MASTER

Degradation of Tryptophan Oxygenase

In Vivo and In Vitro*

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SUMMARY

The loss of tryptophan oxygenase from rat livers after induction by hydrocortisone treatment was studied in living rats, isolated perfused livers, liver slices and cell-free preparations of these livers. Measurements of the enzyme by its activity and as an antigen were parallel except for apparently non-specific inactivations in some incubations of cell-free preparations and the temporary, early appearance of a catalytically inactive antigen during accumulation of the enzyme after hydrocortisone treatment. The enzyme was lost at similar rates in all of the systems studied, including cell-free soluble fractions of liver. In all systems, the antigen was preserved by the substrate analog, α -methyltryptophan. The rate of loss was proportional to the concentration of the enzyme and was the same for added or endogenous enzyme and the same in non-induced or induced liver preparations. Labeled, purified enzyme added to cell-free preparations was used to demonstrate that the loss of antigenic material was not significantly associated with its proteolysis to small fragments. The results are compatible with an initial step in the degradation of this enzyme that is akin to a denaturation, and which can be prevented by combination of the protein with its substrate.

The occurrence of intracellular protein degradation as part of the turnover of proteins in mammals has been known since 1942 (1). This important aspect of protein metabolism has been neglected in comparison with the interest shown in protein synthesis. Most of the experimental data available concerns the role of protein degradation in determining the levels of enzymes in animal tissues, and in this connection, the rates of turnover have been measured for a number of rat proteins (2, 3). However, there is little known about the actual mechanism of intracellular protein degradation, or about the nature of the proteolytic reactions involved.

Rat liver tryptophan oxygenase (EC 1.13.1.12) was one of the first enzymes whose level was found to be regulated in vivo by adrenocortical hormones and its substrate, L-tryptophan (4). This enzyme was chosen for in vivo and in vitro studies on the mechanism of specific enzyme degradation because of its short half-life in vivo of 2.5 hour (5) compared to 3 days for the average liver proteins (3); its inducibility by hydrocortisone and L-tryptophan; and its protection from degradation by L-tryptophan and DL- α -methyltryptophan (6-8). The ability to inhibit its degradation in a specific manner should help identify the physiological process, and the rapidity of its disappearance offered the opportunity to follow it over short time periods. The disappearance of the enzyme after its induction in liver was therefore followed in intact animals, perfused livers, liver slices and cell-free preparations, using a rapid assay of the enzyme protein as an antigen (9), as well as activity assays of the several forms of the enzyme (10).

MATERIALS AND METHODS

All rats used were inbred NADH adult males (200-250 g). Bilateral adrenalectomies were performed on some rats 5-10 days before use. To increase the level of tryptophan oxygenase in livers, rats were treated with 0.25 mg hydrocortisone phosphate per 100 g body weight and sacrificed at 5 hours (with mean and SD of 16.2 ± 6.4 units/g liver), or treated with 2.5 mg hydrocortisone phosphate and 100 mg L-tryptophan per 100 g body weight at 0 and 4 hours and sacrificed at 8 hours (with 38.1 ± 14.4 units/g liver). The livers were promptly removed and used as donor livers for perfusion experiments, or chilled, and then sliced or homogenized for enzyme studies.

Liver slices were cut 0.5 mm thick on a Stadie-Riggs microtome. Approximately 250 mg of slices and 2.25 ml of Eagles tissue medium (Grand Island Biochemical Co.) containing 0.01 M phosphate buffer, pH 7.0, were incubated at 37° with gentle shaking in a stoppered 25 ml flask, with oxygen-carbon dioxide (95:5) as the gas phase. The final pH was 7.2-7.4. At the end of the incubation the entire flask contents were homogenized and centrifuged before assay. In some parallel experiments homogenates and soluble fractions (25%, w/v, final tissue concentration) were incubated like the slices, except that air replaced oxygen.

Liver homogenates were prepared with a glass teflon homogenizer in 2 volumes of Eagles tissue culture medium or in 3 volumes of 0.14 M KCl-0.02 M potassium phosphate buffer, pH 7.0 (the standard 25% homogenate). All liver preparations were centrifuged for 40 min at 50,000 rpm in a Spinco Model L, rotor 50 before the soluble fractions were assayed. The total tryptophan oxygenase activity and the proportion of holoenzyme were measured by the

methods of Piras and Knox (10), with addition of 10 mM GSH to the preincubation (9); the tryptophan oxygenase antigen was determined by an immunoinhibition titration previously described (9). Other enzyme activities measured in whole liver homogenates were catalase (EC 1.11.1.6 (11)) and ornithine aminotransferase (EC 2.6.1.13 (12)); those in soluble fractions of liver were glucokinase (EC 2.7.1.2 (13)), hexokinase (EC 2.7.1.1 (13)) and tyrosine aminotransferase (EC 2.6.1.5 (14)).

Liver proteins were labeled in vivo with [U-¹⁴C]leucine or with a mixture of uniformly labeled [U-¹⁴C]amino acids (New England Nuclear). For maximum labeling of the soluble protein fraction and minimal counts in the trichloroacetic acid soluble fraction, 50 μ C was administered intravenously or intraperitoneally 2-3 hours before sacrificing the rat. The total counts were obtained by dissolving 0.2 ml homogenates in 1.8 ml of 100% formic acid or 0.5 ml of Hyamine (Packard Instrument Corp.) and counting 0.2 ml in 10 ml of Bray's scintillation fluid (15). The trichloroacetic acid soluble counts were measured in duplicate aliquots of 0.5 ml of the homogenate or soluble fraction, plus 0.5 ml water and 1.0 ml 20% trichloroacetic acid. 0.2-0.5 ml of this supernatant was counted in 10 ml of Bray's scintillation fluid. Counts per min were corrected for quenching by means of an external standard on the Packard Tri-Carb scintillation counter.

To prepare labeled tryptophan oxygenase, mixed [U-¹⁴C]amino acids (14 μ C/100 g body weight) were injected intraperitoneally 1 hour before the end of an 8 hour hydrocortisone-tryptophan induction period. The enzyme was purified according to Knox et al. (16). The final specific activities were 36.8 units/mg protein and 324 cpm/unit of activity.

Isolated liver perfusions were performed using the method of Miller et al. (17) as modified by Knox and Sharma (18). The perfusate contained 90 ml of whole, heparinized rat blood, 60 ml of Krebs-Ringer bicarbonate buffer containing 30 mg of glucose, and 1.68 g bovine serum albumin (Armour, fraction V). Where indicated, the amino acid mixture (about 4 times normal plasma levels) described by Miller et al. (17) or Mallette et al. (19), puromycin at 7 mg/150 ml, or DL- α -methyltryptophan at 1 mM were added to the perfusate. Liver donors were rats treated earlier with hydrocortisone to induce high levels of tryptophan oxygenase.

RESULTS

Induction and Degradation of Tryptophan Oxygenase In Vivo--Time curves of the induction of tryptophan oxygenase activity by hydrocortisone and by the substrate, L-tryptophan, were constructed to determine the relative activity and antigen titer of the enzyme during its induction phase and its subsequent return to basal levels. In the hydrocortisone induction, adrenalectomized rats were injected with 0.25 mg hydrocortisone phosphate per 100 g body weight (20), a dose that has a limited period of action, and they were killed at intervals for assay. The levels of tryptophan oxygenase activity and its antigen are plotted in Fig. 1A as a function of time after the hydrocortisone treatment. At 2.5 hours the antigen reached a maximum level, while the activity had increased to only 50% of its maximum. Synthesis of tryptophan oxygenase protein recognizable as antigen may thus precede its conversion to a form which can be measured catalytically, but additional evidence for this is lacking. Both the activity and antigen were at maxima at 5 hours. After that time, both decreased in parallel and approached the

basal level between 9 and 12 hours, as previously found (20). The holo-enzyme content was low and unchanging in these hydrocortisone inductions.

The induction of tryptophan oxygenase by its substrate was determined in adrenalectomized rats injected with 100 mg L-tryptophan per 100 g body weight. The total activity, holoenzyme, and the antigen were measured. As shown in Fig. 1B, all these measures of the enzyme increased together to maximum levels at 5 hours, and then decreased. Accumulation of the antigen did not precede the activity, as was found with hydrocortisone induction. However, after 5 hours the holoenzyme decreased faster than the total activity and the antigen. After both kinds of induction, the parallel disappearance of enzyme measured by its activity or as an antigen demonstrated the occurrence of some sort of rapid enzyme degradation.

Loss of Tryptophan Oxygenase in Isolated Perfused Livers--Tryptophan oxygenase was induced with hydrocortisone exactly as described for the experiments in Fig. 1A. At the height of the induction the livers were transferred to the perfusion apparatus in order to follow the degradation phase in this simpler system. The results are summarized in Table I. Enzyme activity and antigen decreased from the elevated levels initially present in the donor livers. The fall was faster during control perfusions than it was in vivo. By way of comparison, 80% of the peak levels persisted 2.5 hours later in the experiments in vivo shown in Fig. 1A. Less than half this much remained after 2.5 hour perfusion of the livers without added amino acids.

The rate was greatly reduced, to somewhat less than that seen in vivo, when a complete amino acid mixture was added to the perfusate. Only a small amount of tryptophan was in these mixtures, and complete omission of it did not change these results. More tryptophan could be expected to promote

continued synthesis and specifically to protect the enzyme. This was demonstrated with the unmetabolized analog, DL- α -methyltryptophan. Perfusion with 1 mM added α -methyltryptophan substantially prevented the disappearance of enzyme activity and antigen, and it was more effective in this regard than the mixture of amino acids. Since inclusion of puromycin in the perfusate did not lessen the effect of α -methyltryptophan, its main action did not appear to be promotion of enzyme synthesis but protection. α -Methyltryptophan preserved the enzyme in vivo (6, 8) independently of its effect on enzyme synthesis, and appeared to do the same in this system.

Puromycin itself had a dramatic effect on degradation of the enzyme. Alone or in combination with the amino acid mixture it caused a precipitous loss of enzyme and antigen at a rate much faster than that seen in vivo or in the control perfusion. This effect was blocked by α -methyltryptophan. Induced synthesis of tryptophan oxygenase under the most favorable conditions (18) may not be rapid enough for this effect of puromycin to be ascribed solely to inhibition of protein synthesis.

In these several kinds of perfusions the changes of enzyme measured by its activity and as antigen were always parallel. They appeared to represent changes in the rate of some kind of specific enzyme degradation like that seen in vivo.

A number of other enzymes were also measured in the perfused livers by activity assays to provide control information (Table I). Three of these are known to have relatively long half-lives in vivo: glucokinase, 11 hours (21); ornithine aminotransferase, 20 hours (22); catalase, 1.25 days (23). Since these three and hexokinase did not change significantly during the relatively short perfusions, the changes of tryptophan oxygenase were to

some extent specific. Tyrosine aminotransferase has a half life of 1.5 hours (24), even shorter than tryptophan oxygenase (2.5 hours), but it also did not decrease very much. Only in the perfusions containing puromycin did it decrease significantly, and α -methyltryptophan did not prevent this loss. To this extent the protection of tryptophan oxygenase in the perfusions by α -methyltryptophan was also specific.

Loss of Tryptophan Oxygenase In Vitro--Disappearance of tryptophan oxygenase was measured in slices and cell-free preparations from livers of rats treated earlier with hydrocortisone and examined at the height of the induction, following the same experimental design used with intact animals and perfused livers. Some of the rats were given 50 μ C [U-¹⁴C]-amino acids 2 or 3 hours before sacrifice to label their liver proteins. Table II describes the changes in tryptophan oxygenase activity and antigen, and the release of counts soluble in trichloroacetic acid, after incubation of these preparations for 2 hours. In each of these preparations there were significant and parallel losses of tryptophan oxygenase activity and antigen that were of the same order of magnitude as were seen in perfused livers. An amino acid mixture was included in some of the incubations (in the Eagles medium), but it did not lessen the enzyme disappearance like somewhat larger amounts of amino acids did in the perfused livers. α -Methyltryptophan preserved the enzyme activity somewhat, but it regularly preserved the antigen to a significant extent in comparisons with incubations in its absence.

Irregular losses of the enzyme activity during these incubations were expected and were not necessarily meaningful. Activity losses were always as great or greater than antigen losses, but otherwise the two were

independent. However, the regular loss of significant amounts of the antigen, specifically prevented by α -methyltryptophan in the medium indicated that some kind of enzyme degradation occurred to the same extent in these slices, homogenates and soluble fractions as in the intact organs. Loss of tryptophan oxygenase antigen was observed previously in slices but not in homogenates (8). Its occurrence even in the relatively simple soluble fraction was unexpected. The suspicion that the loss from the soluble fraction was not a proteolytic reaction was supported by supplementing this fraction with an excess of a protease-containing lysosomal preparation (25) made according to Sawant et al. (26). This did not alter the rate of disappearance of the activity or antigen.

There was a significant release of counts from the labeled liver into the acid soluble fraction during incubation of the slices but not the homogenates. In relation to the initial counts that were not soluble in trichloroacetic acid, this represented solubilization of 1 to 2% of the labeled protein in 2 hours. Simpson (27) and Steinberg and Vaughan (28) reported similar rates of release in slices, and also did not detect the release in homogenates. α -Methyltryptophan did not affect this release, and so its preservation of tryptophan oxygenase antigen in the same experiments was not due to any general inhibition of protein breakdown.

More detailed studies of the disappearance of tryptophan oxygenase antigen were restricted to the soluble fractions, utilizing combinations of the induced with non-induced preparations, or addition of purified enzyme to these preparations. The loss of the added, purified antigen was not different from that of the endogenous antigen, and the losses were not different in induced or non-induced preparations -- when allowance was made

for the fact that the loss was generally proportional to the initial antigen level (Fig. 2). As expected and as described in vivo, the time curve of the loss in these cell-free preparations was first order with respect to the antigen (Fig. 3). In these experiments the losses of total activity closely paralleled losses of antigen (half-life, 105 min). This half-life in vitro of 1.75 hours can be compared to the half life of 2.5 hours measured in vivo (5).

The small fraction of holoenzyme present in hydrocortisone-induced preparations could not be measured with assurance, but its loss appeared to be more rapid than the main fraction of the enzyme (Fig. 3). However, this point was studied further with highly conjugated enzyme preparations from rat livers after induction for 5 hours with hydrocortisone plus tryptophan. Hemoglobin was removed from the livers by saline perfusion before they were excised. Globin, 0.2 mg per ml, was added to the incubations to bind free heme. Antigen disappeared equally rapidly from these preparations as from the unconjugated ones. Moreover, as long as no tryptophan was added, there was an even more rapid conversion of the holoenzyme to apoenzyme (29). The conjugated state of the enzyme, which presented no obstacle to degradation of the enzyme in vivo (Fig. 1), also need not do so in vitro.

Degradation of Labeled Tryptophan Oxygenase In Vitro--Since purified enzyme added to non-induced liver preparations behaved in the same way as endogenous enzyme in induced preparations (Fig. 2), it was possible to study the process with addition of labeled enzyme (Table III). Disappearance of labeled and purified tryptophan oxygenase when added to homogenate and to the soluble fraction was similar to that observed with unlabeled, endogenous antigen. Approximately half was lost in 2 hours. α -Methyltryptophan minimized this loss. The acid soluble counts released, however, bore no

relation to the loss of antigen. Fewer counts were released than predicted if the lost antigen became acid soluble. Although α -methyltryptophan preserved a significant fraction of the antigen, its addition did not significantly change the counts released. More counts were released in the homogenate than the soluble fraction, and more in the particulate fraction than the homogenate, as expected from their relative proteolytic activities⁽²⁹⁾. This production of acid soluble counts was small and appeared to be the result of non-specific proteolysis, possibly coming in part from labeled proteins that may have contaminated the tryptophan oxygenase preparation. The disappearance of antigen, even in the homogenate, did not appear to be significantly related to proteolytic processes occurring during the period of these incubations.

To confirm this conclusion, the soluble fractions from the incubations in which antigen had disappeared were chromatographed on Sephadex G-100. Except for the small number of solubilized counts already mentioned, which appeared as amino acids or small peptides, the bulk of the labeled material had retained its high molecular weight and was eluted in the fractions with the active tryptophan oxygenase.

DISCUSSION

Enzyme degradation as studied here is an irreversible loss of activity and antigenicity. This does not include loss of activity due to accumulation of the tryptophan oxygenase apoenzyme or the oxidized holoenzyme, because these forms were routinely activated before assay by the procedure used (10). In addition, reactivation of enzyme after a prolonged incubation was about 10% greater with the 10 mM glutathione added here to the preincubation

mixture. Presumably it protected a sulfhydryl group on the apoenzyme that was necessary for conjugation (10). Even so, irregular losses of activity could not be avoided in cell-free preparations. Except that these losses of activity were always as great or greater than loss of antigenicity, the two were not otherwise related. Loss of the antigen did not appear to be limited by a specific kind of inactivation of the enzyme. Such a catalytically inactive antigen cannot be ruled out as an intermediate, however, since a species of this type appeared to accumulate temporarily during hydrocortisone induction of the enzyme (Fig. 1).

The antibodies used to measure the tryptophan oxygenase as an antigen by immunoinhibition were broad in latitude, since they reacted with apo- and holoenzyme (9) as well as with various inactivated forms of the enzyme. The apoenzyme, which can be formed from the holoenzyme rapidly enough not to limit the observed rate of antigen loss (29), would appear to be the species that is actually degraded and is protected from degradation by the substrate or substrate analog, not necessarily by conjugation.

Degradation of the enzyme is not chemically characterized by these criteria. The evidence is circumstantial that the same initial step occurs in the hierarchy of systems studied. Loss of the antigen in whole rats, perfused livers, slices of liver and cell-free liver preparations appeared, however, to occur by the same process because of the roughly similar rates in all of these systems and the specific inhibition of the losses by α -methyltryptophan in all of them. Special characteristics of the process in perfused livers were that the loss was slowed by mixed amino acids and accelerated by puromycin. Since the same mixture of amino acids was necessary for induced synthesis of tryptophan oxygenase in perfused livers (18), and puromycin

inhibits this same synthesis (30), the observed effects might be ascribed to changes in continued synthesis instead of changes in degradation. However, synthesis or turnover of the enzyme is not known to occur rapidly enough for this explanation. For example, the enzyme level was only maintained and not elevated by α -methyltryptophan, as would be expected if there were both preservation plus on-going synthesis. However, neither of the two special characteristics of the loss from perfused livers were seen in the simpler systems.

Degradation of the enzyme was characterized best in the simplest system, the soluble fraction of liver. In addition to inhibition of the loss by α -methyltryptophan (and L-tryptophan), the rate of loss was proportional to the concentration of enzyme present. It was also independent of the physiological state of the liver from which the fraction was obtained, since added purified enzyme behaved in the same way as endogenous enzyme. The loss was not dependent upon the proteolytic activity in liver that is mainly located in the particulate fractions (25). The loss was, in fact, unaccompanied by any significant proteolysis during the 2 hour periods studied. The characteristics of the apparent first step in this enzyme degradation are, therefore, akin to some form of denaturation of the protein. Its prevention by the presence of substrate or its analog could result from stabilizing a certain configuration of the enzyme protein. When unstable, the catalytically and antigenically inactive species formed could be like that postulated by Paulus and Alpers (31) on other grounds, i.e., an obligatory intermediate in the biosynthesis of this and other oligomeric enzymes that are subject to "preconditioning" by allosteric ligands.

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FOOTNOTES

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LEGENDS

FIG. 1. Changes of tryptophan oxygenase activity and its antigen after hydrocortisone and L-tryptophan treatments. Adrenalectomized male rats were injected intraperitoneally with either 0.25 mg hydrocortisone phosphate (A) or 100 mg L-tryptophan (B) per 100 g body weight at 0 hour. Tryptophan oxygenase total activity (--O--), holoenzyme (--Δ--), and antigen (--X--) were measured. Units are μ moles/hour/g liver or the equivalent antigen units. The vertical bars represent 1 standard error of the mean of 3-13 rats.

FIG. 2. Initial and final amounts of tryptophan oxygenase antigen in incubated soluble liver fractions. Different initial levels of tryptophan oxygenase were obtained by combining soluble liver fractions (25%) from hydrocortisone-induced and non-induced rats, and by additions of partially purified enzyme to both types of preparations. All were incubated for 2 hours at 37°, and the tryptophan oxygenase antigen (TO_{AG}) per ml measured before and after the incubation.

FIG. 3. Loss of tryptophan oxygenase, its antigen and holoenzyme during incubation. Soluble fractions of hydrocortisone-induced livers were incubated at 37°, and the antigen (X), total activity (O) and holoenzyme (Δ) were measured at various time points. Measurements have been divided by their respective initial amounts to normalize them to percent of the initial values. Each point is the average of several values determined in different experiments. The time ($t_{1/2}$) for half of the initial amounts to disappear for antigen and total activity was 105 min, and for holoenzyme 20 min.

TABLE I

Loss of tryptophan oxygenase in isolated perfused liver

Donor livers were from rats induced with hydrocortisone for 3-5 hours before perfusion. Additions to the control perfusate were: amino acid mixture (either Miller *et al.* (17) or Mallette *et al.* (19)), 7 mg of puromycin and 1 mM α -methyltryptophan. Biopsies of the liver were taken at 0 and 2.5 hours and homogenates were assayed for ornithine aminotransferase (OAT) and catalase (Cat.); the soluble fraction was assayed for tryptophan oxygenase (TO) and its antigen (TO_{AG}), tyrosine aminotransferase (TAT), glucokinase (GK) and hexokinase (HK). The values are percent of the initial amounts. All results are averages of several perfusions.

Perfusion	Percent of the Initial Value (100) after 2.5 hour Perfusion						
	TO	TO _{AG}	TAT	GK	HK	OAT	Cat.
Control	37	30	140	84	91	81	96
+ Amino Acids	87	81	111	98	114		
+ Amino Acids (- L-tryptophan)	85	86	93				
+ Puromycin ($\pm$ amino acids)	6	5	72				
+ α -Methyltryptophan ($\pm$ puromycin)	107	84	53 ^a				

^a Decrease occurred only with puromycin present.

TABLE II

Loss of tryptophan oxygenase activity and antigen and proteolysis of labeled protein
in liver slices, homogenates and soluble fractions

Assays of tryptophan oxygenase (TO) activity and as antigen before and after incubation for 2 hours at 37° are expressed as percent of the initial values. TCA soluble counts released from prelabeled liver preparations are expressed as percent of the initial ratio of soluble to total cpm. The means $\pm$ standard deviation are given with the number of experiments in parentheses. All preparations of liver were derived from hydrocortisone-induced rats with 16.2 ± 6.4 units/g liver. Slices, homogenates and the first soluble fraction (a) were incubated in Eagles medium and in oxygen-carbon dioxide atmosphere. The second soluble fraction (b) values were incubated in KCl-phosphate buffer, pH 7, with air as the gas phase.

Liver Preparation	2 Hour Incubation			2 Hour Incubation + 5 mM α -Methyltryptophan		
	TO Activity	TP Antigen	TCA Sol. cpm	TO Activity	TO Antigen	TCA Sol. cpm
Slices	33 $\pm$ 15 (19)	32 $\pm$ 10 (23)	132 $\pm$ 35 (6)	56 $\pm$ 23 (9)	77 $\pm$ 23 (12)	125 $\pm$ 28 (6)
Homogenate (a)	15 $\pm$ 9 (16)	21 $\pm$ 6 (6)	109 $\pm$ 16 (6)	17 $\pm$ 14 (4)	58 $\pm$ 19 (4)	99 $\pm$ 10 (6)
Homogenate (b)	43	50	---	37	69	---
Soluble fraction (a)	35 $\pm$ 9 (11)	23 $\pm$ 7 (11)	---	68 $\pm$ 19 (3)	73 $\pm$ 21 (3)	---
Soluble fraction (b)	33	38	---	98	101	---

TABLE III

Incubation of [U-¹⁴C]labeled tryptophan oxygenase

Tryptophan oxygenase was purified from livers of rats given [U-¹⁴C] amino acid mixture (36.8 units/mg protein and 324 cpm/unit). For stability it was stored with 5 mM L-tryptophan at pH 8 in 0.2 M potassium phosphate, and passed through Sephadex G-25 and neutralized just prior to addition to equivalent amounts of homogenate, soluble and particulate fractions from non-induced rat livers. Units (μmoles/hour) of tryptophan oxygenase (TO) activity and antigen (TO_{AG}) which disappeared, and the counts soluble in 10% trichloroacetic acid which were released per ml of incubated mixture are compared with the counts expected if the enzyme lost became acid-soluble.

Preparation	<u>Loss in 2 hour</u>		<u>Acid-Soluble Counts</u>	
	<u>I. Incubation</u>		<u>Released</u>	
	TO	TO _{AG}	Expected	Found
	(units/ml)		(cpm/ml)	
Homogenate	7.24	5.43	1760	48
Homogenate + α-methyltryptophan	5.34	(-0.50)	0	32
Soluble fraction	--	4.04	1310	13
Soluble fraction + α-methyltryptophan	--	1.95	632	16
Particulate fraction	4.10	--	1328	323
Particulate fraction + α-methyltryptophan	0.10	--	32	188