The Role of Adenosine Triphosphate Deficiency in Ethionine-induced Inhibition of Protein Synthesis*

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Ethionine (1), the ethyl analogue of methionine, is known to produce an inhibition of protein synthesis in the liver of the rat. This defect in protein synthesis is evident as an inhibition of (a) the incorporation of labeled amino acids into protein and (b) the increase in many enzyme activities following fasting-feeding regimens, or following the administration of various drugs or hormones (cf. (2)). Yet, despite the widespread use of this presumed antagonist of methionine, no knowledge of its mechanism of action as an inhibitor of protein synthesis is available. Since the administration of methionine counteracts most of the effects of ethionine, including those related to protein synthesis, it has been assumed that the action on protein metabolism involved some specific metabolic methionine-ethionine interaction. However, no evidence for a site for such interaction has been presented.

During a study of the effects of ethionine upon hepatic glycogen phosphorylase, Shull (3) found that this analogue induced a striking decrease in liver ATP concentration within a few hours after its administration to rats. This finding prompted the present study of the possible relationship of the decreased ATP level to the inhibition of protein synthesis induced by ethionine. The incorporation of leucine into the protein of a liver ribosome system and the increase in a hepatic enzyme following the administration of 3-methylcholanthrene (4) were used as indices of hepatic protein synthesis. The evidence for the conclusion that ethionine inhibits liver protein synthesis indirectly through an effect upon hepatic ATP, a hypothesis first suggested by Stekol et al. (5) several years ago, is presented and discussed.

EXPERIMENTAL PROCEDURE

White female rats of the Wistar strain (Carworth Farms) maintained on Purina laboratory chow and weighing 180 to 220 g, were used in all experiments except the ones in which the activity of 2-acetylaminofluorene hydroxylase was measured. In the latter experiments, rats weighing 60 to 80 g were used.

DL-Ethionine, ATP, and DL-methionine were injected in aqueous solution containing 0.153, 0.160, and 0.153 mmole per ml, respectively. Ethionine and methionine were administered intraperitoneally in a total dose of 1.22 mmoles. ATP, adenine, or one of a series of nucleosides (adenosine, guanosine, inosine, cytidine, and uridine) was administered subcutaneously in a total dose of 0.321 mmole. In most experiments, the rats were deprived of food overnight and then treated with injections of 0.9% sodium chloride solution or DL-ethionine in two divided doses at zero time and 1 hour, with or without ATP, a nucleoside, or methionine at the same time. Most of the animals were killed by a blow on the head at 5 hours. In some experiments the total dose of ethionine, the time of administration of methionine or ATP, or the time the animals were killed were varied from this regimen as indicated in the text.

Incorporation Experiments—The ribosomes were prepared according to Korner's method (6) modified by Wettstein et al.[1] as described (7). The pH 5 fraction was prepared by the method of Keller and Zamecnik (8).

The incubation mixture contained in a total volume of 1 ml: ribosomes from the livers of control or experimental rats (1.5 mg of protein); pH 5 fraction from control rats (2.5 mg of protein); 0.0015 M ATP; 0.0005 M GTP; 0.01 M phosphoenolpyruvate; 30 μg of pyruvate kinase (Boehringer and Sons); 0.0012 M KCl; 0.025 M Tris-HCl buffer, pH 7.6; 0.005 M MgCl$_2$; and 0.2 μc of L-leucine-C^{14}, uniformly labeled with a specific activity of 7.14 mc per mmole (Nuclear-Chicago Corporation). The samples were incubated for 60 minutes at 37.5°. The reaction was stopped with 2 ml of 10% trichloroacetic acid containing 10 mg of nonradioactive leucine per ml. The samples were washed twice with cold 10% trichloroacetic acid; extracted once with a 3:1 ethanol-ether mixture for 5 minutes at 60° and once with 10% trichloroacetic acid for 15 minutes at 90°; and washed once each with ethanol, ethanol-ether, and ether. They were plated and counted in a thin window gas flow counter with an efficiency of 25% for C^{14}. Protein was determined gravimetrically. All samples were corrected for the 25% counting efficiency. The results are expressed in micromicromoles (10^{-12} mole) of leucine incorporated per mg of protein.

For optimal incorporation, the system has a high dependence on the presence of the pH 5 fraction, ATP, an ATP-generating system, and MgCl$_2$ as shown in Table I. In several experiments, incorporation in the absence of both ATP and the ATP-generating system was less than 5% of the control value. Unlike the microsome system, which incorporates amino acids into

[1] F. Wettstein, T. Staehelin, and H. Noll, personal communication.

protein for periods of only 10 or 15 minutes, the ribosome system as used in this study continued to incorporate leucine at essentially the same rate for 1 hour (Fig. 1). The rate dropped to virtually zero after this time.

2-Acetylaminofluorene Hydroxylase Experiments—The conditions as reported by Cramer, Miller, and Miller (4) were used, with minor modifications with regard to the administration of methionine. Rats received 0.3 mmole of ethionine 30 minutes before the intraperitoneal administration at zero time of corn oil with or without 3-methylcholanthrene in a concentration of 4 mg per ml and in a dose of 1 mg per 50 g of body weight. Methionine when injected was given in a total dose of 0.75 mmole: 0.3 mmole at the same time as the ethionine, 0.15 mmole at 7 hours, and 0.3 mmole at 14 hours. ATP was injected at the same time intervals as methionine in doses of 0.08, 0.04, and 0.08 mmole. Control animals were given injections of corn oil alone and 0.9% sodium chloride solution in place of methionine, ATP, or ethionine. All animals were killed at 24 hours.

ATP Determination—Hepatic ATP levels were determined by the luciferin-luciferase reaction (9). The animals for these determinations were kept under the same conditions as those used for the incorporation studies. The timing in the determination was very critically controlled as previously described (3).

TABLE I

Influence of different components of liver ribosome system upon incorporation of radioactive leucine into protein

	Leucine incorporated*	Difference from complete system
	μμmoles/mg of protein	*%*
Complete system†	145	
Minus pH 5 fraction	19	−84
Minus ribosomes	2	−98
Minus ATP	20	−83
Minus GTP	47	−61
Minus MgCl₂	19	−84
Minus phosphoenolpyruvate	11	−90
Zero time	0	−100

* Each value represents the average of two incubated samples.
† The components of the complete system are given in the text.

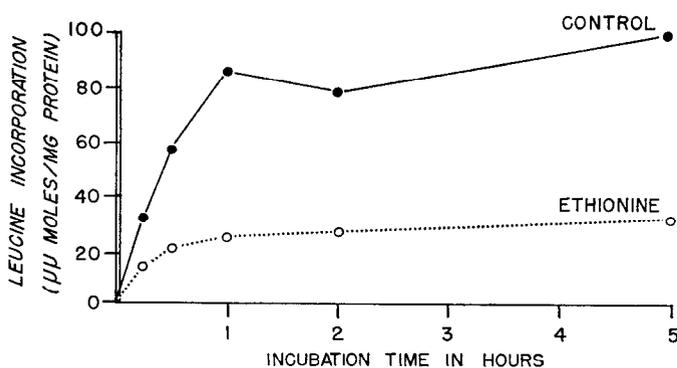
FIG. 1. The incorporation of L-leucine-C¹⁴ into the ribosome system from the liver of a control or ethionine-treated female rat as a function of time of incubation.

TABLE II

Reversal of ethionine inhibition of incorporation in vitro of leucine into proteins by adenosine triphosphate

	Leucine incorporated[a]	Difference from control
	μμmoles/mg of protein	*%*
Experiment 1		
Control	68 ± 5[b]	
Ethionine[c]	16 ± 2	−76
Ethionine plus ATP[d]	68 ± 5	0
Experiment 2		
Control	95 ± 9	
Ethionine	23 ± 6	−76
Ethionine plus ATP	82 ± 7	−14

[a] Each value represents an average of four incubated samples from two animals. All animals were killed 5 hours after the first dose of ethionine or 0.9% sodium chloride solution.

[b] Mean ± standard error of the mean.

[c] The ethionine was injected intraperitoneally in two doses, as detailed in the text.

[d] The ATP was administered subcutaneously in two doses, as described in the text.

RESULTS

The incorporation of leucine into protein in a liver ribosome system prepared from female rats treated with ethionine is very much less than in a similar system from control rats (Table II). This result confirms previous work with microsomes instead of ribosomes (10) and justifies the further exclusive use of ribosomes in this study. The incorporation with ribosomes is almost always considerably greater than with microsomes. Despite this, the day-to-day variation in incorporation is comparable to that with microsomes (10). As observed in Fig. 1, the difference between the ribosomes from control and ethionine-treated rats persists for at least a 5-hour incubation period.

Protection by ATP—The administration of ATP to female rats counteracts the inhibitory effect of ethionine upon amino acid incorporation into protein of the liver ribosome system (Table II) just as effectively as it does the decrease in hepatic ATP concentration induced by the same analogue (3). These results tend to implicate cellular ATP deficiency as being important in the inhibition of protein synthesis, and they pose several questions concerning its action. The first one is whether this effect is specific for ATP or whether it is also a property of other compounds related to adenine or to other purines or pyrimidines. Representative data on this question are recorded in Table III, where it can be seen that adenine is as effective as ATP, whereas cytidine, uridine, or guanosine are without influence upon the inhibition by ethionine. These nucleoside compounds are also ineffective in counteracting the ethionine-induced decrease in hepatic ATP concentration (3).[2] Inosine, like ATP, completely prevents the ethionine-induced inhibition of incorporation, whereas adenosine does so only partially. The results with adenosine have been somewhat variable, in that sometimes it is found to be completely effective whereas at other times only partially so. This may be due to the very low solubility of adenosine in water. Other derivatives of guanine, for example, guanylic acid or GDP, are ineffective. Thus, the ability of compounds to prevent the depression of leucine

[2] K. H. Shull, unpublished results.

TABLE III

Effect of administration of ATP, adenine, or different nucleosides upon inhibition of incorporation in vitro of leucine into protein by ethionine

	Leucine incorporated[a]	Difference from control
	μμmoles/mg of protein	*%*
Experiment 1[b]		
Control	91 ± 9[c]	
Ethionine plus cytidine	8 ± 2	−91
Ethionine plus uridine	8 ± 2	−91
Experiment 2[b]		
Control	24 ± 2	
Ethionine	5 ± 1	−77
Ethionine plus ATP	24 ± 1	0
Ethionine plus adenosine	13 ± 2	−47
Ethionine plus guanosine	6 ± 2	−75
Ethionine plus inosine	26 ± 2	+8
Experiment 3		
Control	30 ± 2	
Ethionine[e]	5 ± 0.2	−83
Ethionine plus ATP[c]	27 ± 5	−10
Ethionine plus adenine sulfate[e]	37 ± 1	+23

[a] Each value represents an average of four incubated samples from two animals.

[b] The ethionine, ATP, or nucleosides in these experiments were injected either intraperitoneally or subcutaneously as described in detail in the text.

[c] Mean ± standard error of the mean.

[d] The ethionine was administered intraperitoneally in a single dose.

[e] The ATP or adenine sulfate was administered subcutaneously in a single dose of 0.321 mmole at zero time.
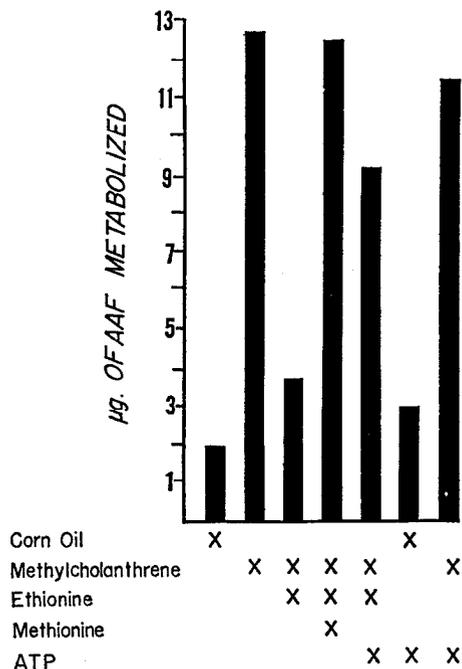

FIG. 2. The effects of injection of ethionine, methionine, or ATP in various combinations upon the hepatic 2-acetylaminofluorene hydroxylase activity in young female rats which received 3-methylcholanthrene in corn oil. *AAF*, acetylaminofluorene.

incorporation into protein in the liver ribosome system appears to be limited to adenine and adenine derivatives or precursors. In most subsequent experiments, ATP rather than adenine was used because the nucleotide appears to be less toxic for the rat kidney than is the free purine. It is noteworthy that the incorporation of leucine into protein in the ribosome system from control animals which received adenine or ATP was usually equal to that in the uninjected controls, although occasional animals receiving these compounds did show an increased level of incorporation.

2-Acetylaminofluorene Hydroxylase—Another important question is the possible effectiveness of ATP administration when a nonisotopic method of measuring protein synthesis is used. For this purpose, the hepatic microsomal 2-acetylaminofluorene hydroxylase system described by Cramer *et al.* (4) was selected. The activity of this enzyme system is increased manyfold after the injection of 3-methylcholanthrene, and this increase is largely prevented by the simultaneous administration of ethionine (4). As can be seen in Fig. 2, the injection of ATP counteracted to a major degree the ethionine-induced inhibition of the increase in hydroxylase produced by 3-methylcholanthrene. The fact that ATP is somewhat less effective than methionine is considered to be due to incomplete knowledge about optimal dosage and time of administration of ATP over a 24-hour experimental time period.

Decrease in Hepatic ATP versus Inhibition of Incorporation—The foregoing results are strongly in favor of the conclusion that the ethionine-induced inhibition of hepatic protein synthesis is somehow linked to cellular alterations in adenine-containing compounds. It naturally follows that the remainder of this phase of the study should be concerned with attempts to understand the mechanism of this link. It was of considerable interest to determine whether the changes in liver ATP preceded or followed the inhibition of protein synthesis and whether a parallelism occurred between these two effects of ethionine when different doses of the analogue were administered. It is
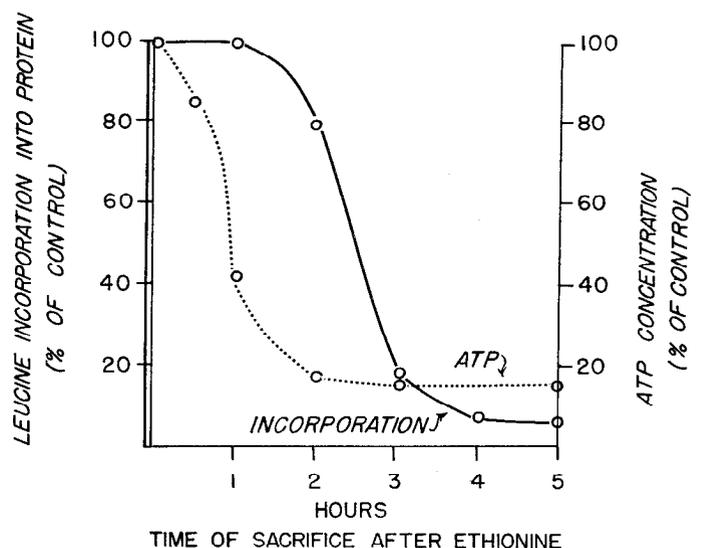

FIG. 3. The extent of incorporation *in vitro* of L-leucine-C[14] into a hepatic ribosome system and the ATP concentration in the liver from female rats treated by injection of ethionine at different time intervals before being killed. Each point represents the mean of the value from two ethionine-treated rats as compared to the mean of the values from two control animals.

readily apparent from Fig. 3 that the ATP level begins to decrease at least 1 hour before the beginning of any detectable decrease in the incorporation of leucine into protein in the ribosomal system. By 3 hours each of these has decreased to its minimal level. These results are consistent with the hypothesis that the change in protein metabolism is secondary to the change in ATP concentration. In Fig. 4 is seen a close parallel between the relative ATP concentration and the degree of inhibition of amino acid incorporation into protein induced by the administration of different doses of ethionine. It is note-
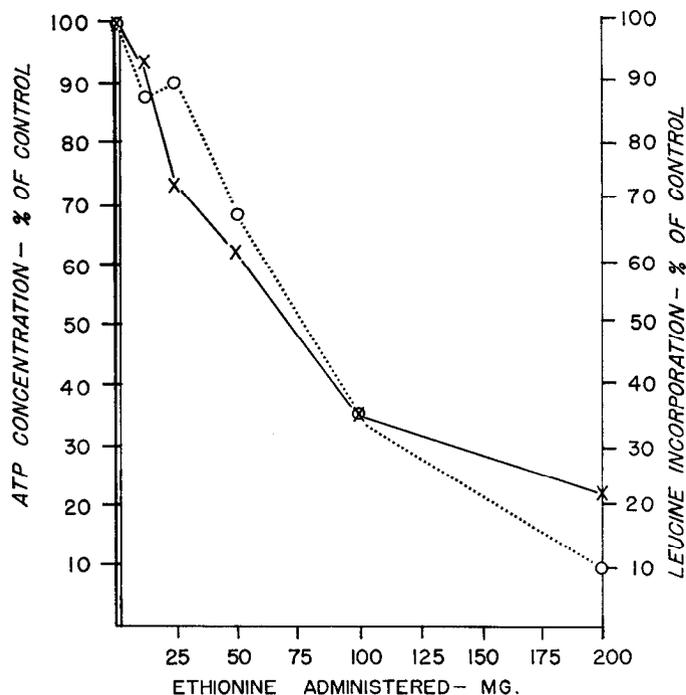

Fig. 4. The hepatic ATP concentration ($\times$——$\times$) and the extent of incorporation of L-leucine-C^{14} into a liver ribosome system ($\circ$---$\circ$) from female rats as a function of the dose of ethionine administered. Each point represents the mean from two ethionine-treated rats as compared to the mean from two control animals.

TABLE IV

Effect of ATP administered at different times after ethionine upon incorporation in vitro of leucine into protein

| Group | Injection schedule* | | Leucine incorporated† | Difference from control |
	Ethionine	ATP		
	hrs		*μμmoles/mg of protein*	*%*
Control.			91 ± 6‡	
Ethionine.	0, 1		6 ± 1	−92
Ethionine plus ATP. . .	0, 1	0, 1	81 ± 4	−11
Ethionine plus ATP. . .	0, 1	1, 2	76 ± 7	−16
Ethionine plus ATP. . .	0, 1	2, 3	86 ± 8	−5
Ethionine plus ATP. . .	0, 1	3, 4	53 ± 5	−41

* Ethionine (1.2 mmoles) was administered intraperitoneally in two divided doses at zero time and 1 hour; 0.32 mmole of ATP was administered subcutaneously in two divided doses at the indicated times; all animals were killed at 5 hours.

† Each value represents the average of four incubated samples from two animals.

‡ Mean ± standard error of the mean.

TABLE V

Effect of adenine on hepatic ATP levels of ethionine-treated and control female rats

| Treatment* | | No. of rats | ATP levels† | Relative ATP levels |
Ethionine	Adenine			
−	−	5	0.273 ± 0.012	100
+	−	5	0.050 ± 0.008	18
+	+	5	0.393 ± 0.024	144
−	+	5	0.379 ± 0.027	139

* Ethionine was administered as described in the text. All animals were killed 5 hours after the first dose of ethionine or 0.9% sodium chloride solution. Adenine was injected subcutaneously in a total dose of 0.116 mmole at 2 hours after the first injection of ethionine or sodium chloride solution.

† Micromoles per g of liver ± standard error of the mean.

TABLE VI

Effect of administration of methionine or ATP 5 hours after ethionine upon inhibition of leucine incorporation into protein in ribosome system by ethionine

Group*	Animal No.	Leucine incorporated	Difference from control
		μμmoles/mg of protein	*%*
Control	1	123	
Ethionine	2	23	−80
Ethionine plus ATP	3	22	−82
	4	20	−84
	5	75	−39
	6	89	−27
	7	50	−60
Ethionine plus methionine	8	62	−49
	9	87	−29
	10	86	−30
	11	61	−50
	12	93	−25
	13	73	−40

* Ethionine (1.2 mmoles) was administered at zero time and 0.321 mmole of ATP or 1.2 mmole of methionine were administered at 5 hours. All animals were killed at 10 hours after the time of ethionine injection.

worthy that in the dosage range of 12.5 to 100 mg of ethionine (0.077 to 0.61 mmole), the ATP level appears to be a linear function of the dose. This observation is consistent with the presence of some form of stoichiometric relationship between ethionine and ATP.

Effects of ATP upon an Already Established Inhibition—The next problem is concerned with whether ATP would protect against the ethionine-induced interference in protein metabolism *only* when administered *at the same time as* ethionine or whether it would be effective also in reversing an already established inhibition. The results of the first type of experiment designed to test this are recorded in Table IV. It is apparent from this table that ATP is completely protective when administered up to 2 hours after the injection of the first dose of ethionine. When administered at 3 or 4 hours, it also reverses the inhibitory effect but to a lesser degree. At 2 hours, the inhibition of incorporation of leucine into protein is already apparent and it reaches its maximal value at 3 hours (Fig. 3). In this series of

experiments, the effect of adenine injection upon the ATP concentration in the liver after it had decreased to a very low level was measured also. As is apparent in Table V, the ATP level of ethionine-treated female rats at 5 hours is even higher than that of the control animals if adenine is administered 2 hours after the first dose of ethionine. At 2 hours, the ATP level is at a minimum (Fig. 3). The results in Tables IV and V are what one would expect if the decrease in ATP level is the basis for the inhibition of protein synthesis.

In the second type of experiment, the ATP was administered 5 hours after the first dose of ethionine and the rats were killed 5 hours thereafter. Methionine was administered to some animals in place of ATP. The results of one such experiment are recorded in Table VI. Leucine incorporation into the protein of the ribosome system from an ethionine-treated female was 80% less than that from a control animal. This result is similar to that obtained in well over 75 rats in which the incorporation of leucine into protein in the liver ribosome system was inhibited consistently to the extent of at least 70% by ethionine at the same dosage level. This inhibition is regularly decreased to a value of 50% or less of the control by methionine (Table VI). With ATP the response is more variable, some animals showing no effect and others showing a considerable degree of reversal. These results with ATP and methionine indicate that whatever may be the nature of the metabolic lesion in protein synthesis induced by ethionine, it can be partially reversed even when the lesion has been present for 5 hours.

ATP in Vitro—Although ATP and an ATP-generating system are added to the ribosome system incorporating leucine into protein *in vitro*, it became of interest to observe whether the addition of larger amounts of ATP or of adenine would counteract the inhibition of this system induced by ethionine. As reported by Korner (11), increasing amounts of ATP are not inhibitory to the system so long as equivalent amounts of Mg^{++} are added. As may be seen in Table VII, increasing the ATP levels from 1 to 2 or to 4 μmoles per flask did not increase the low level of incorporation observed with the ribosomes from an ethionine-treated female. Similarly, the addition of adenine did not reverse the inhibition.

TABLE VII

*Effect in vitro of extra ATP or of adenine upon ethionine inhibition of incorporation of L-leucine-C^{14} into protein**

Source of ribosomes	Compound added		Leucine incorporated	Difference from control
		μmoles/flask	$\mu\mu$moles/mg of protein	%
Control	ATP	2	109	
Ethionine-treated	ATP	2	31	−71
Control	ATP	4	120	
Ethionine-treated	ATP	4	24	−80
Control	Adenine	2	148	
Ethionine-treated	Adenine	2	31	−79
Control	Adenine	4	137	
Ethionine-treated	Adenine	4	34	−75

* Extra ATP or adenine plus an equimolar amount of MgCl$_2$ were added to the regular incubation medium for incorporation *in vitro* of amino acid into protein (see the text). A single ribosome suspension prepared from a female rat was used for all the control flasks and a second one prepared from an ethionine-treated female rat for all the "ethionine-treated" flasks.

DISCUSSION

It is evident from the results of this study that a close parallelism exists between the ethionine-induced alterations in the hepatic ATP level and the degree of inhibition of protein synthesis by this analogue, and that the administration of adenine or ATP counteracts the inhibition of protein synthesis as effectively as it does the decrease in ATP concentration in the liver (3). Of the compounds tested, only those known to be converted to adenine or an adenine derivative were found to be effective. It is probable that all the effective adenine compounds are hydrolyzed, possibly to adenine, before being utilized by the cells for adenine nucleotide synthesis. These findings suggest that the primary effect of ethionine is on the cellular ATP level and that the inhibition of protein synthesis is a secondary phenomenon. Consistent with this is the failure of ethionine to have a direct effect on amino acid incorporation into protein in a liver microsome system (10, 12).

If this conclusion is correct, two basic questions naturally come to mind: (*a*) How does ethionine affect the cellular ATP level and by what mechanism does adenine or ATP counteract this effect? (*b*) How does a decrease in ATP concentration damage the protein synthetic system in such a way as to make it evident in assay systems both *in vivo* and *in vitro*?

With regard to the first question, two hypotheses have been suggested by Stekol *et al.* (5, 13). The first one (5) is based on the original observations of Cantoni (14) with regard to the *S*-activation of methionine and ethionine by the methionine-activating enzyme to form *S*-adenosylmethionine and *S*-adenosylethionine. Although the activation of ethionine occurs to a considerable extent, it appears that the product, the *S*-adenosyl derivative, is utilized to a much lesser degree than is the corresponding derivative of methionine in transalkylation and in other metabolic reactions (*cf.* (2)). In addition, ethionine is excreted in part in the urine in the form of the *S*-adenosyl derivative. Both these properties have the effect of decreasing the metabolically available adenine compounds, either by excretion or by combination. Schmidt *et al.* (15) have described a similar adenine-trapping effect of ethionine in yeast. This mechanism would presuppose that the rates of removal of ATP or derivatives from metabolic availability following ethionine administration are considerably faster than the rates of synthesis of ATP from adenine precursors. According to this hypothesis, the administration of adenine, adenine precursors, or derivatives counteracts the decrease in ATP concentration by supplying the essential substrates for ATP synthesis. On the other hand, methionine presumably counteracts the effects of ethionine by preventing competitively the initial *S*-activation of the analogue. The second hypothesis (13) is based upon the observation that the addition *in vitro* of *S*-adenosylethionine but not of *S*-adenosylmethionine inhibits mitochondrial respiration. Such an action *in vivo* could interfere with the mitochondrial synthesis of ATP through oxidative phosphorylation.

Although no final conclusion is possible as yet on the validity of either hypothesis, the following observations lend strong support to the first rather than the second one: (*a*) the magnitude of the decrease in hepatic ATP concentration is approximately proportional to the dose of ethionine administered in the dosage range of 12.5 to 100 mg; (*b*) the administration of sufficient ethionine to induce a fatty liver is without effect upon liver

mitochondrial oxidative phosphorylation (16, 17), even though the administration of ATP effectively prevents the ethionine-induced fatty liver;[3] (c) the administration of adenine is as effective as that of ATP; and (d) adenine, ATP, or methionine are effective in counteracting the effects of ethionine upon hepatic ATP levels and protein synthesis *even after ethionine has induced its maximal effects.*

The last observations lend no support for the possibility that adenine or ATP are effective by virtue of accelerating the excretion of ethionine from the whole organism or preventing the uptake of ethionine by the liver. Against this idea are also observations made on the distribution of radioactive ethionine (ethyl-1-C[14]) in animals that received toxic doses of ethionine with or without ATP. At the end of 5 hours female rats that received ethionine and ATP had exactly the same distribution of ethionine in the plasma and in the liver, kidney, pancreas, adrenal, and skeletal muscle acid-soluble fractions as did the animals that received ethionine alone. In addition, the 5-hour urinary excretion of ethionine was approximately equal in the two groups of rats. These results are consistent with the thesis that adenine or ATP counteracts the ethionine effect on ATP by facilitating the synthesis of ATP from adenine.

Natori *et al.* (18) have suggested that the inhibition of protein synthesis induced in the liver by ethionine may be the result of ethionine becoming incorporated into protein with the production of abnormal protein molecules containing ethionine in place of some methionine. We have found that the administration of adenine or of ATP has no measurable influence upon the extent of ethionine incorporation into liver or plasma protein even though the same amounts of these compounds completely protected the animals against the inhibitory effects of ethionine.[4, 5] Similar findings with ATP and adenine have been obtained with regard to the ethylation of transfer RNA by ethionine (19, 20). It is, therefore, concluded that neither of these properties of ethionine are related to the acute inhibitory effects of ethionine upon protein synthesis and that the most probable mechanism is through the effect upon ATP metabolism.

With respect to the second basic question, *viz.*, the mechanism whereby ATP deficiency inhibits protein synthesis, our understanding is still fragmentary. Assuming that the known steps in amino acid incorporation into protein in the ribosome-supernatant system (*cf.* (21)) are truly steps in the protein synthetic system in the intact cell, an ATP deficiency may act on at least three known levels: the activation of the amino acid and its esterification to transfer RNA, the interaction of the amino acid-transfer RNA complex with the ribosome, and the synthesis and interaction of "messenger RNA" with the ribosome. Previous work on the mechanism of inhibition of protein synthesis by ethionine has indicated a probable effect upon the microsome (10) or ribosome (7) fraction rather than upon the components in the nonparticulate cell fraction. For example, with either ribosomes or microsomes from ethionine-treated animals, substitution of the pH 5 fraction from an ethionine-treated animal with one from a control animal or from an ATP-treated animal (7) has no detectable influence upon the rate of incorporation of amino acid into protein. Also, the addition of increased amounts of ATP, with sufficient extra Mg^{++} to prevent

inhibition, has no effect upon the extent of incorporation. In addition, we have found that the pH 5 fraction prepared from ethionine-treated animals activates and binds leucine or methionine to transfer RNA as actively as does the fraction from normal animals.[4] These results tend to rule out components of the pH 5 fraction as sites of inhibition.

If the ribosome is the site of inhibition, then a possible mechanism for the effect of ethionine is the interference in the synthesis of "messenger RNA." ATP and the other nucleoside triphosphates which, in part, are derived from ATP are the probable substrates for "messenger RNA" synthesis. If "messenger RNA" in mammalian cells is labile and short-lived as it is in some microorganisms (22, 23), and this appears likely (24–27), and if combination with this RNA is essential for ribosomal amino acid incorporation into protein, then the ATP deficiency induced by ethionine could inhibit protein synthesis by interfering with the synthesis of "messenger RNA."

SUMMARY

1. The administration of adenine or adenosine triphosphate protects female rats against the inhibition of hepatic protein synthesis induced by ethionine. The indices for protein synthesis were (a) incorporation *in vitro* of radioactive leucine into protein in a ribosome system; and (b) the increase in 2-acetylaminofluorene hydroxylase activity induced by the injection of methylcholanthrene. An adenosine triphosphate precursor, inosine, is also effective, whereas guanine, uracil, or cytosine derivatives are without effect.

2. A close parallelism has been found between the magnitude of the decrease in hepatic adenosine triphosphate concentration induced by ethionine and the degree of inhibition of incorporation *in vitro* of radioactive leucine into protein. The drop in adenosine triphosphate level occurs about 1 to $1\frac{1}{2}$ hours before any measurable change in amino acid incorporation into protein.

3. The administration of adenosine triphosphate at varying times after the injection of ethionine counteracts the effects of ethionine upon both the adenosine triphosphate concentration in the liver and protein synthesis. However, the degree of protection decreases as the time interval between the injection of ethionine and of adenosine triphosphate is lengthened up to 5 hours.

4. The available evidence indicates that the inhibition of protein synthesis in the liver by ethionine is secondary to a decrease in the concentration of adenosine triphosphate. The most likely hypothesis is that ethionine affects the adenosine triphosphate level by reacting with this nucleotide to form S-adenosylethionine and, thereby, acts as an adenosine triphosphate-trapping agent. Adenine or adenosine triphosphate counteracts this effect by supplying the necessary precursors for adenosine triphosphate synthesis.

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