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# Effect of Ethanol on Polyribosomes and Protein Synthesis of Transplantable Hepatomas and Host Livers of Rats (40773)[1]

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For a number of years our laboratory has been studying the effects of selected hepatotoxic agents as well as the mechanisms by which they act on hepatic polyribosomes and protein synthesis in experimental animals (1–10). More recently we have also become concerned with how selected hepatotoxic agents act on transplantable hepatomas and host livers of rats (11, 12). In general, we have found that the acute administration of hepatotoxins, such as actinomycin D, aflatoxin $B_1$, $CCl_4$, dimethylnitrosamine, ethionine, puromycin, or sparsomycin, affect the polyribosomes and protein synthesis of the host livers more adversely than those of the hepatomas (11, 12).

Recently, we have studied the effect of acute administration of ethanol on the livers of normal rats (13). Extension of these studies to rats bearing intrahepatically transplanted hepatomas revealed that the acute administration of ethanol caused a more severe effect on polyribosomes and protein synthesis of hepatoma than of host liver. The results of these experiments are the subject of this communication.

*Materials and methods.* Female inbred Buffalo rats were used to maintain Morris hepatoma 5123 induced by the ingestion of *N*-2-fluorenylphthalamic acid (14) by serial sc transplantations. For these studies the hepatomas were transplanted intrahepatically into the left lobes of livers of 7- to 9-week-old Buffalo rats and were removed 21 to 30 days after transplantation. Rats were kept on a commercial diet (Wayne Lab-Blox; Allied Mills, Inc., Chicago, Ill.) throughout, except that the food was removed overnight before killing. Rats were tube fed ethanol (0.75 g/100 g body wt) as a 50% (v/v) solution in saline 1, 2, or 3 hr before killing. Rats were killed by decapitation and tumor tissues were dissected free of surrounding liver tissue and of necrotic tissue before being homogenized in a suitable buffer. Host livers, after removal of tumor tissues, were obtained from the same animals that had borne the hepatomas.

The tissues were rapidly homogenized and postmitochondrial supernatants were prepared as described earlier (10) and were used for size distribution analysis of polyribosomes after addition of deoxycholate (0.7% final concentration) (4, 10). In some experiments free and membrane-bound polyribosomes were prepared as described earlier (3). In *in vitro* incorporation experiments, using [$^{14}$C]leucine, uniformly labeled (10 mCi/mmol) microsomes of homogenates of pooled host livers or hepatomas were used, similar to those described earlier (12).

The status of the polyribosomes was evaluated from the patterns obtained by sucrose density gradients. This was conducted by calculating the relative distribution of monomer–dimers in relation to total ribosomes by measuring the area under the monomer and dimer peaks and the area under the entire pattern (monomer–dimers plus the other polyribosome fractions) of each gradient pattern.

*Results.* In one experiment rats were killed at 1, 2, or 3 hr after tube feeding ethanol and the status of polyribosomes in host liver and hepatoma 5123 was evaluated. The results were similar after each of the three intervals and subsequently only the 3-hr interval was used. Rats treated with ethanol 3 hr before being killed revealed little change (a slight improvement in degree of aggregation) in the status of hepatic polyribosomes of host livers (Table 1). In contrast, the polyribosomes of hepatoma 5123 revealed disaggregation (a 13% increase in the monomer–dimer fraction in relation to total ribosomes) (Table 1). The

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TABLE 1. CHANGES IN TOTAL POLYRIBOSOMES OF HOST LIVER, HEPATOMA 5123, AND HEPATOMA 19 OF RATS TREATED WITH ETHANOL

| Treatment with ethanol | No. of experiments | Status of polyribosomes (Monomer–dimers/total ribosomes ×100) | |
|---|---|---|---|
| | | Host liver | Hepatoma 5123 |
| − | 9 | 40.0 ± 1.17 [a] | 39.9 ±1.01 [a] |
| + | 9 | 36.3 ± 1.44 | 45.2 ± 1.02 [b] |
| | | | Hepatoma 19 |
| − | 1 | 41.9 | 48.8 |
| + | 1 | 40.2 | 53.7 |

[a] Mean ± SEM.
[b] $P < 0.01$.

response of the host livers to ethanol was similar to that reported earlier in livers of normal rats (13). In four experiments the status of free and of membrane-bound polyribosomes of host liver and hepatoma 5123 of control and ethanol-treated rats was investigated. The results expressed as a monomer–dimer fraction in relation to total ribosomes are summarized in Table 2. Ethanol treatment affected the monomer–dimer fractions of free and membrane-bound ribosomes minimally in the host livers (−0.6% for free and +8.2% for membrane-bound) and appreciably more in the hepatoma 5123 (+6.1% for free and +15.5% for membrane-bound).

Table 3 summarizes the *in vitro* incorporation of [$^{14}$C]leucine into proteins using microsomes of host liver or hepatoma 5123 of rats treated with ethanol 3 hr before being killed. There was little change in [$^{14}$C]leucine incorporation into protein between microsomes of host livers of control and ethanol-treated rats. In contrast, there was a significant decrease (35%) in [$^{14}$C]leucine incorporation into protein using microsomes of hepatomas of ethanol-treated rats compared to using microsomes of hepatomas of control rats. In control animals, [$^{14}$C]leucine incorporation into protein of hepatoma was significantly increased in comparison to that of host liver (Table 3). Similar results have been reported earlier (15).

In the experiments summarized in Table 3, the microsomes of host livers or hepatomas were incubated in each case in a medium containing cell saps from host livers of control or ethanol-treated rats. From these data we determined that the cell sap from livers of ethanol-treated rats caused only a 2.7% decrease in [$^{14}$C]leucine incorporation into protein in comparison with the cell sap from livers of control rats when used with comparable microsomes.

In one experiment we used hepatoma 19 (16) instead of hepatoma 5123. The status of the polyribosomes expressed as monomer–dimers/total ribosomes is presented in Table 1. [$^{14}$C]Leucine incorporation into protein using microsomes of host livers revealed a 3.5% decrease in the ethanol treated in comparison to the control rats, while microsomes of hepatoma 19 re-

TABLE 2. CHANGES IN FREE AND MEMBRANE-BOUND POLYRIBOSOMES OF HOST LIVER AND HEPATOMA 5123 OF RATS TREATED WITH ETHANOL

| Group | Treatment with ethanol | Status of polyribosomes [a] (Monomer–dimers/total ribosomes × 100) | |
|---|---|---|---|
| | | Free | Membrane-bound |
| Host liver | − | 32.5 ± 0.32 | 31.8 ± 2.68 |
| | + | 32.3 ± 1.28 | 34.4 ± 1.99 |
| Hepatoma 5123 | − | 32.8 ± 2.04 | 34.9 ± 2.37 |
| | + | 34.8 ± 2.48 | 40.3 ± 3.63 |

[a] Results are means of four experiments.

TABLE III. *In Vitro* PROTEIN SYNTHESIS OF HOST LIVER AND HEPATOMA 5123 OF RATS TREATED WITH ETHANOL

| Treatment with ethanol | No. of experiments | [$^{14}$C]Leucine incorporation into protein (cpm/mg RNA) | |
|---|---|---|---|
| | | Host liver (%) | Hepatoma 5123 (%) |
| − | 8 | 100 | 204.8 ± 29.4[a,b] |
| + | 8 | 92.9 ± 7.69 | 132.7 ± 8.57[c] |

[a] Mean ± SEM.
[b] $P < 0.01$, compared with untreated host liver.
[c] $P < 0.01$, compared with treated host liver or with untreated hepatoma.

vealed a 23.7% decrease in the ethanol treated in comparison to the control rats. Thus, these findings are similar to those dealing with hepatoma 5123 reported in Tables 1 and 3.

*Discussion.* In an attempt to explain why ethanol acts to cause polyribosomal disaggregation and decreased protein synthesis in hepatomas (5123 or 19) but not in host liver, one must consider a number of possibilities. Differences in the abilities of certain tissues to respond to or to metabolize or detoxify ethanol or its metabolites must be considered. An example of this is that protein synthesis in the liver and heart are affected differently by ethanol and acetaldehyde (17–18) and this difference may be similar to that encountered in host liver and hepatoma. Differences in the concentrations of ethanol or its metabolites in tissues must also be considered. Since the blood supply to the hepatoma differs from that to the liver (19–21), this difference may be important in explaining our present results. While the host liver receives blood predominantly via the portal venous system, the hepatoma is supplied predominantly by arterial blood. According to this, ethanol received orally by stomach tube would reach the host liver via the portal circulation more rapidly than it would reach the hepatoma via the systemic circulation. Thus the timing in terms of when ethanol reaches the tissues and the duration it remains in the tissues may be of importance. Yet the effects of ethanol after 1, 2, or 3 hr on polyribosomes of host liver and hepatoma were different and remained similar at these three intervals.

Earlier studies by Cederbaum *et al.* (22) have revealed that tissue slices of hepatoma 5123C act similarly to those of normal liver in metabolizing ethanol. They measured NADPH-dependent ethanol oxidation, ethanol oxidation plus catalase, NADH-dependent oxygen uptake, and NADPH-dependent oxygen uptake and found similar effects with the hepatoma and liver. Perin *et al.* (23) have presented evidence that ethanol decreases the incorporation of labeled amino acids into cell proteins of rat liver slices and that this effect is not due to ethanol per se but is a consequence of ethanol metabolism. They found that acetaldehyde at much lower concentrations than ethanol inhibited protein synthesis. A report by Oratz *et al.* (24) suggests that the high level of acetaldehyde released by the liver following ethanol metabolism may prove inhibitory to protein synthesis in another organ, the heart. A similar situation may occur in host liver and hepatoma. In preliminary experiments (unreported results), we have added acetaldehyde to postmitochondrial supernatants, microsomes, or ribosomes of host liver or hepatoma 5123 and then measured *in vitro* [$^{14}$C]leucine incorporation into protein. Our results revealed similar degrees of inhibition in the preparations of host liver as in those of hepatoma 5123.

Earlier studies by Rothschild *et al.* (25) have revealed that the nutritional status of an animal influences the response of the liver perfused with ethanol; livers from fasted rabbits, at a low level of albumin synthesis, revealed little or no further decrease due to ethanol while livers from fed rabbits responded with diminished albumin synthesis and polyribosomal disaggrega-

tion. Also, Perin *et al.* (23) using liver slices from fasted and fed rats reported that the addition of ethanol decreased the incorporation of amino acids into cell proteins appreciably more in tissue from fed than from fasting rats. Thus, in our present experiments using overnight-fasted tumor-bearing rats, the host livers responded more like the perfused livers from fasted rats, while the hepatomas responded more like the perfused livers from fed rabbits. Consistent with this view was the finding that the concentrations of the free amino acids in the hepatomas were appreciably higher than those in the host livers of rats fasted overnight before the administration of ethanol (unreported results). However, other studies have revealed that in the presence of a mixture of amino acids (26) or of excess tryptophan (25), the effects of ethanol on albumin synthesis are prevented in the liver of normal animals. Thus, even though the nutritional status of the animal may be of importance in influencing the response of the normal liver to the administration of ethanol, it is difficult to extrapolate this to the hepatoma which, in general, is less responsive or responds differently to nutritional manipulations (15, 27). Also, it is difficult to evaluate results based upon albumin synthesis in liver with protein synthesis in hepatoma since transplantable hepatomas synthesize albumin at a reduced rate which is not secreted into the blood (28) while much (13%) of total protein synthesis in liver is albumin but only 1.8% occurs in hepatoma 5123tc (29). Also, it has been reported that albumin synthesis (cell-free protein synthesis) in hepatoma 5123 is by free polyribosomes, which make up 79% of the total ribosomes, but this is not the case in normal liver where the free polyribosomes constitute 38% of the total ribosomes and where albumin synthesis is by membrane-bound polyribosomes (30).

*Summary.* The effect of ethanol administration on polyribosomes and *in vitro* protein synthesis of host liver and hepatoma was investigated. When Buffalo rats bearing hepatoma 5123 intrahepatically were tube-fed ethanol (0.75 g/100 g body wt as a 50% (v/v) solution in saline) 3 hr before killing, there was disaggregation of total polyribosomes and a decrease in *in vitro* protein synthesis ([$^{14}$C]leucine incorporation into protein) in hepatoma but not in host liver in comparison with control groups. Evaluation of the disaggregation of the polyribosomes of the hepatoma of the ethanol-treated rats revealed that the membrane-bound fraction was affected more than the free fraction.


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