gentle washing in 0.01*M* phosphate buffer. Immediately prior to treatment, these three organisms were diluted in a 10- to 100-fold volume of sterile distilled water. The number of viable cells (or spores) was determined by duplicate plate counts on growth medium.

Bacteriophage T2 was cultured in nutrient broth on its host organism *E. coli* B and harvested at 18 hours by filtration through a Seitz filter. Following dilution in sterile distilled water, the number of phage particles was determined by plaque counts on nutrient agar seeded with the host organism.

For testing, 1200 ml of a suspension of the microorganism in sterile distilled water was put in the sterilizing tank. An initial sample was withdrawn and placed in an ice bath for determination of the number of organisms present prior to treatment. The tank was then sealed and the contents were subjected to a multiplicity of electrohydraulic discharges with varying voltage and capacitance.

After treatment, samples of bacteria or yeast were plated on nutrient agar or Sabouraud's glucose agar, respectively, in duplicate, and the count of viable organisms was determined after 48 hours of incubation. The effect on bacteriophage T2 was assayed in the same manner by plating serial tenfold dilutions on nutrient agar seeded with the host organism *E. coli* B.

In order to have a basis for comparison of different voltage levels and capacitances, the total electrohydraulic input energy was held as close as possible to 2.6 watt-hours per liter for *E. coli*, *B. subtilis* spores, and the phage. Because of the greater resistance of *S. cerevisiae*, tests were performed at an electrohydraulic input energy as close as possible to 6.6 watt-hours per liter. The total energy for each single electrohydraulic discharge is given by $J = \frac{1}{2} CE^2$, where J is the energy in joules or watt seconds; C, the capacitance in farads; and E, the voltage. Therefore, as C and E are varied, the energy per discharge J varies. Constant input energy was maintained by varying the total number of discharges fired into the 1.2-liter volume of organisms suspended in sterile distilled water in the sterilizing tank. In all cases, total treatment time was less than 1 minute.

The experimental conditions and results are given in Table 1. Variation in total input energy for any one organism is caused by the necessity of firing whole numbers of discharges at any given joule input level. In all cases, increased disinfection at constant input energy occurs at lower capacitance and lower voltage.

The equipment utilized in this study was limited to a low capacitance of 6 μfarad, and data below that level could not be taken. The equipment was capable of operation at voltage levels below 5 kv, but oscilloscope traces of the electrical characteristic (voltage and current versus time) of the electrohydraulic discharge showed a rapid deterioration as voltage was lowered below about 4.5 kv. This deterioration of electrical characteristic, a function of electrode design, would have introduced other variables into the experiment, so that testing was limited to a low voltage level of 5 kv.

The fact that disinfection by electrohydraulic treatment is maximized at low voltages and low capacitances means that equipment is less expensive to frabricate and the costs of power are minimized. As a result, this disinfection technique continues to look attractive.

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Turnover of Rat Liver Tyrosine Transaminase: Stabilization after Inhibition of Protein Synthesis

Abstract. *Turnover of the rat liver tyrosine transaminase in vivo was measured by a label and chase procedure under conditions where the amount of enzyme undergoes no change. Half-life of the ^{14}C-labeled enzyme in this basal condition was found to be 1.5 ± 0.3 hours. Inhibitors of protein synthesis (cycloheximide or puromycin) do not appreciably influence the basal enzyme level over a 5-hour period, although these drugs will block hormonal induction of this enzyme. In pulse-labeling experiments, cycloheximide blocked transaminase synthesis almost completely. The conclusion that enzyme degradation, as well as synthesis, must be blocked when protein synthesis is stopped was confirmed in experiments showing that labeled enzyme is stable in the liver of rats treated with cycloheximide. The participation of a continuously synthesized polypeptide in the degradative phase of transaminase turnover is suggested.*

The tyrosine α-ketoglutarate transaminase of rat liver is generally considered to undergo unusually rapid turnover in vivo. This was first shown by measurements of the rate of decay of the elevated enzyme level in hydrocortisone-treated animals (*1*) and later confirmed by a crude isotopic analysis of turnover in the steady-state or basal condition (*2*), in which the enzyme level undergoes no change. These and subsequent measurements based on the rate of hormonal-induced change (*3*) have yielded half-life estimates ranging from 1.7 to 3.5 hours. The widely used inhibitors of synthesis of RNA and of protein (actinomycin D, puromycin, and cycloheximide) do not appreciably affect the basal level of this enzyme (*4*) except in some special circumstances (*5*), but these inhibitors are all effective in blocking changes in the rate of transaminase synthesis brought about by various hormones (*4*). The failure of actinomycin to lower the basal enzyme level has been interpreted as indicating that synthesis of this enzyme occurs on stable templates (*6*), but no explanation for the similar results with inhibitors of protein synthesis has been presented. The present experiments were done (i) to determine the rate of transaminase turnover in the basal condition with the increased precision that is now possible due to improved immunological techniques and (ii) to resolve the apparent discrepancy which arises in the failure of inhibitors of protein synthesis to lower the level of an enzyme which normally must be maintained by continual enzyme synthesis.

The data of Fig. 1 are from two experiments in which the normal rate of transaminase turnover was determined. Livers of adrenalectomized, fasted (18 hours) rats, killed at hourly intervals after a single injection of

^{14}C-leucine, were homogenized in 4 volumes of a mixture of 0.15*M* KCl and 0.001*M* ethylenediaminetetraacetate and the soluble fractions were collected after centrifugation at 100,000*g* for 60 minutes. Aliquots of each soluble fraction were assayed for tyrosine transaminase activity (*7*), protein content (*8*), and both acid-soluble and protein-bound radioactivity. For the latter determinations, duplicate 0.1-ml aliquots were pipetted onto filter paper disks. One set was washed to remove nonprotein radioactivity (*9*) and the other was counted directly. After correction for quenching, these duplicates yielded essentially identical count rates, reaffirming previous results which indicated that virtually no ^{14}C-leucine remains in the hepatic amino acid pool after 1 hour under these conditions (*2*). These measurements then constitute a "chase" analysis of turnover rate. Tyrosine transaminase was partially purified by passing each soluble fraction through a column of diethylaminoethyl cellulose (*7*), after which equal amounts (activity units) of the enzyme preparations were analyzed immunochemically (*10*). Briefly, this consists of precipitating the ^{14}C-labeled enzyme with antitransaminase serum after addition of sufficient unlabeled, purified carrier enzyme to form a measurable precipitate. The transaminase-free supernatant of this first precipitation is again treated with unlabeled carrier enzyme and antiserum to form a control precipitate (*11*), which gives a measure of the extent of contamination by coprecipitation of radioactive, nontransaminase proteins. In these experiments, radioactivity of the first precipitate ranged from 820 (1 hour) to 120 (5 hours) count/min, while that of the second was 20 to 50 count/min; the difference was taken as transaminase radioactivity. Since individual variation in the extent of labeling of the total soluble proteins is reflected in transaminase labeling, the enzyme radioactivity data are expressed relative to soluble protein radioactivity in Fig. 1.

The transaminase level remains essentially constant in these experiments, showing only the usual variation encountered in measurements of this metabolically labile enzyme. Radioactivity of the total soluble proteins is similarly constant, as expected from the slow turnover rate of the bulk of the liver proteins [half-life = about 3 days (*12*)]. Radioactivity of tyrosine transaminase, plotted in semilogarithmic fashion in Fig. 1, drops precipitously, with the experimental points generally falling into the expected first-order relationship. Analysis of these data by the least squares procedure yields a half-life of 1.5 ± 0.3 hours for rat liver tyrosine transaminase.

Measurements made during the 1st and 4th hours after treatment with the inhibitor of protein synthesis, cycloheximide, at a dose sufficient to block any of the hormonal effects on synthesis of this enzyme, are presented in Table 1. In agreement with previous reports, this inhibitor did not appreciably alter the transaminase level during this interval. With the rapid turn-

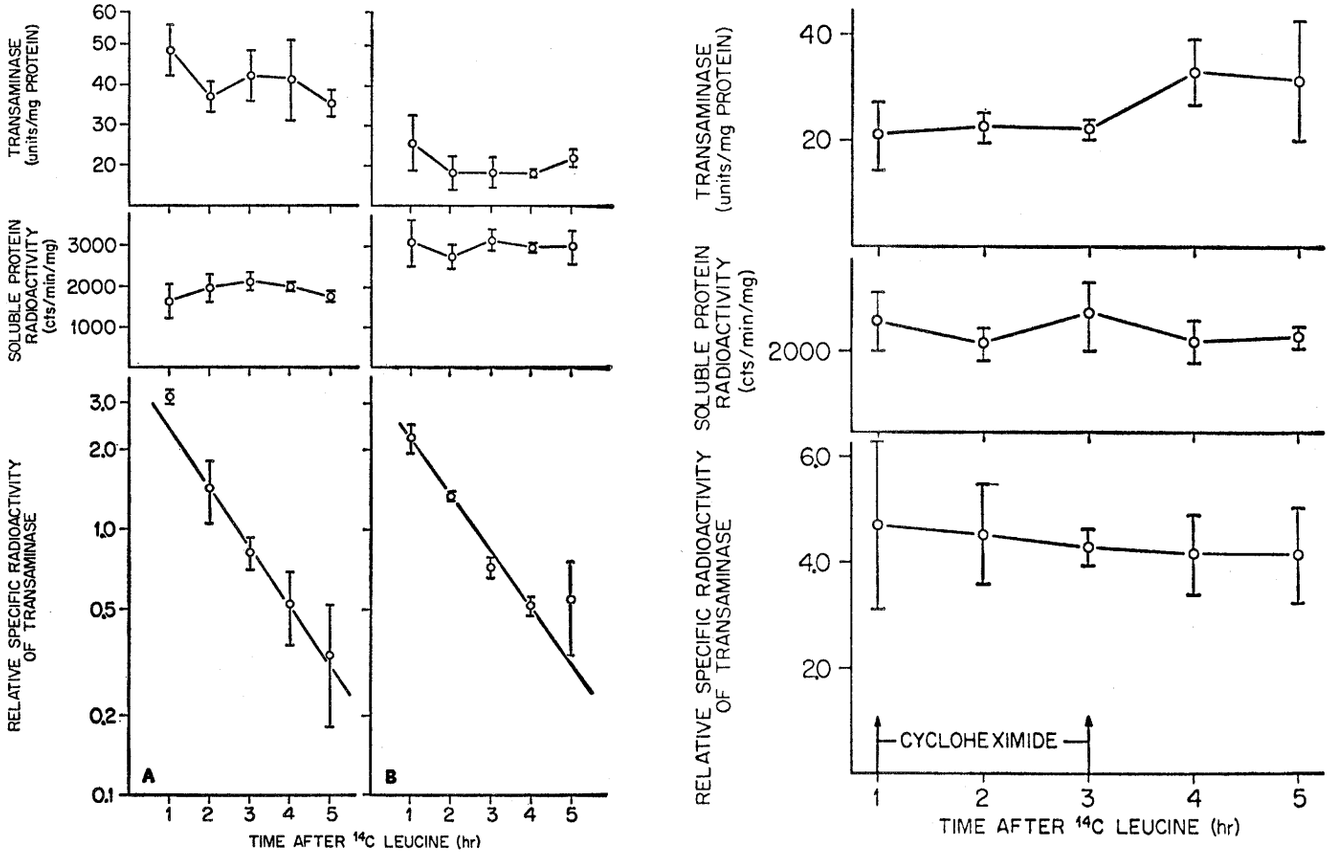

Fig. 1. (left). Turnover measurements of rat liver tyrosine transaminase in vivo. The data are the mean ± standard deviation for three animals at each point. Male rats were adrenalectomized and fasted for 24 hours before the experiments began. At zero time each rat was given 50 μc of ^{14}C-leucine intraperitoneally (200 mc/mmole in A, 275 mc/mmole in B); subsequent measurements are described in the text. Fig. 2 (right). Inhibition of turnover in cycloheximide-treated rats. Experimental details as in Fig. 1, except that animals killed after the first hour received cycloheximide (100 μg/100 g) intraperitoneally at 1 hour and again at 3 hours after ^{14}C-leucine.

Table 1. Rate of transaminase synthesis in cycloheximide-treated rats. Data are the mean ± range of experimental values for three or four animals. Male rats were adrenalectomized 24 hours before the experiment and fasted overnight in the second experiment but not the first. Those treated with cycloheximide were given 100 μg/100 g at the start of the experiment and again 2 hours later. In experiment 1, ^{14}C-valine (40 μc, 200 mc/mmole) was given 10 minutes before the animals were killed, and in experiment 2, 30 μc was given 20 minutes before. Abbreviation: cpm, counts per minute.

Expt. No.	Cycloheximide treatment (min)	Transaminase activity (units/mg protein)	Radioactivity	
			Soluble proteins (cpm/mg)	Transaminase (cpm)
1	Untreated	54 ± 15	524 ± 115	150 ± 30
1	240	43 ± 16	12 ± 5	7 ± 7
2	Untreated	47 ± 12	1090 ± 280	260 ± 80
2	50	52 ± 11	27 ± 9	5 ± 3
2	240	53 ± 6	69 ± 60	23 ± 20

over demonstrated above, it is clear that the failure of cycloheximide to depress the enzyme level must mean either that it does not, in fact, block transaminase synthesis or that both synthesis and degradation are inhibited to an equivalent extent. These rats were given ^{14}C-amino acids in a brief "pulse" exposure, the labeling time being short relative to the half-life of the enzyme. Under these conditions the contribution of turnover is negligible, and the extent of isotope incorporation into the enzyme is a measure of its rate of synthesis. Isolation methods used in these experiments were as described above, except that preliminary purification was done by heating liver soluble fractions (stabilized by addition of α-ketoglutarate or pyridoxal phosphate) rather than by ion exchange columns. The labeling data show clearly that transaminase synthesis was effectively blocked over virtually the entire period of cycloheximide treatment. Similar results were obtained when protein synthesis was blocked by puromycin (10 mg/100 g, every 2 hours) instead of cycloheximide.

With enzyme synthesis stopped, the transaminase level would be expected to drop to 10 to 15 percent of the control level if the normal degradative rate was maintained. Since the enzyme level was in fact unchanged, it can be concluded that degradation as well as synthesis must be blocked in the inhibitor-treated animals. This was confirmed by measurements of transaminase turnover in animals treated with cycloheximide after labeling of the enzyme had been completed. In Fig. 2 are presented combined data from two experiments similar to those of Fig. 1 except that cycloheximide was given at the 1st and 3rd hours after isotopic leucine. The variation is large (owing partially to combining data from separate experiments) but the trends are clear—there is no significant change in any of the parameters measured, including transaminase radioactivity. Thus inhibition of protein synthesis stops degradation of tyrosine transaminase as well as its synthesis.

This result clearly demonstrates that removal of the active enzyme in normal turnover is not due simply to the action of stable intracellular proteases, although such activity may ultimately be involved. These turnover measurements indicate only that the enzyme is removed as an enzymically and immunologically detectable entity; the nature of the reactions involved is unknown. One explanation which cannot be excluded from consideration is the possibility that the ribosomal mechanism for transaminase synthesis is able to function in both directions, effecting both the synthesis of the enzyme and its degradation. However, stabilization of the normally labile tyrosine transaminase by inhibition of protein synthesis is not a reflection of a general facet of liver metabolism, since other rat liver enzymes which undergo rapid turnover are not stabilized under these conditions (*6, 13*). This element of specificity leads me to suggest that transaminase turnover may require the participation of a specific polypeptide, which could either catalyze or be a reactant in the process leading to removal of the enzyme. Such a component would necessarily have an extremely short half-life, for transaminase removal ceases very quickly when protein synthesis is stopped.

Grossman and Mavrides (*3*) have recently presented data which suggest that the removal of tyrosine transaminase following hydrocortisone induction represents a specific inactivation. This is not inconsistent with my results, and it implies that the synthetic mechanism for transaminase removal may play an important role in modulating the response of the enzyme level to changes in enzyme synthesis brought about by various hormones.

Phenomena similar to that described here have also been observed in nonmammalian systems. Thus, disappearance as well as synthesis of uridine diphosphate–galactose polysaccharide transferase in *Dictyostelium discoideum* is blocked by cycloheximide (*14*). Becher et and Wiame have suggested the stoichiometric operation of a specific binding protein in the inactivation of yeast ornithine transcarbamylase which results when synthesis of this enzyme is repressed by arginine (*15*). This suggestion, too, was based partially on the finding that removal of the enzyme was blocked by cycloheximide, but in these experiments it was clear that a reversible inactivation process is involved in removal of the enzyme.

It is apparent that the participation of synthetic events in enzyme turnover adds uncertainty to experiments involving the effects of inhibitors of protein or nucleic acid synthesis on enzyme levels, from which information on either enzyme or template stability is deduced. For example, the stability of tyrosine transaminase in actinomycin-treated rats may reflect a dual inhibition of both synthesis and removal, instead of the stability of the transaminase template as has been assumed. The demonstration that small doses of either cycloheximide or actinomycin lead to increased transaminase levels after 12 to 24 hours (*5*) lends credence to this possibility, for these effects could be interpreted as due to a differential sensitivity of the synthesis of the macromolecules involved in forming the transaminase and those required for its removal. Small differences in sensitivity could lead to marked changes in the enzyme level over a period of time encompassing several half-lives.

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Blastomyces dermatitidis: Production of the Sexual Stage

Abstract. *Growth of five combinations of strains of* Blastomyces dermatitidis *in paired cultures on yeast-extract agar, plus bone meal, yielded the perfect stage for the first time. These cleistothecia, apparently of a new genus, produced eight spored asci. Monoascospore cultures developed hyphae and conidia typical of* B. dermatitidis. *Mice were successfully infected with inocula from a monoascospore culture.*

The pathogenic fungus *Blastomyces dermatitidis* and the disease it causes, North American blastomycosis, are of medical, mycological, and general interest. The life history of the fungus and its taxonomic position are inadequately known (*1*) and, in spite of reported isolation of the pathogen from nature (*2*), it is generally recognized that the reservoir of infection has yet to be demonstrated (*2, 3*). Our report of the discovery of the sexual stage should contribute to solution of these problems and open up a new field of study of the genetics of this dimorphic, pathogenic organism.

First indications of sexuality were observed during study of the growth characteristics of paired strains of *B. dermatitidis* (*4*). All possible combinations of 34 isolates (595 pairings) were made at 25°C on Sabouraud's dextrose agar in sealed, plastic petri dishes. No sexual structures were seen, but 185 of the plates developed a raised type of growth at the juncture of the two colonies. The ridge-forming pairs were replated on cornmeal agar, a medium that had been employed effectively by Emmons in stimulating cleistothecial formation by *Allescheria boydii* (*5*). Sterile cleistothecia-like structures resulted from six different pairings in separate cultures. These irregularly shaped structures were characterized by being produced near the line of juncture of the two colonies, and by the presence of radiating hyphae in the form of spiral coils reminiscent of the coiled appendages observed in the cleistothecia of some members of the Gymnoascaceae (*6*). Such sterile cleistothecia-like bodies were also obtained in cultures on yeast-extract agar (*7*) with either horse hair or bone meal added.

More recently we have grown fertile cleistothecia at will, using yeast-extract agar (*7*), with or without antibiotics, and bone meal. Yeast-extract agar was used because Smith (*7*) found that it supported slight mycelial growth while stimulating spore production. Bone was used because our own unpublished experiments had demonstrated that it stimulated growth of *B. dermatitidis*. In preparing the medium, steamed bone meal (Armour) is pulverized, sterilized with ethylene oxide, and placed along the midline of a plate of yeast-extract agar before the two inocula for each pairing are placed 2 cm apart near the center of the plate and equidistant from the bone meal.

At 25°C, fruiting bodies become visible by the unaided eye within 2½ to 5 weeks. The most conspicuous structures characterizing these cleistothecia at all ages are thick-walled, tightly coiled spirals radiating from a common source at the base of the young ascocarp (Fig. 1A). In later stages the coils become obscured to some extent by hyphae that arise, at least for the most part, as branches from the coils (Fig. 1, B and D). Dark-field illumination, however, makes it easier to follow the course of the coil in the fruiting body (Fig. 1C).

The offshoots of the spiral hyphae, which become much more abundant in the outer part of the ascocarp as it matures, consist of frequently branched thin-walled hyphae, the cells of which are constricted where they meet at the cross walls. A group of these cells resembles the spongy parenchyma of higher plants.

A mature cleistothecium (Fig. 1D) is 200 to 350 μ in diameter and has a tan color resulting from a pigment located in the coils and in the thin-walled spongy tissue–like cells. Usually young fruiting bodies contain many clusters of ascogenous hypae which are most abundant at the proximal ends of the coils, near the lower-central part of the ascocarp. It appears that the distal ends of the spiral hyphae are forced outward by pressure exerted by growing intercoil cells.

Mature asci contain eight smooth, spherical, hyaline or very light tan, uni-

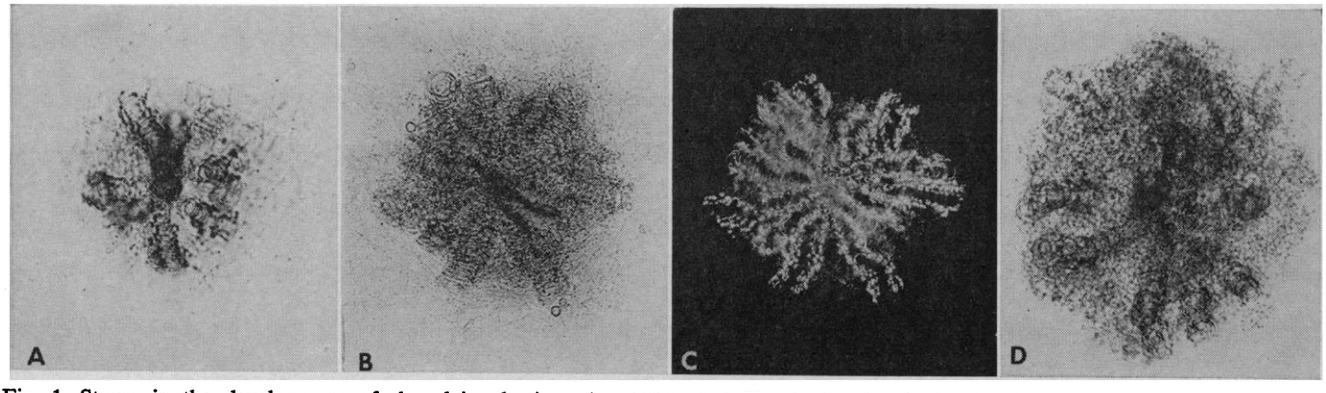

Fig. 1. Stages in the development of the cleistothecium (× 200). (A) Young fruiting body showing characteristic tightly coiled spirals. (B) An intermediate stage of the ascocarp. (C) Same intermediate stage but with dark-field illumination. (D) A young but fully developed cleistothecium containing ascospores. In some instances the coils can still be followed to their place of origin. [Stanislaus Ratajczak, Biology Department, Marquette University]