Effect of Cycloheximide on Polysomes and Protein Synthesis in the Mouse Liver*

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ABSTRACT: Administration of cycloheximide (actidione) to male mice of the C3H/HeJ strain inhibited the *in vivo* incorporation of L-[U-^{14}C]leucine into liver proteins. This inhibition was approximately 20% with 0.2 mg of cycloheximide/100 g of body weight and 62–65% with 0.4 mg/100 mg of body weight. A maximum inhibition of 94–96% was reached with 8–10 mg of cycloheximide/100 g of body weight. The inhibitory effect of cycloheximide was observed almost immediately after injection, reached a maximum after 1–2 hr, and was still pronounced after 24 hr. Experiments with a cell-free amino acid incorporating system containing normal mouse liver supernatant demonstrated that liver ribosomal preparations isolated from cycloheximide-treated animals or homogenates performed as well as those isolated in the absence of this antibiotic. In contrast, a decreased capacity of normal liver ribosomal preparations to incorporate L-[U-^{14}C]leucine was noted when the liver supernatant utilized in the incorporation assay was prepared from a cycloheximide-treated animal. The breakdown of liver polysomes following administration of actinomycin or ethionine was inhibited by injection of cycloheximide. Similarly, the reassembly of polysomes produced by reversing the effect of ethionine by methionine and adenosine triphosphate was inhibited in the presence of cycloheximide.

It has been reported that cycloheximide (actidione), an antibiotic isolated from *Streptomyces griseus*, inhibited protein synthesis in *Saccharomyces carlsbergensis* (Kerridge, 1958). Similar effects were observed in rabbit liver proteins (Young *et al.*, 1963), mammalian cell cultures (Bennett *et al.*, 1964), and rabbit reticulocytes (Colombo *et al.*, 1965). Studies in cell-free systems from *Saccharomyces pastorianus* and rat liver suggested that cycloheximide does not inhibit amino acid activation or transfer of activated amino acids to s-RNA, but prevents the transfer of amino acids from s-RNA to the nascent polypeptide chain (Siegel and Sisler, 1963, 1964; Ennis and Lubin, 1964). Wettstein *et al.* (1964) have shown that this antibiotic blocks the read-out mechanism of m-RNA and prevents polysome breakdown during protein synthesis *in vitro*. Our studies are concerned with the effect of this antibiotic on the breakdown of polysomes observed after administration of actinomycin (Staehelin *et al.*, 1963b; Korner and Munro, 1963; Penman *et al.*, 1963; Trakatellis *et al.*, 1964a) and ethionine (Villa-Trevino *et al.*, 1964) and with the effect of cycloheximide on the reassembly of polysomes observed upon reversal of the ethionine effect with methionine and adenine (Villa-Trevino *et al.*, 1964). Furthermore, we investigated the mechanism of action of this antibiotic on protein synthesis in the mouse liver.

Materials and Methods

Animals. Male, adult mice of the C3H/HeJ strain utilized in these experiments were purchased from the Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine.

Tracer Compound. L-[U-^{14}C]Leucine with a specific activity of 223 mc/mmole was purchased from New England Nuclear Corp., Boston, Mass.

Compounds. Cycloheximide (actidione) was a generous gift from Dr. Robert M. Smith of the Upjohn Co., Kalamazoo, Mich., and actinomycin D (C_1 in the new nomenclature) was a generous gift from Dr. Elmer Alpert of Merck Sharp and Dohme, West Point, Pa. Aqueous solutions of DL-ethionine, L-methionine, and adenosine triphosphate (ATP)[1] were prepared for injection.

Procedures. Methods for preparation of standard ribosomes (Korner, 1961) and purified ribosomes (Wettstein *et al.*, 1963), fractionation of ribosomal preparations into polysomes of varying aggregate size (Wettstein *et al.*, 1963), and the techniques for determining amino acid incorporation *in vivo* have been described in a previous publication (Trakatellis *et al.*, 1965).

Cell-Free Amino Acid Incorporating System. Ribosomes were suspended carefully in 0.5 ml of an ice-cold buffer solution (0.045 M Tris, 0.0075 M $MgCl_2$, 0.12 M KCl, and 0.075 M NaCl, pH 7.2). A 0.2-ml portion of this suspension was utilized per incorporation assay and 0.1 ml for determination of RNA content. To this ribosomal suspension (0.2 ml) were added 0.3 ml of a

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[1] Abbreviation used: ATP, adenosine triphosphate.

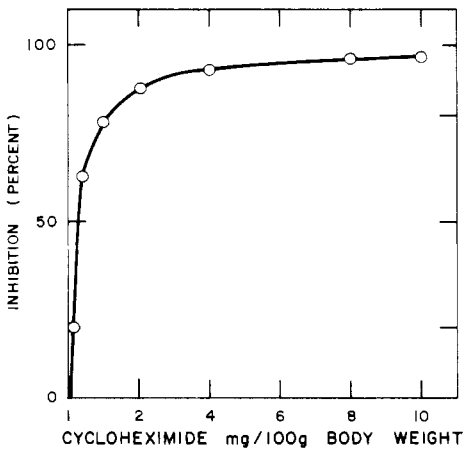

FIGURE 1: Effect of cycloheximide on the *in vivo* incorporation of L-[U-^{14}C]leucine into liver proteins. Animals were injected with various doses of cycloheximide intraperitoneally and, after 20 min, received 20 μc/100 g of body weight of L-[U-^{14}C]leucine. Animals were sacrificed 30 min after the intraperitoneal administration of the labeled amino acid. Controls, which did not receive cycloheximide, were also employed. The average cpm/mg of protein of 4 control animals was taken as 100% incorporation (0% inhibition). Each point represents average values of four animals. Cycloheximide was dissolved in 0.9% NaCl to give a stock solution of 10 mg/ml.

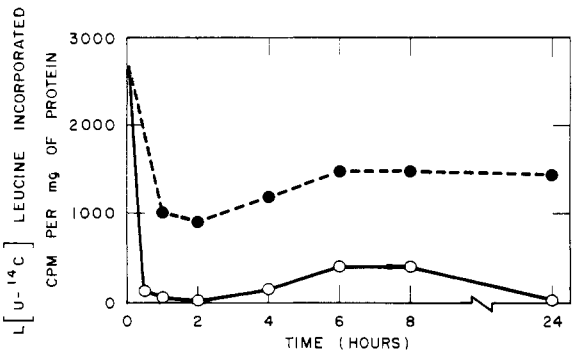

FIGURE 2: Effect of cycloheximide on the *in vivo* incorporation of L-[U-^{14}C]leucine into liver proteins at various times after the administration of the antibiotic. Cycloheximide was injected at zero time in all animals in a dose of 0.4 mg/100 g of body weight (dash line) or 8 mg/100 g of body weight (solid line). Each point represents the average cpm of L-[U-^{14}C]leucine incorporated per mg of protein of four animals. L-[U-^{14}C]-Leucine was injected in a dose of 20 μc/100 g of body weight 30 min prior to the killing of the animals.

mouse liver supernatant and 0.5 ml of a buffer solution (0.02 M Tris, 0.005 M $MgCl_2$, 0.080 M KCl, and 0.050 M NaCl, pH 7.2) containing 0.825 μmole of β-mercaptoethanol, 10 μmoles of phosphoenolpyruvic acid (tricyclohexylamine salt purchased from the Sigma Chemical Co.), 30 μg of pyruvate kinase (purchased from the Sigma Chemical Co.), 1.0 μmole of ATP, 0.6 μmole of GTP, and 0.005 mmole of L-[U-^{14}C]leucine. The liver supernatant was prepared from a 10% homogenate of normal mouse liver in Hoagland's salt buffer containing 0.44 M sucrose. All operations were conducted at 1–2°. The supernatant obtained by centrifugation of the homogenate at 4000 rpm for 10 min was centrifuged in a Spinco rotor No. 40 at 20,000 rpm for 20 min. The resulting postmitochondrial supernatant was centrifuged at 40,000 rpm in a Spinco rotor No. 40 for 150 min to yield the postmicrosomal supernatant utilized in the incorporation assay. Methods employed for the precipitation of protein and measurement of radioactivity after incubation have been described (Wettstein *et al.*, 1963).

Results

In Vivo Incorporation Experiments. It has been demonstrated that acetoxycycloheximide greatly inhibits incorporation of [^{14}C]amino acids into mouse and rabbit liver proteins and that cycloheximide produces a similar inhibition of incorporation into rabbit liver proteins (Young *et al.*, 1963).

Our results (Figure 1) show that a 20% inhibition of incorporation of L-[U-^{14}C]leucine into mouse liver proteins can be observed with a very low dose of cycloheximide (0.2 mg/100 g of body weight), that the inhibition increases to approximately 62–65% with a dose of 0.4 mg/100 g of body weight, and that the inhibition reaches a maximum of 94–96% with a dose of 8–10 mg/100 g of body weight. This inhibition can be observed almost immediately after cycloheximide injection (Figure 2) and reaches a maximum after 1–2 hr. Although a very small decrease of inhibition can be observed 4–6 hr after injection of cycloheximide, the inhibition of protein synthesis cannot be overcome with time even when the cycloheximide dose is small (Figure 2). With this small dose the inhibition can still be observed after 24 hr. As with ethionine and puromycin (Farber *et al.*, 1950; Robinson and Seakins, 1962), cycloheximide produces rapid development of a fatty liver as noted by gross examination (4–12 hr after injection) especially with doses of 8–10 mg/100 g of body weight. Although the possibility exists that cycloheximide may inhibit amino acid uptake by the intact liver, this seems unlikely in view of the fact that this inhibition can be observed in a cell-free system.

Studies with a Cell-Free Amino Acid Incorporating System. Ribosomal preparations isolated from livers of mice injected with various amounts of cycloheximide show no decrease in ability to incorporate L-[U-^{14}C]-leucine, even when mice were injected with large doses of 10–40 mg of cycloheximide/100 g of body weight (Table I). It should be noted that the liver post-microsomal supernatant used in the incorporation assay was obtained from normal mice.

TABLE I: Effect of *in Vivo* Injected Cycloheximide on the Ability of Standard Liver Ribosomes to Incorporate L-[U-^{14}C]Leucine *in Vitro*.[a]

Cycloheximide (mg/100 g of body wt)	L-[U-^{14}C]Leucine Incorporated (cpm/mg of RNA)
None	11,938
None	12,375
0.4	11,235
1.0	12,826
2.5	12,481
4.0	13,205
10.0	11,307
10.0	12,836
40.0	11,921

[a] Animals were sacrificed 45 min after cycloheximide injection. RNA was estimated from absorbancy at 254 mμ (20 absorbancy units = 1 mg). Assay samples containing 0.4–0.5 mg of RNA were incubated at 37° for 30 min. A 0.3-ml portion of normal mouse liver supernatant was used per assay.

In a second experiment, 3 g of liver tissue from normal mice was homogenized in 30 ml of Hoagland's Medium A. The homogenate was divided into six equal parts. Cycloheximide (1 mg) was added to each of two samples and 5 mg of cycloheximide to each of another two samples. Standard ribosomes were prepared from all six homogenates, and the ribosomal preparations were tested for their capacity to incorporate L-[U-^{14}C]leucine *in vitro* with normal liver supernatant. Cycloheximide addition did not affect this ability, and the results of two experiments were similar to those listed in Table I.

In contrast, the incorporation capacity of ribosomal preparations from normal animals was inhibited when the supernatant used in the incorporation assay (see Materials and Methods) was derived from cycloheximide-injected animals. This was demonstrated in an experiment in which six equal ribosomal fractions from normal animals (containing 0.4 mg of RNA) were tested *in vitro* for incorporation ability with supernatant obtained from normal animals and cycloheximide-injected animals (Table II).

The inhibitory effect upon L-[U-^{14}C]leucine incorporation was studied by adding varying amounts of cycloheximide to homogenate samples prior to preparation of supernatant. Homogenate (30 ml) containing 3 g of liver tissue (in Hoagland's salt buffer containing 0.44 M sucrose) from normal mice was divided into three equal parts. Cycloheximide (2 mg) in 0.5 ml of normal saline was added to one part, 5 mg of cycloheximide in 0.5 ml of normal saline was added to the second part, and 0.5 ml of normal saline was added to the third part. The supernatant was prepared from these three aliquots and utilized in an experiment in which six

TABLE II: Effect of Supernatants from Cycloheximide-Injected Animals on the Ability of Standard Liver Ribosomes to Incorporate L-[U-^{14}C]Leucine *in Vitro*.

Supernatant Derived from Animals Injected with Cycloheximide (mg/100 g of body wt)	Sample No.	L-[U-^{14}C]-Leucine Incorporated (cmp/mg of RNA)	Av % Inhibition
None	1	13,912	0
None	2	13,723	
20	3	5,729	59.1
20	4	5,574	
40	5	4,316	69.5
40	6	4,108	

equal samples of ribosomes from normal animals (containing 0.38 mg of RNA) were studied for their incorporation ability. Results were similar to those listed in Table II and the average per cent inhibition of incorporation of L-[U-^{14}C]leucine was 25.1 and 67.5%, respectively.

Additional experiments were carried out in which cycloheximide was added directly to the complete mixture utilized for *in vitro* incorporation. Marked inhibition was observed with low concentrations of cycloheximide (100 μg/assay sample) if 0.1 ml of supernatant was used. When the amount of supernatant was increased to 0.2 or 0.3 ml, the inhibitory effect was very small. These results are summarized in Table III.

TABLE III: Effect of Various Amounts of Supernatant on the Incorporation of L-[U-^{14}C]Leucine by Purified Ribosomes *in Vitro* in the Presence and Absence of Cycloheximide.[a]

	Expt. No.	Supernatant (ml) 0.1	0.2	0.3
Control	1	19,185	22,738	20,752
	2	19,377	22,434	20,184
Cycloheximide (100 μg/assay)	1	1,611	21,988	20,164
	2	2,002	21,154	19,450
Inhibition, %[b]	1	90	3.3	2.8
	2	87.7	5.8	3.6

[a] Purified liver ribosomes were prepared from normal mice. Each assay tube contained 0.20 mg of RNA. Values are expressed as cpm/mg of RNA. [b] The corresponding control values were taken as 100% incorporation or 0% inhibition.

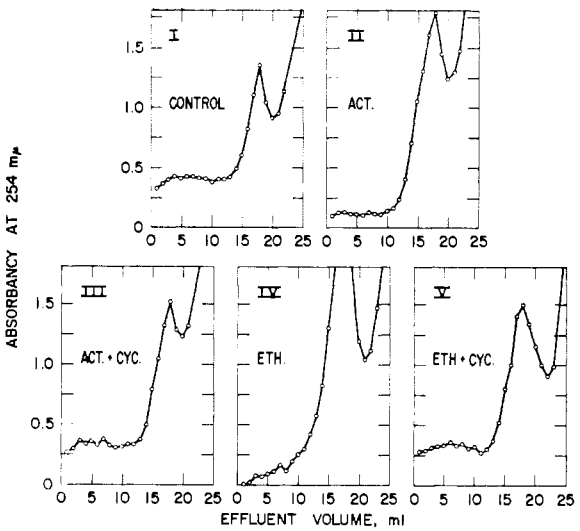

FIGURE 3: Zone centrifugation analysis of mouse liver ribosomes. (I) Control animal. (II) Animal injected with actinomycin (200 μg/ml of 0.9% NaCl) at a level of 200 μg/100 g of body weight and sacrificed by decapitation 8 hr later. (III) Animal injected with cycloheximide at a level of 10 mg/100 g of body weight and 15 min later with actinomycin (200 μg/100 g of body weight) and sacrificed 8 hr later. (IV) Animal injected with ethionine (24.8 mg/ml of aqueous solution) at a dose of 100 mg/100 g of body weight. The injection was repeated 4 hr later and the animal was sacrificed 8 hr after the first injection. (V) Animal injected with cycloheximide as in (III) plus ethionine as in (IV) and sacrificed 8 hr after the first injection of ethionine. Ribosomes obtained from 0.5 g of liver tissue were utilized in all experiments. All injections were given intraperitoneally.

control animal (Figure 3, I). A similar effect following ethionine injection is shown in Figure 3, IV. The polysome breakdown after injection of actinomycin or ethionine may be greatly inhibited by cycloheximide injection (Figure 3, III and V). Experiments with a cell-free amino acid incorporating system utilizing ribosomal preparations isolated from animals treated with actinomycin, ethionine, actinomycin plus cycloheximide, and ethionine plus cycloheximide gave results (Table IV)

TABLE IV: Effect of Injections into Mice of Various Substances on the Ability of Standard Liver Ribosomes to Incorporate L-[U-^{14}C]Leucine *in Vitro*.[a]

Treatment	L-[U-^{14}C]-Leucine Incorporated (cpm/mg of RNA)	% Inhibition
None	12,437	
None	13,106	
Actinomycin	3,729	70.9
Actinomycin	4,350	66.0
Actinomycin + cycloheximide	9,316	27.0
Actinomycin + cycloheximide	8,414	34.1
Ethionine	2,620	79.5
Ethionine	3,859	69.8
Ethionine + cycloheximide	7,674	40.6
Ethionine + cycloheximide	8,635	32.4

[a] The schedule of injections and doses of various drugs as in Figure 4. The average of the two controls has been taken as 100% (0% inhibition). Supernatants from normal mice were used in the assay.

Effect of Cycloheximide on Polysome Breakdown in Vivo. It has been reported that the injection of actinomycin produces a breakdown of polysomes in rat liver (Staehelin *et al.*, 1963b; Korner and Munro, 1963), in HeLa cells (Penman *et al.*, 1963), in the mouse liver (Trakatellis *et al.*, 1964a), and in mouse mammary adenocarcinoma (Trakatellis *et al.*, 1965). This effect was accompanied by a corresponding loss of ability of the ribosomes to incorporate amino acids in a cell-free system (Staehelin *et al.*, 1963b; Korner and Munro, 1963; Trakatellis *et al.*, 1964a, 1965) and a parallel decay of radioactively labeled m-RNA (Trakatellis *et al.*, 1964a, 1965). Similar effects have been observed in rat liver with the amino acid analog ethionine (Villa-Trevino *et al.*, 1964). These effects have been attributed to degradation of m-RNA due to rapid turnover of this component without compensatory replacement because of the inhibition of RNA synthesis by actinomycin or ethionine.

The extensive breakdown of mouse liver polysomes 8 hr after administration of actinomycin (Figure 3, II) is contrasted with the normal polysome pattern of a

supporting the above observations. The incorporation capacity of ribosomal preparations from actinomycin- or ethionine-treated animals is greatly reduced. Animals treated with cycloheximide plus either actinomycin or ethionine show less decrease in ability to incorporate L-[U-^{14}C]leucine *in vitro*.

Effect of Cycloheximide on the Reassembly of Polysomes in Vivo. It has been demonstrated that the reversal of the ethionine effect may be accomplished with methionine and adenine which results in reassembly of polysomes (ergosomes) and restoration of protein synthesis (Villa-Trevino *et al.*, 1964). The effect of ethionine on mouse liver polysomes 6 hr and 30 min after injection (Figure 4, II) is contrasted with the polysome pattern of a control animal (Figure 4, I).

The ethionine effect is reversed by injection of methionine and ATP, and it can be seen that the reassembly of polysomes proceeds rapidly (Figure 4, IV). Two hours after administration of methionine and adenine, a considerable amount of polysomes is formed. However, in the presence of cycloheximide, the reassembly

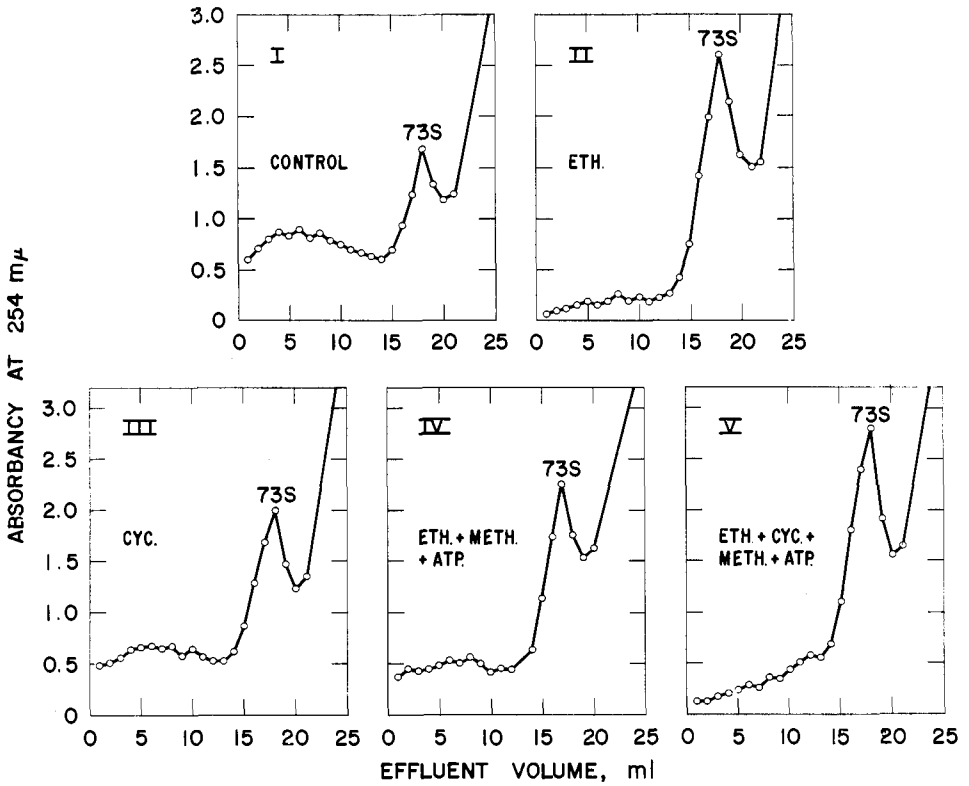

FIGURE 4: Zone centrifugation analysis of mouse liver ribosomes. (I) Control animal. (II) Animal injected with ethionine at a level of 100 mg/100 g of body weight. The injection was repeated 3 hr later and the animal was sacrificed at 6.5 hr after the first injection. (III) Animal injected with cycloheximide at a level of 10 mg/100 g of body weight and sacrificed 2.5 hr later. (IV) Animal injected with ethionine as in (II) and 6.5 hr later with L-methionine at a level of 100 mg/100 g of body weight and ATP (100 mg/100 g of body weight) and sacrificed 2 hr later. (V) Animal injected with ethionine as in (II) and 6 hr later with cycloheximide as in (III). Thirty minutes after cycloheximide injection the animal was injected with L-methionine and ATP as in (IV) and sacrificed 2 hr later. Ribosomes obtained from 0.75 g of liver tissue were utilized in all experiments. All injections were given intraperitoneally.

procceds much more slowly, especially in the area of heavy aggregates (Figure 4, V).

On the other hand, the polysome content of a cycloheximide-injected animal is approximately 84% of that of the control animal (Figure 4, III). However, this slight effect of cycloheximide was not observed in experiments with 20 animals and accordingly we concluded that cycloheximide alone does not produce any significant change in the polysome population. However, the inhibitory effect of cycloheximide on reassembly of polysomes was consistently found in four experiments similar to the experiment of Figure 4.

Experiments with a cell-free amino acid incorporating system with ribosomal preparations isolated from mice treated as in the experiment of Figure 4 gives results (Table V) in agreement with the observed absorbancy profiles of Figure 4. It can be seen that 2 hr after the administration of methionine and adenine restoration of protein synthesis *in vitro* is very marked. In contrast, only partial restoration is achieved when the reversal occurs in the presence of cycloheximide.

Discussion

According to the hypothesis of the tape mechanism of protein biosynthesis (Gierer, 1963; Gilbert, 1963; Watson, 1963) which has been verified in experiments on polysome (ergosome) function (Noll *et al.*, 1963; Goodman and Rich, 1963; Staehelin *et al.*, 1963a; Hardesty *et al.*, 1963a,b; Williamson and Schweet, 1965), the read-out of genetic information begins with the attachment of single ribosomes to the beginning of an m-RNA strand and *via* a moving process; this strand passes through the condensing site of each ribosome with successive exposure of codons. A polypeptide chain grows continuously at that condensing site until the reading of the message is completed. The ribosome now at the end of the m-RNA strand is released with the complete polypeptide chain.

Since cycloheximide does not inhibit the activation of amino acids or the transfer of activated amino acids to s-RNA, it has been postulated that it interferes with the transfer of amino acid to the nascent polypeptide chain. Wettstein *et al.* (1964) have shown that this

TABLE V: Incorporating Ability of Standard Liver Ribosomes after Injections of Various Substances.[a]

Treatment	L-[U-^{14}C]-Leucine Incorporated (cpm/mg of RNA)	% Inhibition
None	13,235	
None	13,507	
Ethionine	3,969	71.3
Ethionine	4,174	68.8
Cycloheximide	12,437	6.8
Cycloheximide	12,896	3.4
Ethionine +methionine + ATP	9,876	26.1
Ethionine + methionine + ATP	8,964	33.0
Ethionine + cycloheximide + Methionine + ATP	5,236	60.8
Ethionine + cycloheximide + methionine + ATP	6,413	51.6

[a] The schedule of injection and doses of various substances as in Figure 4. The average of the two controls has been taken as 100% (0% inhibition). Supernatants from normal mice were used in the assay.

antibiotic blocks the read-out mechanism of m-RNA and prevents polysome breakdown during protein synthesis *in vitro*. The same authors, analyzing their data, conclude that cycloheximide acts primarily by reducing the initial reaction rate rather than the final yield and that this precludes the possibility of an inactivation of ribosomes by irreversible binding to a critical site.

Our experiments showed that polysomes isolated from cycloheximide-treated animals or homogenates performed as well in a cell-free incorporating system containing normal liver supernatant (Table I) as those isolated in the absence of this antibiotic. This does eliminate the possibility of an inactivation of ribosomes due to an irreversible binding of cycloheximide to a critical site and agrees with the conclusion of Wettstein *et al.* (1964). On the other hand, our experiments clearly showed that when supernatant used in the incorporation assay was derived from cycloheximide-treated animals or homogenates, an inhibitory effect on the *in vitro* incorporation was produced (Table II). Furthermore, the experiments showed that the inhibitory effect of cycloheximide depends upon the amount of supernatant used (Table III). These data strongly suggest that the site of action of cycloheximide is located in the system responsible for the transfer of amino acids from s-RNA to the nascent polypeptide chain and is in agreement with previous conclusions (Siegel and Sisler, 1963, 1964; Wettstein *et al.*, 1964; Ennis and Lubin, 1964; Schweet *et al.*, 1964; Williamson and Schweet, 1965). It has been reported (Allen and Zamecknik, 1962; Gilbert, 1963; Nathans, 1964) that the amino group of puromycin can react with the activated acyl group of the nascent polypeptide and bring about detachment of the polypeptide chain from the condensing site. It has been postulated that the same enzyme catalyzes peptide bond formation and also the puromycin reaction (Nathans, 1964) and, if this enzyme is inhibited by cycloheximide, resultant peptide bond formation would be greatly diminished. On the other hand, puromycin could still detach the nascent polypeptides at a slower rate (Colombo *et al.*, 1964) since this reaction involves formation of only one bond. Therefore, we suggest that the cycloheximide probably acts either by inhibiting the enzymatic system responsible for the transfer of amino acid to nascent polypeptide or by interfering with attachment of charged RNA to the decoding site. The latter possibility is in agreement with the above-mentioned puromycin data since puromycin does not need to be attached at the decoding site. It is probably that the transfer of the amino acid to the nascent polypeptide chain is closely related to the mechanism of ribosome movement relative to m-RNA (see also Noll *et al.*, 1963), and thus a block of the transfer mechanism would result in a slow-down of the reading process.

The attachment of ribosomes to the beginning of the m-RNA strand and the read-out process are extremely rapid *in vivo*, and these processes are repeated until m-RNA breaks down. As a consequence, one m-RNA molecule serves as a template for many polypeptide chains (Villa-Trevino *et al.*, 1964). The half-lives of rat liver m-RNA (Villa-Trevino *et al.*, 1964; Noll *et al.*, unpublished observations) and mouse liver m-RNA (Trakatellis *et al.*, 1964b) have been found to be 48–60 min and 2 hr, respectively. In actinomycin- or ethionine-treated animals the rapid m-RNA breakdown without replacement is manifested by a progressive breakdown of polysomes (Staehelin *et al.*, 1963b; Korner and Munro, 1963; Trakatellis *et al.*, 1964a; Villa-Trevino *et al.*, 1964). However, in our experiments this breakdown was inhibited in the presence of cycloheximide (Figure 3), and this suggests a slow-down of the reading mechanism in the presence of this antibiotic as proposed by Wettstein *et al.* (1964). The experiments on polysome (ergosome) reassembly, upon reversal of the ethionine effect with methionine and ATP, lead to the same conclusion. These experiments showed that upon reversal of the ethionine effect the reassembly of polysomes (a very fast process) was inhibited in the presence of cycloheximide (Figure 4). This effect can be attributed to the very slow movement of ribosomes relative to the m-RNA strand after they have attached to it. This obviously results in a very slow reassembly. In these experiments it can be seen that reassembly of heavy polysomes (which requires more ribosomes per m-RNA strand) was very poor, while some formation of lighter aggregates had taken place (Figure 4).

These data are in agreement with those observed in the reassembly of polysomes in reticulocytes (Colombo *et al.*, 1965). In these experiments reticulocyte polysomes were disaggregated by NaF and reassembled by washing away NaF. However, in the presence of cycloheximide the reassembly failed to take place.

There is the possibility that in the above experiments a defect in attachment of ribosomes was in operation together with a slowed-down reading process.

Also an effect of cycloheximide upon RNA synthesis (especially m-RNA) should be considered. Further experiments to elucidate the points discussed above are now in progress.

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