Reformation of Functional Liver Polyribosomes from Ribosome Monomers in the Absence of RNA Synthesis

Abstract. *The administration to rats of the ethyl analog of methionine, ethionine, results in the rapid decrease in the hepatic concentration of adenosine triphosphate followed by an extensive disaggregation of polysomes to ribosome monomers and a concomitant inhibition of protein synthesis. These effects are readily reversed by the injection of methionine or precursors of adenine nucleotides such as adenine. The reformation of liver polyribosomes in such animals following the administration of adenine plus methionine was found to occur under conditions in which new RNA synthesis was markedly inhibited. Free messenger RNA without attached ribosomes must be capable of remaining functionally active in the liver cytoplasm for many hours.*

Ethionine, the ethyl analog of methionine (*1*), when injected into female rats, induces a marked inhibition of hepatic RNA (*2*) and protein (*3*) synthesis and a rapid accumulation of triglycerides in the liver (*4, 5*). These effects of ethionine are the consequences of a rapid decrease in the hepatic concentration of adenosine triphosphate (ATP) (*6, 7*). The main site of ethionine interference with protein synthesis is the polysome (*3, 8, 9*) which shows a progressive disaggregation into monomers within a few hours after the administration of the analog (*10, 11*). All of these effects of ethionine, including the breakdown of the polysomes and the inhibition of protein synthesis, are reversed by the administration of methionine or of ATP precursors such as adenine, inosine, or 5-amino-imidazole-4-carboxamide (*12*) which effectively restore the ATP concentration to control amounts.

Since the inhibition of RNA precedes the inhibition of protein synthesis (*2*), it was thought possible that the basis of polysome changes after the treatment with ethionine is an interference with the synthesis of messenger RNA (mRNA) (*3, 5, 10*). If the initial cause for the disaggregation of polysomes is the unavailability of adequate amounts of mRNA, the reformation of the polysomes from monomers following the administration of adenine or adenine plus methionine should be dependent upon the restoration of RNA synthesis. The results of our study clearly show that such is not the case, since the reaggregation of the ribosomes was readily induced by adenine, or methionine, or both, in the presence of a dose of actinomycin D sufficiently large to inhibit RNA synthesis by 90 to 95 percent.

White female rats (Wistar strain, Carworth Farms) maintained on Wayne Lab Blox and weighing from 180 to 220 g were deprived of food overnight and were given aqueous solutions of 0.153*M* DL-ethionine, 0.153*M* DL-methionine, 0.053*M* adenine sulfate or actinomycin D (500 μg per ml) (*13*) as indicated in the figure legends. Control animals received equal volumes of 0.9 percent NaCl solution. Ribosomes were prepared from the fraction of homogenates centrifuged for 10 minutes at 17,000*g* with the use of deoxycholate and were analyzed as previously described (*10*). The synthesis of RNA was measured by the incorporation of orotic acid-6-C^{14} (5 mc/mmole, New England Nuclear) into material precipitated from total liver homogenates, with trichloroacetic acid (TCA), washed as previously described, with the omission of the hot TCA washes (*3*). The radioactive orotic acid was injected intravenously as a single dose of 5 μc 15 minutes before the animals were killed. A Packard liquid scintillation spectrometer measured the radioactivity.

Table 1. The effect of actinomycin D, ethionine, adenine, and methionine upon the incorporation of leucine-C^{14} into rat liver protein in vivo. Female rats received 1.22 mmole of DL-ethionine intraperitoneally 6 hours before being killed, 500 μg actinomycin D intravenously 3 hours before being killed, 0.161 mmole adenine and 1.22 mmole DL-methionine intraperitoneally 2 hours before being killed, or 5 μc L-leucine-C^{14} (uniformly labeled, specific activity 10.45 mc/mmole) intravenously 30 minutes before sacrifice. Each value is the mean of the values for six animals ± standard error of the mean.

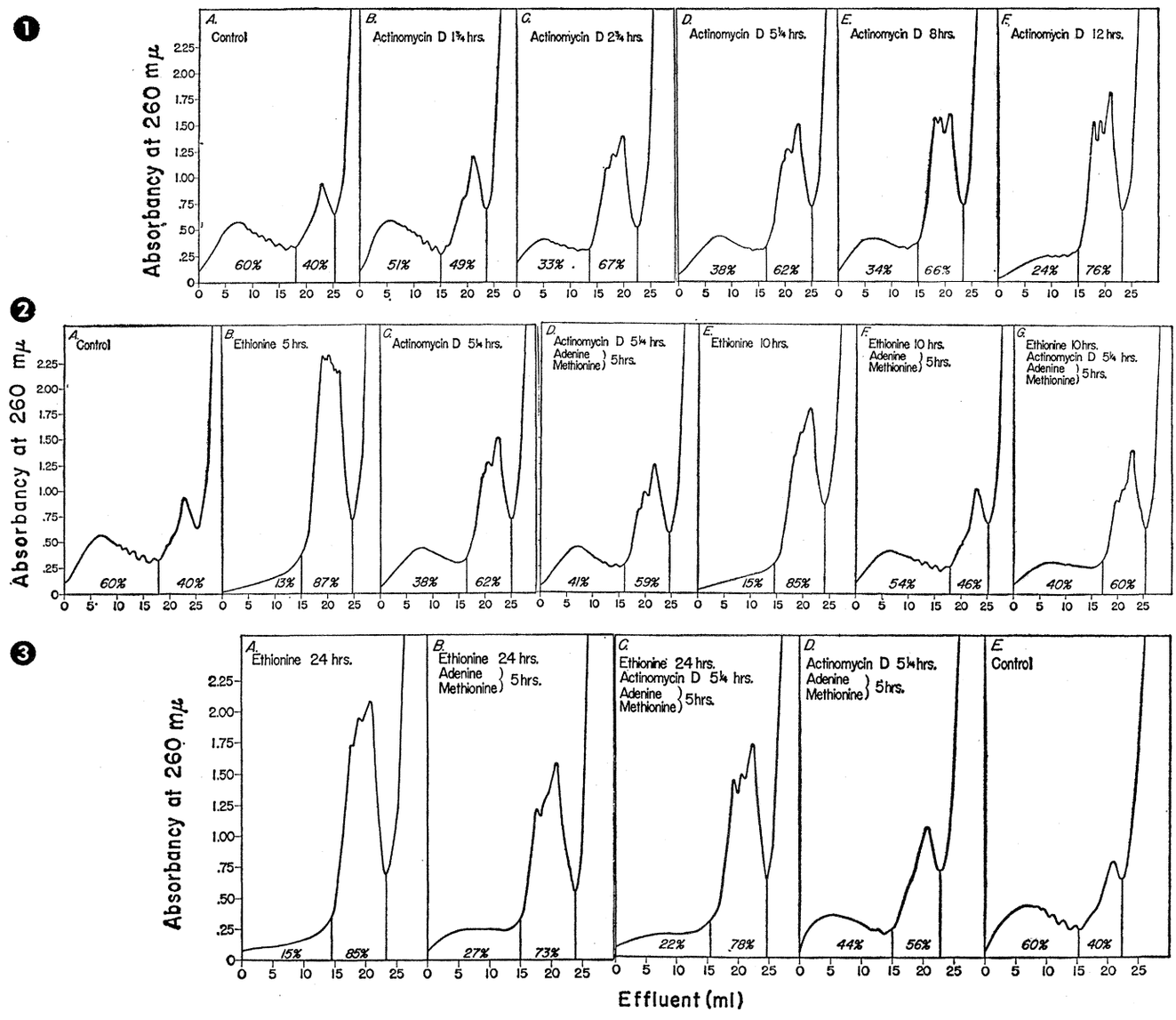
Treatment	Leucine incorporated (count/min per milligram protein)	Difference from control 1 (%)
Saline (control 1)	832 ± 76	
Ethionine	78 ± 8	−91
Actinomycin D	535 ± 61	−36
Ethionine-actinomycin D	120 ± 11	−85
Adenine-methionine (control 2)	1095 ± 108	+32
Ethionine, adenine-methionine	1123 ± 112	+35
Actinomycin D, adenine-methionine (control 3)	876 ± 102	+5
Ethionine, actinomycin D, adenine-methionine	852 ± 84	+2

We observed the polysome patterns at different times after the administration of actionomycin D in order to test the feasibility of the study. As is apparent from Fig. 1, the rate of breakdown of polysomes to monomers occurs fairly slowly in comparison to that observed when ethionine was used (Fig. 2). As found by Staehelin *et al.* (*14*), the disaggregation of the polysomes is progressive with time and a considerable proportion of the ribosomal population is present as polysomes even 5 to 8 hours after the injection of actinomycin.

In Fig. 2 is recorded the extensive breakdown of the polysomes to monomers induced within 5 hours after the administration of ethionine and the virtually complete reversal within an additional 5 hours after the injection of adenine plus methionine. Actinomycin at a dosage which markedly inhibited incorporation of orotic acid into RNA allowed the almost complete reformation of polysomes with adenine plus methionine in the animal treated with ethionine. The polysome pattern with actinomycin plus adenine and methionine is similar to that with actinomycin alone. The dose of actinomycin used produced the following inhibition of the incorporation of orotic acid-6-C^{14} (5 μc) into RNA: 90 percent from 45 to 60 minutes after the intravenous injection of actinomycin D (31,066 ± 2780 count/min per milligram of nuclear RNA) in the control group versus 3,025 ± 288 in that given actinomycin and 94 percent from 3 hours 45 minutes to 4 hours (13,228 ± 1125 count/min per milligram of total liver RNA in the control group versus 729 ± 108 in the group treated with actinomycin). The administration of adenine or adenine plus methionine had no detectable influence upon the degree of inhibition of RNA synthesis induced by actinomycin. Adenine plus methionine was used in most of the experiments because this combined treatment is more effective than either compound alone in the complete restoration to normal of both protein synthesis and the polysome pattern (*10*). However, similar results were obtained with adenine or methionine alone or with 5-amino imidazole-4-carboxamide. The reformation of polysomes is accompanied by a return of protein synthesis to normal (Table 1). The animals treated with ethionine show about a 90 percent inhibition of amino acid incorporation, while those receiving actinomycin have only a 36 percent depression. After the

administration of adenine plus methionine, the incorporation becomes larger than the control value since the concentration of ATP is increased over the control value observed previously (*3, 6*). The animals treated with ethionine receiving adenine plus methionine and actinomycin D show a return of incorporation only to the control value, probably due to a residual depression of protein synthesis by actinomycin. At 19 hours after the injection of ethionine, the administration of adenine plus methionine is much less effective in reversing the breakdown of the polysomes (Fig. 3). However, even under these conditions, actinomycin does not completely prevent the reformation. In all the experiments with ethionine and actinomycin, RNA synthesis is almost completely inhibited from about 3.5 hours after the time of injection of ethionine to the time of termination of the experiment, since the dosage of ethionine used inhibits liver RNA synthesis from 90 to 95 percent (*2*) and actinomycin at the dosage used shows maximal inhibition within 10 minutes after its injection (*15*).

The results of this study clearly show that new RNA synthesis is not necessary for the reformation of polysomes from ribosome monomers in the liver. If the current concepts of polysome structure are valid (*16*) in that the basic organization consists of a strand of mRNA to which are attached the ribosomes and if the reformed polysome is similar structurally to the original polysome, it naturally follows that free mRNA essentially devoid of most or all of its ribosomes may exist in the liver cell for many hours without being irreversibly degraded. Since protein synthesis is restored to the control level



Figs. 1–3. Fig. 1. Ribosome patterns at various time intervals after the intraperitoneal injection of actinomycin D (2.5 mg/kg of body weight). The control animal received an equal volume of saline. The polysomes increase in size as the volume of the effluent decreases. The percentages of monomers-dimers and of polysomes with three or more ribosomes are recorded. Fig. 2. Ribosome patterns at different times after the administration of ethionine (1.23 mmole intraperitoneally) at zero time, actinomycin (2.5 mg/kg of body weight) at 4.75 hours, or adenine (0.16 mmole) plus methionine (1.23 mmole) at 5 hours, or both. Note the almost complete reformation of the polysomes induced by the addition of adenine plus methionine within 5 hours after the striking loss of polysomes following administration of ethionine. Note also how little influence the administration of actinomycin has on this reformation. Fig. 3. Polysome patterns at 24 hours after administration of ethionine (1.25 mmole intraperitoneally) at zero time and the minimal reversal of the breakdown with adenine (0.16 mmole) plus methionine (1.23 mmole) at 19 hours. Note that even at this time interval, the reformation is not completely prevented by actinomycin at 18.75 hours.

it follows that the reformed polysomes are functionally competent.

The mechanism whereby the decrease in the concentration of ATP induces the redistribution of ribosomes from polysomes to monomers is in doubt. There are three known roles of ATP in protein synthesis in acellular systems: (i) in the activation of amino acids for binding to transfer RNA, (ii) in the synthesis of GTP, and (iii) as a substrate for the synthesis of mRNA. Our study has virtually eliminated the third role as a probable basis for the disaggregation of polysomes and inhibition of protein synthesis induced by low concentrations of ATP. Previous studies in vitro with microsomes (*8*) or ribosomes (*9*) from female rats treated with ethionine failed to show any reversal of the inhibition of protein synthesis by optimum concentrations of ATP, guanosine triphosphate and an ATP-generating system. Therefore, we tentatively conclude that ATP plays some additional but unknown role in the function or stabilization of the polysome and in protein synthesis in the liver. This could conceivably be through an effect on amounts of cofactors necessary for chain initiation (*17*). Reported studies with reticulocytes (*18*) or with sea-urchin eggs (*19*) suggest the possibility that low concentrations of ATP may have similar effects on polysomes in other cells.

Our results offer new possibilities for the study of the metabolism of mRNA in the cells of higher animals under reasonably well controlled experimental conditions. It has frequently been suggested that the mRNA molecules may be very susceptible to degradation when they are not stabilized by attached ribosomes. Our results suggest that this may not be the case in the liver cell, unless ethionine also inhibits the mechanism or mechanisms for mRNA degradation. Is the mRNA in the disaggregated form attached to the endoplasmic reticulum or is it free in the cytoplasm? Does the reformation of polysomes after their breakdown mimic the suggested pathway of their initial formation from mRNA attached to a 40 to 45*S* particle (*20*)? Hopefully, the animal treated with ethionine in which the ATP deficiency and its consequences may be turned on and off at will, may offer a novel system for the study of such questions in the intact cells of a higher organism.

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Adenovirus Endocarditis in Mice

Abstract. *Viral endocarditis developed in 24 percent of 50 newborn mice 6 to 8 days after intraperitoneal inoculation with murine adenovirus. Typical adenovirus intranuclear inclusions were seen in heart-valve lesions, and high titers of virus were recovered from heart tissue. Furthermore, adenovirions were directly visualized by electron microscopy within endothelial cells and fibroblasts of the heart valves.*

The occurrence of viral endocarditis in man is uncertain, but chronic valvular disease appears in many patients having no history of rheumatic fever (*1*). This fact has led to the suspicion that other agents (*2*), including viruses (*3*), may cause endocarditis and subsequent valvular scarring in man. Burch *et al.* (*4*) recently reported endocarditis in mice infected with coxsackie virus B4. Moreover, others have noted endocarditis in experimental animals infected with virus III (*5*) or encephalomyocarditis virus (*6*). We now describe significant incidence of endocarditis in mice infected with murine adenovirus (*7*). Furthermore, direct visualization by electron microscopy revealed viral invasion and replication within cells of cardiac valve tissue.

Approximately 200 newborn white mice were inoculated intraperitoneally each with 0.1 ml of a suspension containing 0.3×10^4 plaque-forming units (PFU) of murine adenovirus. The animals either died or were killed at intervals between 2 and 21 days after inoculation. Virus was first recovered from heart, kidneys, and blood on day 6. Highest titers (greater than 10^5 PFU per gram of tissue) occurred in the heart on days 9 and 11; concurrently they were high in kidney (8.5×10^4 PFU/g) and blood (1.7×10^4 PFU/ml).

Heart valves of 50 mice that either died or were killed between days 8 and 12 were examined by light microscopy. The heart was bisected along its long axis and fixed in 10-percent neutral buffered formalin; several sections were cut and stained with hematoxylin and eosin. In 12 of these 50 animals foci of necrosis were seen in the valves; the foci contained intranuclear inclusion bodies, characteristic of adenovirus, in endothelial cells and in fibroblasts (Fig. 1). In these areas subendothelial edema and a scanty cellular infiltrate were present. Usually the infiltrate was of mononuclear cells, but in one instance there was necrosis in the valve leaflets with a polymorphonuclear leukocytic infiltrate. Extensive myocarditis accompanied the valve lesions. The lesions will be described in detail later (*8*).

By electron microscopy, adenovirions were directly visualized in nuclei of endothelial cells (Fig. 2) and fibroblasts of the valves; in some of these nuclei, virus particles were sparsely scattered, while in others there were large masses of adenovirions in crystalline arrays.