibited by chronic alcohol treatment [14]. The exact means by which alcohol–sucrose ingestion may change these cell processes is not known, but hormonal changes may play a role [30].

In alcohol–sucrose-treated mice, 62.5 per cent of the newly made RNA was poly(A)mRNA, whereas

this value was only 37.5 per cent in controls. This increase in the amount of newly made poly(A)mRNA may well explain the observation that there was 1.23 times more total poly(A)mRNA present in alcohol–sucrose-treated animals than in controls. Selective mRNA degradation may have contributed to maintaining the high level of poly(A)mRNA in alcohol–sucrose-treated mice as well. As the proportion of poly(A)mRNA increased, the amount of poly(A⁻)mRNA may have declined. The possibility is supported by the lesser amount of newly made poly(A⁻)mRNA entering polysomes of experimental mice and by the observation that the amount of polysomes recovered per milligram of PMSP was the same in experimental and control groups. Since there were ample quantities of monosomes available in both groups, then an increase in the poly(A)mRNA in alcoholic mice would be accompanied by a corresponding decrease in poly(A⁻)mRNA. Changes in the amount of poly(A)mRNA relative to poly(A⁻)mRNA have been reported [31] for regenerating rat liver, where the amount of poly(A)mRNA declined from control values of 30–40 per cent to 8 per cent; this decline was attributed to selective degradation of poly (A)mRNA. In the present study, there was 1.5 times more newly made RNA in alcohol–sucrose livers entering the mRNA pool. An increased overall rate of degradation in alcohol–sucrose may explain why both groups had the same amount of mRNA. The addition of poly(A) to RNA is catalyzed by poly(A) polymerase. This enzyme is post-translationally regulated by phosphorylation by protein kinase, and it has been shown that rat hepatoma cells have higher rates of polyadenylation than normal liver cells [32].

Although the ratio of poly(A)mRNA to poly-(A⁻)mRNA was altered in this and other systems, the function of poly(A) has yet to be elucidated [33]. Poly(A)mRNA varied qualitatively from poly-(A⁻)mRNA in mouse brain [34], and one report suggests that the same is true for liver [35]. Recent research indicates however, that both RNA populations in the mouse liver are transcribed from the same genes (J. Van Ness, University of Colorado Medical Center, personal communication). This latter work is similar to recent reports that poly(A)mRNA and poly(A⁻)mRNA are transcribed from the same genes in mouse kidney [36] and sea urchin embryos [37].

The [5-^{3}H]poly(U) hybridization data suggest that not only was there more poly(A)mRNA present in alcoholic mice but that the tract size of the poly(A) may have been longer by a factor of 1.5. In *Xenopus* oocytes undergoing maturation, there was a similar lengthening of the poly(A) tracts [38]. Other experiments, such as gel electrophoresis of the poly(A) tracts, need to be performed in order to provide additional proof of this lengthening process.

Since the number of polysomes/mg PMSP was found not to vary between experimental and control groups, and the mRNA content appeared also to be equal, a decrease in liver protein synthesis due to alcohol treatment would need to be due to changes in the ability of ribosomes to bind to mRNA or to reduced translational movement. Using brain ribosomes from rats fed alcohol, Tewari and Noble [10] found altered binding properties and reduced *in vitro* translational activity. If this was the case in liver under the present experimental conditions, then when mRNA is present in large quantities, protein synthesis can still be repressed.

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