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Protein Turnover and the Control of Enzyme Levels in Animal Tissues

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Regulation of cellular processes is essential to life. Successful regulation is achieved, as the biochemist sees it, by variation of the rates of specific chemical reactions. Inasmuch as most significant biochemical reactions depend on catalysis by enzymes, a large measure of control can be effected by regulating the levels of enzyme protein in the cell. This type of regulation, as studied in the author's laboratory, is the subject of this Account.

Microbial systems are especially suitable to provide information about the regulation of enzyme concentrations, and most of what we know is derived from studies of them.[2a] In mammals, enzyme concentrations may also undergo changes in response to various stimuli. However, the regulation requirements of uni- and multicellular organisms are different, and it turns out that the regulatory systems which have evolved are more complex for higher organisms.

In bacteria, the regulatory mechanisms are seemingly geared to achievement of a maximal rate of cell proliferation consistent with available nutritional sources. Enzyme levels are controlled by the rate of synthesis of the messenger RNA (mRNA) for that enzyme.[2b] In contrast, the individual cell of a multicellular organism is usually dividing more slowly, if at all. Also, it is generally specialized in structure and function and in its relationship to similar and dissimilar cell types. In such specialized cells, organized into tissues, organs, and the whole animal, new regulatory problems have arisen, and new solutions have been found or superimposed on those mechanisms common to all organisms.

Among the regulatory problems encountered by higher organisms are those involved in the communication among diverse cell types that is essential for the integrated functioning of the entire organism. One solution to this problem is the complex system of hormones, many of which are important in regulating the content of specific enzymes in specific target tissues. Another problem is that of how to effect changes in the metabolic machinery, *i.e.*, in specific enzymes, in response to environmental and nutritional changes or as part of a developmental sequence. Such changes include removal of unneeded enzymes as well as synthesis of those newly required. In bacteria, the removal process can occur by dilution during phases of rapid growth. In animal tissues, where little cellular division takes place, the process of intracellular protein degradation becomes increasingly significant as a means of removing unneeded metabolic machinery and for controlling enzyme levels.[3]

The interaction between synthesis and degradation may have different overall consequences in animal tissues and in bacteria, as sketched in Figure 1. In bacteria, the total enzyme activity in a culture increases following the introduction of an inducing agent, such as a particular substrate, an increase that results from *de novo* enzyme synthesis. In animal tissues, also, enzyme levels can be increased by hormones, changes in diet, and administration of various other agents, including drugs, or specific substrates. However, in contrast to the stability of induced enzymes in exponentially growing bacteria, enzyme activities in animal tissues return to basal levels once the stimulus is removed as a result of metabolism, excretion, etc.

Such changes in mammalian tissues do not reflect simply activation and inactivation of the enzyme protein, since agents that inhibit protein synthesis can prevent the increases in enzyme activity.[4] More convincing are studies using combined immunologic and radioisotope techniques in demonstrating both increased content of immunologically reactive protein and an active net uptake of isotopic amino acids into specific enzyme proteins[5,6] (see below).

Most important, and central to an understanding of the dynamics of enzyme regulation in mammalian tissues, is the fact that changes in enzyme levels take place against a background of continual synthesis and

(1) Recipient of the 1969 American Chemical Society Award in Enzyme Chemistry, sponsored by Chas. Pfizer & Co., Inc.

(2) (a) F. Jacob and J. Monod, *J. Mol. Biol.* **3**, 318 (1961); (b) W. Gilbert and B. Muller-Hill, *Proc. Natl. Acad. Sci., U. S.*, **58**, 2415 (1967).

(3) R. T. Schimke, *Bull. Soc. Chim. Biol.*, **48**, 1009 (1966).

(4) O. Greengard, M. A. Smith, and G. Acs, *J. Biol. Chem.*, **238**, 1548 (1963).

(5) J. P. Jost, E. A. Khairallah, and H. Pitot, *ibid.*, **243**, 3057 (1968).

(6) F. T. Kenney, *ibid.*, **237**, 1610 (1962).

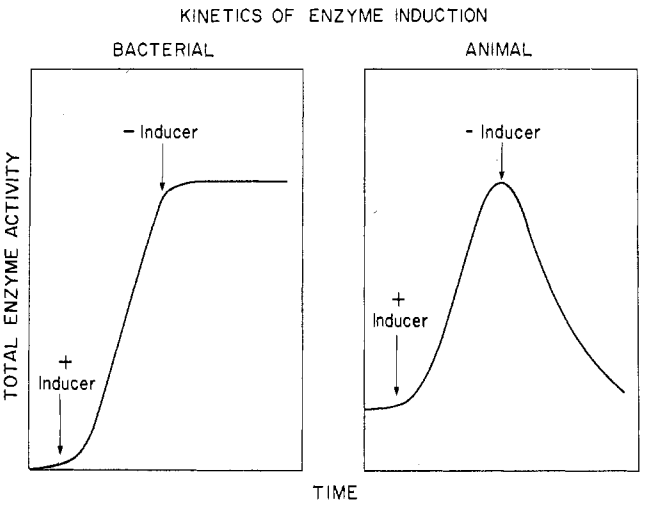

Figure 1. Schematic time courses of enzyme induction in bacteria and animal tissues.

degradation of protein, *i.e.*, turnover, as documented so elegantly by Schoenheimer and his coworkers in the early 1940's[7] and studied more recently in other laboratories.[8,9]

Properties of Protein Turnover in Rat Liver

Since regulation of enzyme levels takes place against a background of continual synthesis and degradation, certain properties of this overall process are presented as a basis for subsequent discussions.

Studies from a number of laboratories have shown that replacement of liver protein is rapid and extensive. One such experiment, designed to answer questions about the rate and extent of turnover of total liver protein and one specific enzyme, arginase, is shown in Figure 2.[10] Rats were fed an amino acid diet containing [^{14}C]L-lysine of a constant specific activity for up to 28 days. The rate and extent of protein turnover were estimated from how rapidly and to what extent total cellular protein and arginase were replaced from the dietary source. As shown in Figure 2, the free lysine pool (counts soluble after trichloroacetic acid precipitation of protein) approached maximal labeling in 24 to 36 hr. Lysine incorporation into total liver protein (counts per minute per milligram of protein) was initially rapid, and then slowed markedly after 5–6 days. The rate of incorporation of lysine into arginase (total counts per minute in enzyme precipitated with a specific antiarginase antibody) was slower.

A measure of the extent to which the cellular proteins are replaced can be obtained by comparing the specific activity of the lysine in the protein with that of the dietary source as shown in Table I. The specific radioactivity of the lysine isolated from the original diet was 1105 cpm/μmol. After 20 days of labeling, approximately 75% of the lysine residues of total liver had been replaced, *i.e.*, the specific activity of the lysine of liver protein was 829 cpm/μmol. In addition, virtually all of the lysine of arginase had been replaced in this time.

Table I

Specific Activity of [^{14}C]L-Lysine Isolated from Total Liver Protein and Arginase Following 20 Days of Continuous Administration of [^{14}C]L-Lysine Diet[a]

Source of lys	% radioactivity recovered as lys	Lys spec act. cpm/μmol	% replacement of lys
Diet	98	1105	..
Total liver protein	95	829	75
Arginase	66	1006	91

[a] The liver from three rats maintained on a [^{14}C]L-lysine diet for 20 days as described in Figure 2 was pooled and arginase purified as outlined elsewhere.[10] [^{14}C]L-lysine of the purified arginase and a sample of all discarded protein (total liver protein), as well of the initial [^{14}C]L-lysine amino acid mixture, was isolated following hydrolysis in 6 *N* HCl by chromatography on Amberlite CG-50 (NH_4^+ form) columns.

From this experiment we can conclude the following. (1) The replacement of protein in rat liver is extensive and rapid. At least 50% of the protein is replaced in 4–5 days. Similar conclusions have been drawn by Swick[8] and Buchanan.[9] (2) The majority of the protein degradation is intracellular, rather than intercellular. The lifespan of cells in liver is from 160 to 400 days.[9] It follows, then, that since most of the liver protein is replaced within 20 days, the degradation that occurs must be largely intracellular rather than the result of cell replacement. (3) There is a marked heterogeneity of degradation rate constants of different cell organelles and individual intracellular enzymes. This is already evident from the experiment of Figure 2, since arginase is replaced more slowly than a large portion of the protein.

Table II shows the heterogeneity of degradation rate constants of several cell fractions and organelles as isolated by standard centrifugation techniques. Of particular interest is the finding that the proteins of the membrane fractions, *i.e.*, smooth and rough endoplasmic reticulum and the plasma (cell surface) membrane, are in the greatest state of flux, with a mean half-life of about 2 days. There is an even more marked heterogeneity of degradation rate constants among individual enzymes (Table II). There is no correlation between the intracellular localization of an enzyme and its half-life. Thus, δ-aminolevulinate synthetase has a half-life of only 1 hr, but is localized on mitochondria ($t_{1/2}$ 6–7 days). Likewise, the range of half-lives of soluble proteins is from 1.5 hr (tyrosine transaminase) to 5 days (arginase).

Theoretical Formulation of Changing Enzyme Levels

In view of the fact that there is a continual synthesis and degradation of essentially all proteins of liver, any formulation must consider both synthesis and degradation. Thus, a change of an enzyme level can be expressed by eq 1, where E is the content of enzyme (units

$$dE/dt = k_s - k_dE \quad (1)$$

(7) R. Schoenheimer, "Dynamic State of Body Constituents," Harvard University Press, Cambridge, Mass., 1942.
(8) R. W. Swick, *J. Biol. Chem.*, **231**, 751 (1958).
(9) D. L. Buchanan, *Arch. Biochem. Biophys.*, **94**, 501 (1961).
(10) R. T. Schimke, *J. Biol. Chem.*, **239**, 3808 (1964).

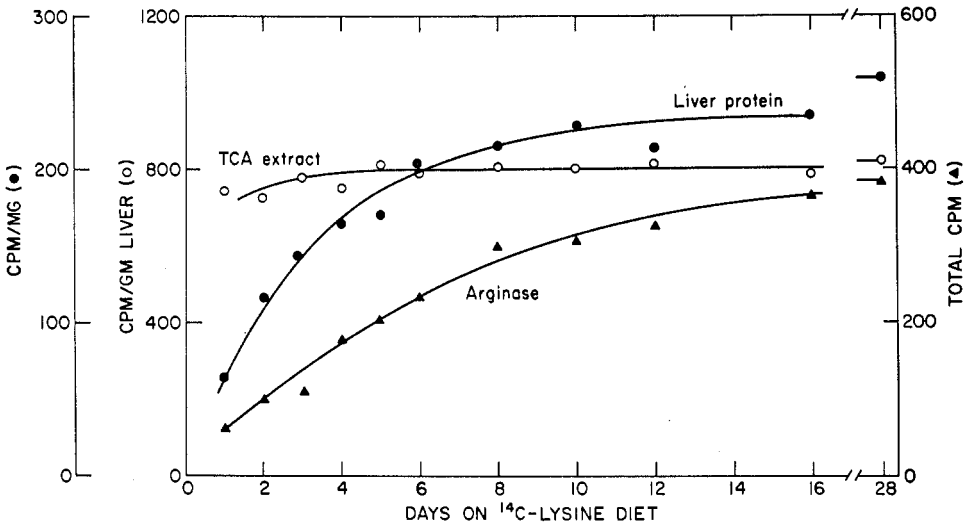

Figure 2. Incorporation of continuously administered [^{14}C]L-lysine into total protein, arginase, and trichloroacetic acid soluble extracts of rat liver. Osborne–Mendel rats, weighing 250 to 275 g each, were maintained for 7 days on a diet consisting of 25% complete amino acid mixture. Following a 12-hr period without food, they were placed on a diet containing [^{14}C]L-lysine (specific activity 1100 cpm/μmol). At the intervals specified, one rat was killed; the liver was then removed and divided into two weighed portions. One sample was made into acetone powder from which arginase was isolated by immunological techniques; the other was treated with 10% Cl_3CCOOH and divided into protein and supernatant fractions. Radioactivity of the trichloroacetic acid soluble counts is expressed as counts per minute per extract from 1 g of liver, wet weight (○—○). Counts in total liver protein are expressed as counts per minute per milligram of protein (●—●). Counts in the arginase represent the total number of counts precipitated (▲—▲). Data from ref 10.

Table II
Half-Life Estimates of Subcellular Fractions and Specific Enzymes of Rat Liver

Fractions	Mean half-life, days	Localization	Ref
Nuclear	5.1		
Mitochondrial	6.8		
Supernatant	5.1		*a*
Smooth endoplasmic reticulum	2.1		
Rough endoplasmic reticulum	2.0		
Plasma membrane	1.8		
Enzymes			
δ-Aminolevulinate synthetase	0.04	Mitochondrial	*b*
Alanine aminotransferase	0.7–1.0	Mitochondrial	*c*
Catalase	1.2	Peroxisomal	*d*
Tyrosine aminotransferase	0.06	Soluble	*e*
Tryptophan oxygenase	0.10	Soluble	*f*
Glucokinase	1.25	Soluble	*g*
Arginase	4–5	Soluble	*h*
Glutamic–alanine transaminase	2–3	Soluble	*i*

[a] I. Arias, D. Doyle, and R. T. Schimke, *J. Biol. Chem.*, **244,** 3303 (1969). [b] H. S. Marver, A. Collins, D. P. Tschudy, and M. Rechcigl, Jr., *ibid.*, **241,** 4323 (1966). [c] R. W. Swick, A. K. Rexroth, and J. L. Stange, *ibid.*, **243,** 3581 (1968). [d] V. E. Price, W. R. Sterling, V. A. Tarantola, R. W. Hartley, and M. Rechcigl, Jr., *ibid.*, **237,** 3468 (1962). [e] F. T. Kenney, *Science*, **156,** 525 (1967). [f] P. Feigelson, T. Dashman, and F. Margolis, *Arch. Biochem. Biophys.*, **85,** 478 (1959). [g] H. Niemeyer, *Natl. Cancer Inst. Monograph*, **27,** 29 (1967). [h] See ref 10. [i] H. L. Segal and Y. S. Kim, *Proc. Natl. Acad. Sci., U. S.*, **50,** 912 (1963).

× mass^{-1}), k_s is a zero-order rate constant of synthesis[11] (units × time^{-1} × mass^{-1}), and k_d is a first-order rate constant for degradation[12] (time^{-1}). In general there is little, if any, change in total mass of a tissue, *e.g.*, liver, during an experimental time period, and consequently an expression for a change in total tissue mass is not included.

In the steady state, *i.e.*, when $dE/dt = 0$, then

$$k_s = k_d E \tag{2}$$

and

$$E = \frac{k_s}{k_d} \tag{3}$$

Thus, in the steady state the amount of enzyme is a function both of the rate of synthesis and the rate of degradation. An alteration in either rate can affect the level of E.

Consider, now, the time course of change in enzyme level that may result from various hormonal, nutritional, or physiological manipulations or treatments, where k_s is changed to k'_s and k_d is changed to k'_d.

The time course describing the approach of E to a new steady state, defined by the new values k'_s and k'_d, is given by eq 4, where E_t is the activity at any time

$$\frac{E_t}{E_0} = \frac{k'_s}{k'_d E_0} - \left(\frac{k'_s}{k'_d E_0} - 1\right) e^{-k'_d t} \tag{4}$$

t and E_0 is the enzyme activity under steady-state conditions defined by k_s and k_d. As written, if E_0 were taken as 1, the equation would represent the "fold" increase in enzyme activity, an expression commonly

(11) The rate of synthesis of a specific protein is determined by a number of factors, including the number of ribosomes, amount of messenger RNA, levels of amino acids and tRNA, availability of initiation and transfer factors, etc. In this simplified model the separate roles of such variables have not been factored, since they are largely unknown in mammalian tissues. Hence, all such variables have been included under a general notation of a rate of enzyme synthesis.

(12) The rate of degradation of a protein is expressed in terms of a first-order rate constant because, in all cases studied in liver, the degradation of specific intracellular proteins has followed first-order kinetics. Rate constants of degradation are often expressed in terms of a half-life where $t_{1/2} = \ln 2/k_d$.

used in studies on mammalian tissues. Equation 4 shows that, although a new steady state is defined by the new values for k'_s and k'_d, the time course of change from one steady state to another is determined only by the rate constant of degradation, k'_d.

Several theoretical conclusions can be drawn from this formulation. (1) Whereas, in bacteria, changes in enzyme level involve an acceleration in the rates of synthesis, increases of enzyme levels in mammalian tissues may result from two general mechanisms: (a) an increase in the rate of enzyme synthesis and/or (b) stabilization of existing enzyme in the presence of continued enzyme synthesis. (2) The fact that an agent causes an increase in the activity of one enzyme relative either to total protein or to another enzyme at some finite time does not constitute proof of a specific effect on that enzyme. Thus, as indicated in eq 4, the time course of approach to a new steady state is solely a function of the rate constant of degradation. Since there is a great heterogeneity of values for k_d among different proteins, as discussed previously, the time course of increase in activity of each enzyme will vary, even if the rates of synthesis of all proteins are increased to the same extent. The significance of these generalizations will be illustrated with several experimental systems.

Control of Synthesis and Degradation of Specific Enzymes

In studying the mechanisms involved in changes of enzyme levels, the first question to be answered is whether the differences in assayable enzyme activity result from differences in content of enzyme protein. Once this has been established, the question of whether the rate of synthesis, the rate constant of degradation, or both have been altered can be answered. We have attempted to answer these questions as directly as possible by isolating the specific enzyme protein in question. Generally this has involved the use of immunologic techniques as adjuncts to isolation and purification of the protein.

Nutritional Control of Liver Arginase in Steady-State Conditions. As is the case with many liver enzymes that are involved in the catabolism of amino acids,[13] a direct relationship exists between the levels of such enzymes and the caloric intake provided in the form of protein. Among such enzymes are those of the urea cycle, *i.e.*, carbamyl phosphate synthetase, ornithine transcarbamylase, argininosuccinate synthetase, argininosuccinase, and arginase.[14] We have studied arginase particularly because it can be purified readily to a homogeneous state and is capable of eliciting precipitating antibodies when administered to rabbits. As shown in Table III, there is a 2–3-fold difference in the specific activity of liver arginase between animals maintained 14 days on a diet containing either 8 or 70% of the diet by weight. That this difference in activity results from a difference in the amount of enzyme protein is supported by studies using an antibody specific for arginase. The arginase used to immunize rabbits was purified to the point of homogeneity as indicated by sedimentation velocity studies in an analytical ultracentrifuge and by the presence of a single protein band on acrylamide gel electrophoresis. The antiserum obtained specifically precipitates only arginase.

Table III
Effect of Diet on Steady-State Levels, Synthesis, and Degradation of Rat Liver Arginase[a]

Diet	Activity, μmol/g wet weight × 10^{-3}	Half-life,[b] days	k_d, day^{-1}	k_s, units/g per day × 10^{-3}
8% casein	20.2 ± 1.0	5.2	0.13	2.6
30% casein	36.7 ± 1.3	4.8	0.14	5.2
70% casein	56.1 ± 1.1	4.6	0.15	8.4

[a] Data from ref 10. [b] See Figure 3.

Figure 3 shows a typical immunotitration of the antiserum with highly purified enzyme (5480 units/mg), as well as with crude liver extracts with specific activities varying by 4-fold (10 and 39 units/mg of protein). These crude extracts were from livers of animals maintained on 8 or 70% dietary protein, respectively. The amount of enzyme activity neutralized (precipitated) by a constant amount of antiserum was the same, despite the fact that the total amount of protein added varied over a 500-fold range in the three arginase preparations. Furthermore, as indicated by the amount of protein precipitated, the precipitation reactions were quantitatively similar with the highly purified enzyme and with the crude extracts of differing specific activities. These results, then, demonstrate that the differences in the amount of assayable enzyme activity in livers of animals maintained on diets with differing protein contents represent differences in the amount of enzyme protein as determined immunologically.

The next question to be answered, then, is whether the difference in enzyme content resulting from maintaining the animals on diets with differing proportions of protein results from a more rapid rate of synthesis, or a less rapid rate of degradation, *i.e.*, from eq 3, is k_s or k_d altered? This question was answered by determining the values for k_d at three different steady-state levels of arginase. In this experiment, the details of which are given in the legend to Figure 4, animals were pulse labeled with [^{14}C]guanidinoarginine, and thereafter the decay of radioactivity of labeled total protein and arginase was determined with time. The degradation rate constant of arginase, here indicated by a half-life value, is essentially the same in the three steady states, *i.e.*, $t_{1/2}$ = 5 days. The calculations of Table III indicate that variations in dietary protein content affect the rate of enzyme synthesis, rather than the rate of enzyme degradation.

Hormonal and Substrate Control of Tryptophan Oxygenase (Pyrrolase). As studied extensively by

(13) W. E. Knox and O. Greengard, *Advan. Enzyme Regulation*, **3**, 247 (1965).
(14) R. T. Schimke, *J. Biol. Chem.*, **237**, 459 (1962).

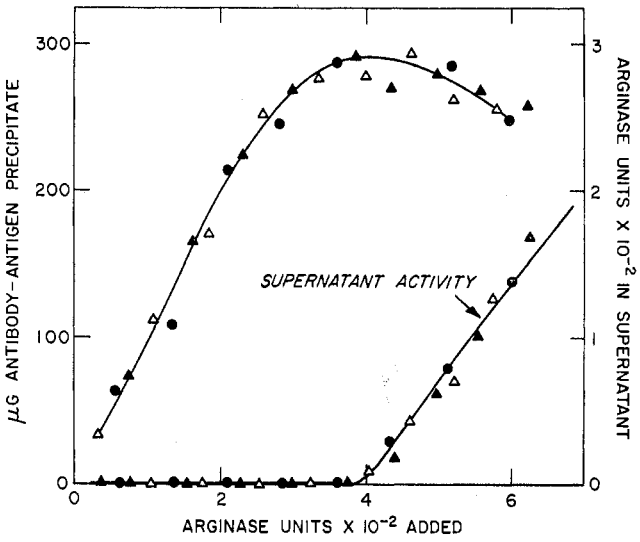

Figure 3. Quantitative precipitin reactions of purified arginase and liver extracts. Arginase preparations were of three sources: (a) purified arginase (specific activity 5480 units/mg), ▲—▲; (b) a crude arginase preparation from animals maintained on 8% dietary casein (specific activity 10 units/mg), Δ—Δ; (c) a crude arginase from animals maintained on 70% dietary casein (specific activity 39 units/mg of protein), ●—●. Volumes and protein concentrations were made constant by equalizing the specific activity of all preparations by addition of suitable amounts of bovine serum albumin. See ref 10 for details.

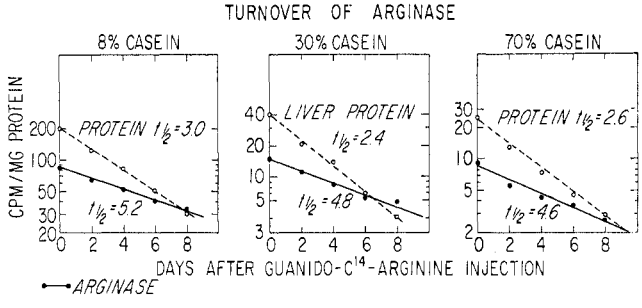

Figure 4. Turnover of total liver protein and arginase determined by the decay of radioactivity after single administration of [^{14}C]guanidino-L-arginine. Following maintenance of 20 rats for 14 days on each of three diets containing 8, 30, or 70% casein, each rat was given a single intraperitoneal injection of 25 μCi of [^{14}C]guanidino-L-arginine. One hour later, and at 2-day intervals, four animals from each dietary group were killed and the four pooled livers were subjected to purification of arginase. The protein fractions discarded during the arginase purifications were pooled to constitute total liver protein. Results are expressed as counts per minute per milligram of protein. O----O, total liver protein; ●—●, arginase. See ref 10 for details.

Knox and his collaborators[15] as well as by Feigelson and his group,[16] the activity of tryptophan oxygenase can be increased by the administration of either hydrocortisone and other glucocorticoids, or by tryptophan or certain tryptophan analogs. We became interested in the mechanisms of the hormonal and substrate induction of tryptophan oxygenase because induced enzyme levels returned to basal levels with a half-life of 2–3 hr, suggesting both rapid synthesis and degradation. Summarized below are certain experiments supporting the thesis that the accumulation of tryptophan oxygenase following hydrocortisone (hormone) results from an increased rate of enzyme synthesis, whereas the major effect of the substrate is to prevent enzyme degradation in the presence of continued enzyme synthesis.[17]

In order to assess the effect of hydrocortisone and tryptophan on the rate of enzyme synthesis (k_s), the short-term incorporation of [^{14}C]L-leucine into tryptophan oxygenase was studied. Hydrocortisone or tryptophan, or both, were administered to rats for varying time periods, followed by [^{14}C]leucine. Extracts of livers were prepared 40 min after isotope administrations and enzyme was isolated by means of an antibody that specifically precipitates tryptophan oxygenase. These results are summarized in Table IV. With no treatment a total of about 1400 cpm was incorporated into tryptophan pyrrolase of livers of two animals during a 40-min period. When hydrocortisone had been administered 4 hr previously, and the enzyme level had increased 3-fold, total incorporation into tryptophan oxygenase increased some 4-fold (5640 cpm). After repeated doses of hydrocortisone at 4-hr intervals for 12 hr, the extent of incorporation during a 40-min period remained about 4-fold greater than with no treatment at a time when the accumulation of enzyme was seven times that of the control. Tryptophan administration, in contrast, increased only slightly the short-term incorporation (1620 cpm *vs.* 1400 cpm) into enzyme, although enzyme level increased 2-fold. Hydrocortisone plus tryptophan increased the incorporation in untreated animals about 5-fold. These changes in extent of isotope incorporation into tryptophan oxygenase are to be contrasted with the lack of comparable effects with total protein. This contrast indicates the hydrocortisone effect to be highly specific. These results,

Table IV

Incorporation of (40-min) [^{14}C]Leucine into Rat Liver Tryptophan Oxygenase[a]

Treatment	Enzyme act., units/g of liver	[^{14}C]Leu incpn — Tryptophan oxygenase, total cpm	[^{14}C]Leu incpn — Supernatant protein, cpm/mg
None	4.2	1368	1190
Hydrocortisone			
4 hr	13.6	5640	1320
12 hr	31.4	6502	1491
Tryptophan			
4 hr	8.2	1620	1564
12 hr	14.1	1670	1165
Hydrocortisone + tryptophan			
4 hr	28.3	7680	1491
12 hr	72.0	7280	1018

[a] Rats were given repeated doses of hydrocortisone or L-tryptophan, or both, at 4-hr intervals for the times indicated. Each rat was given, 40 min before death, a single intraperitoneal injection of 20 μCi of [^{14}C]leucine in 1.0 ml of 0.85% NaCl. Results of [^{14}C]L-leucine incorporation into tryptophan oxygenase are reported as total net counts per minute in the precipitate from the total DEAE-cellulose extract of two rats. See ref 17 for details.

(15) W. E. Knox and M. M. Piras, *J. Biol. Chem.*, **242**, 2959 (1967).
(16) P. Feigelson, M. Feigelson, and O. Greengard, *Recent Progr. Hormone Res.*, **18**, 491 (1962).
(17) R. T. Schimke, E. W. Sweeney, and C. M. Berlin, *J. Biol. Chem.*, **240**, 322 (1965).

then, indicate that hydrocortisone increased the rate of synthesis of tryptophan oxygenase about 4- to 5-fold, whereas tryptophan, which also results in the accumulation of enzyme, does not so stimulate its synthesis.

Evidence that tryptophan prevents the breakdown of the active, immunologically reactive enzyme is shown in Figure 5. In this experiment, liver proteins were prelabeled by the administration of [^{14}C]leucine 60 min before the time indicated as zero. We have found that, within 40 min after the administration of a single dose of [^{14}C]leucine, incorporation of radioactivity into total liver protein is essentially complete, and therefore any protein synthesized after this time will be derived from unlabeled amino acids. In control animals the amount of enzyme activity and radioactivity present in total protein remained essentially constant over a 9-hr period. However, the radioactivity present in prelabeled enzyme decreased progressively. This shows that there is a continual degradation of the enzyme under basal conditions. When tryptophan was administered, on the other hand, total enzyme increases and there was no decrease in the total amount of prelabeled enzyme. Thus, the substrate, tryptophan, resulted in accumulation of enzyme in large part by preventing degradation of the enzyme in the presence of continued synthesis.

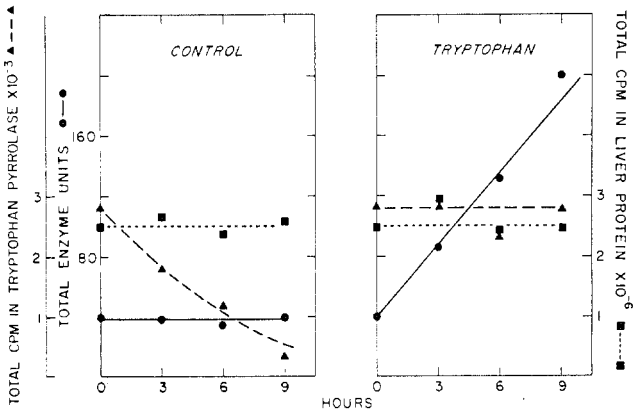

Figure 5. Effect of L-tryptophan administration on the loss of tryptophan oxygenase (pyrrolase) prelabeled with [^{14}C]L-leucine. Rats were given single injections of 20 μCi of [^{14}C]L-leucine. Sixty minutes later, two animals were killed. The remainder were given 10 ml of 0.85% NaCl or 10 ml of 0.85% NaCl containing 150 mg of L-tryptophan. These injections were repeated in the remaining animals after 4 and 8 hr. At the times specified, the livers of two animals in each group were removed and frozen immediately. At the end of the experiment extracts of the livers were prepared, and the radioactivity that was incorporated into tryptophan oxygenase and protein was determined. The values given are for totals of combined extracts of two animals. ●—●, enzyme activity; ▲---▲, total radioactivity in protein precipitated by the tryptophan oxygenase antiserum; ■----■, radioactivity in total cellular protein. See ref 17 for details.

Effects of Degradation Rate Constants on the Response of Enzymes to Altered Rates of Enzyme Synthesis

The heterogeneity of degradation rate constants of individual enzymes is of significance in considering the rapidity and specificity of response to agents which increase rates of enzyme synthesis. Thus, several enzymes of rat liver increase in quantity following corticosteroid administration, and for them an increased rate of enzyme synthesis has been implicated. Among such enzymes, tryptophan oxygenase and tyrosine transaminase respond with up to 8-fold increases 4 hr after a single administration of cortisone, whereas glutamic–alanine transaminase and arginase increase very little in this time (see Figure 2). Such results suggest that cortisone specifically stimulates the synthesis of the two enzymes which respond rapidly and dramatically to the glucocorticoid. However, as discussed previously, the time course of change of an enzyme will depend on its rate constant of degradation (see eq 4), and hence the apparent specificity can be explained by the marked differences in the rate constants of degradation.

As shown in Figure 6, when cortisone is administered repeatedly for 4 days, increased levels of arginase and glutamic–alanine transaminase, as well as tryptophan oxygenase and tyrosine transaminase, occur. From these data and a knowledge of the half-lives of these enzymes and the basal amount of enzyme, estimates of the relative effects of cortisone in increasing rates of synthesis of all four enzymes can be made (Table V). In the second column of Table V are listed estimates of the half-lives as obtained by a variety of techniques. In the third column are given enzyme levels under basal or

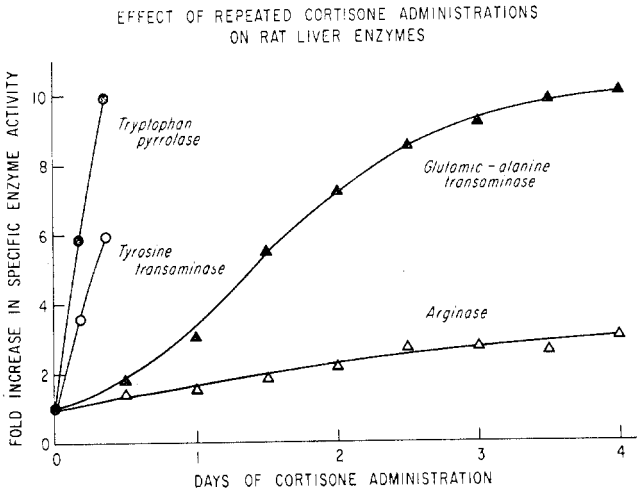

Figure 6. Time course of the increase in tryptophan pyrrolase, tyrosine–glutamic transaminase, glutamic–alanine transaminase, and arginase with cortisone administration. Animals received 10 mg of cortisone acetate every 8 hr intramuscularly. Each value is the mean of three animals. See C. M. Berlin and R. T. Schimke, *Mol. Phamacol.*, **1**, 149 (1965), for details.

steady-state conditions. From the half-life and the basal enzyme level, a rate of synthesis can be calculated (eq 3). The rate of synthesis during cortisone administration is estimated from the earliest linear portion of the accumulation of enzyme activity as shown in Figure 6.

In the last column of Table V are the ratios of rates of synthesis with cortisone to those under basal conditions. These ratios are very similar, varying from 4 to 7. Such an analysis demonstrates that in spite of the marked difference in response of the enzyme levels to cortisone, the rates of synthesis of the four enzymes are

Table V

Comparison of Rates of Enzyme Synthesis under Basal Conditions and during Cortisone Treatment[a]

Enzyme	Half-life, hr	Basal enzyme act., units[b]	Enzyme synthesized— Basal, units/hr	Cortisone, units/hr	Ratio cortisone:normal
Tryptophan pyrrolase	2.5	3.0	0.84	3.4	4.0
Tyrosine–glutamic transaminase	2.0	78	27	114	4.2
Glutamic–alanine transaminase	84	252	2.0	14.4	7.2
Arginase	96	13,800	138	534	3.9

[a] Data as in C. M. Berlin and R. T. Schimke, *Mol. Pharmacol.*, **1**, 149 (1965). [b] Enzyme unit/g wet weight.

increased to approximately the same extent. Kenney[18a] and Segal and Kim[18b] have concluded from isotope incorporation data that the rates of synthesis of liver tyrosine transaminase and glutamic–alanine transaminase, respectively, are increased about 3–5-fold by administration of glucocorticoids to the intact animal.

The pattern that emerges from these studies is one of continual change, in which rates of synthesis and degradation of enzymes change in response to nutritional, hormonal, or genetic variations, the summation of which determines the given enzyme level. These studies, of course, raise numerous questions of the nature of molecular events underlying regulation of altered rates of both synthesis and degradation. Unfortunately, an understanding of mechanism in the depth and clarity known for microbial systems is not available at present for animal tissues. This is in large part due to difficulties in using intact animals as experimental systems, difficulties which include the differences in cell populations, the complex interrelationships between nutritional variables and multiple hormones, and the lack of suitable mutants.

Although there is considerable evidence that invokes alterations in RNA metabolism in regulation of enzyme synthesis in animals, there are certain properties of animal cells which allow for means of control other than the strict genetic transcription model described for bacteria where protein synthesis is controlled by immediate and continued synthesis of mRNA. For instance, there is an extensive and rapid synthesis and degradation of RNA within the nucleus of animal cells such that 80% of the RNA synthesized never enters the cytoplasm,[19] where protein synthesis occurs. Hence, the regulation of protein synthesis may be at the level of whether mRNA is transported out of the nucleus, rather than at the level of its initial synthesis. In addition, there is increasing evidence for reduplication of genes in animal cells[20]; this allows for the possibility that the rate of synthesis of a protein may be controlled by the number of copies of that particular gene.[21] Lastly, mRNA in animal cells is more stable than in bacteria,[22] a fact that allows for control at the point of mRNA stability. Stability of mRNA also allows for control by availability of tRNA or amino acids. At this time there are no experiments that allow a clear distinction from among the above possibilities in any particular enzyme studied.

On balance, degradation is equally as important as synthesis in determining the amount of an enzyme. Yet little is known about the regulation of this process. A theory of "aging" of protein molecules as a signal for degradation seems generally excluded, since the degradation of specific proteins follows first-order kinetics. This finding implies that once a particular protein molecule is synthesized, its chance of being degraded from the population of like molecules is a random event. How, then, do we account for the random nature of the degradation, the general property of heterogeneity of degradation rate constants among different proteins, and the finding that degradation rate constants of individual enzymes can be changed? Two possible mechanisms can be considered.

(1) The properties of the protein molecule as a substrate. Theoretically, protein molecules can exist in a number of different thermodynamic states. A protein might be subject to degradation only when the molecule assumes certain conformations. Moreover, a heterogeneity of degradation rates could exist depending on the number and nature of particularly labile peptide bonds exposed in particular conformations.

Shifting distributions and concentrations of substrates, cofactors, etc., as might occur under various hormonal and physiologic conditions, may lead to a variety of effects on specific enzymes, either to stabilize or labilize by ligand interaction, and thereby affect enzyme levels by altering rates of degradation.

(2) Alterations in activity of degradative processes. In the above discussion, the activity of the degradative mechanism was assumed to be in excess. It is also conceivable that the rate of degradation may also be dependent on the activity of the degrading system. Thus degradation may be controlled by activation, inhibition, translocation within a cell, or *de novo* synthesis of degrading enzymes.

One clear candidate for a degradative system is the lysosome, which occurs in essentially all cells.[23] Lysosomes are intracellular organelles that contain acid hydrolases and are currently conceived as involved in the autophagy of discrete areas of cytoplasm. It is difficult to conceive that lysosomes are involved in

(18) (a) F. T. Kenney, *J. Biol. Chem.*, **237**, 3495 (1962); (b) see Table II, footnote *i*.
(19) R. W. Shearer and B. McCarthy, *Biochemistry*, **6**, 283 (1967).
(20) R. J. Britten and D. E. Kohne, *Science*, **161**, 529 (1968).
(21) D. M. Brown and I. B. Dawid, *ibid.*, **160**, 272 (1968).
(22) M. Revel and H. Hiatt, *Proc. Natl. Acad. Sci., U. S.*, **51**, 810 (1964).
(23) C. deDuve and R. Wattiaux, *Ann. Rev. Physiol.*, **28**, 435 (1966).

protein degradation which involves a heterogeneity of degradation rates among different proteins, since some mechanism would be required for the recognition of whether a protein molecule is degraded, perhaps involving transport into a lysosome, acetylation, or formylation, etc.

Another possibility is that there are specific degrading enzymes for specific proteins. At the extreme, degradation of each protein would require a specific protein (enzyme). This, however, is logically impossible, since in liver there is continual replacement of essentially all proteins. Thus, if a protein were necessary to degrade each specific protein, then it would follow that a protein would be necessary to degrade a protein..... *ad infinitum.*

It might be most reasonable to consider that there are a number of mechanisms for degrading or otherwise inactivating enzymes. Lysosomes may be important when cell involution or gross changes in rates of total protein degradation occur, whereas the degradation that occurs in normal, steady-state conditions involves a system or systems that are not understood well or defined at present.

Clearly, the regulation of both synthesis and degradation of proteins in animal tissues is an area in which much work is needed.

The Juvenile Hormone of *Hyalophora cecropia*

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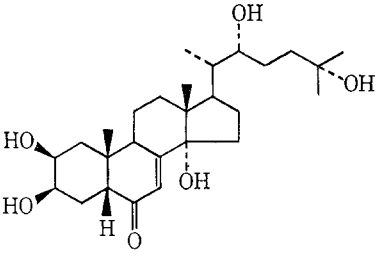
The molting process of insects involves the transformation of sexually immature individuals into reproducing adults of different form and structure. In some insects, this developmental sequence consists of a series of larval stages followed by pupal stage and finally the adult, whereas others do not have a pupal stage but molt directly from the larva to the adult. Control of these events of periodic molting rests with three different hormones (or hormonal systems).[1]

Neurosecretory cells secrete the brain hormone or prothoracotropic hormone whose action on the prothoracic gland initiates the synthesis and release of the prothoracic gland hormone also known as ecdysone. Ecdysone induces the events associated with each molt. Acting in contrast to ecdysone is a third hormone produced by a small gland known as the corpora allata. This hormone plays a regulatory role in the molting process. The type of molt that occurs appears to be a function of the concentration of this hormone which is called the juvenile hormone (henceforth abbreviated JH) since it induces the retention of juvenile characteristics.

Of the three hormones, prior to our work only ecdysone had been isolated and characterized. Karlson and his group[2] identified the hormone from silk worm pupae



ecdysone
2β,3β,14α,22β$_F$,25-pentahydroxy-5β,Δ7-cholesten-6-one

as a pentahydroxycholestenone derivative. The detailed structure was derived from X-ray data[3] and was confirmed by synthesis.[4] Molting hormones from other sources whose structures have been elucidated resemble ecdysone closely, differing mainly in the pattern and number of hydroxyl groups. It appears that all arthropods employ essentially the same compound as the molting hormone.

During the past few years, reports appeared describing attempts to isolate the juvenile hormone.[5] None of the active principles isolated and characterized possessed all the biological properties of the hormone itself. However, the finding that the active substances were farnesol or its derivatives led to a general testing of a wide range of acyclic terpenes in the hope of learn-

(1) (a) P. Karlson, *Angew. Chem., Intern. Ed. Engl.*, **2**, 175 (1963). (b) P. Karlson, *Pure Appl. Chem.*, **14**, 75 (1967). (c) N. A. Tamarina, *Usp. Sovrum. Biol.*, **62**, 415 (1966). (d) C. M. Williams, *Sci. Am.*, **217**, 13 (1967). (e) K. D. Highnam, *J. Endorcrinol.*, **39**, 115 (1967). (f) For simplicity in the subsequent discussion, reference is made to three hormones controlling insect development. At present, it is unknown whether the control of these processes involves three discrete chemical entities or whether each "hormone" is a mixture of compounds. The former case seems the more likely at present. It is unlikely that the same chemical entities serve as the various hormones in all species. C. E. Berkoff, *Quart. Rev.* (London), **23**, 372 (1969).

(2) P. Karlson, *Naturwissenschaften*, **53**, 445 (1966).

(3) (a) R. Huber and W. Hoppe, *Chem. Ber.*, **98**, 2403 (1965); (b) W. Hoppe, *Angew. Chem.*, **77**, 484 (1965).

(4) (a) V. Kerb, P. Hocks, R. Wiechert, A. Furlenmeier, A. Furst, A. Tangemann, and G. Waldvogel, *Tetrahedron Letters*, 1387 (1966); (b) J. B. Siddall, J. P. Marshall, A. Bomers, A. D. Cross, J. A. Edward, and J. H. Fried, *J. Am. Chem. Soc.*, **88**, 379 (1966); (c) J. B. Siddall, A. D. Cross, and J. H. Fried, *ibid.*, **88**, 862 (1966).

(5) (a) M. Gabe, P. Karlson, and J. Roche, *Comp. Biochem.*, **6**, 246 (1964); (b) P. Schmialek, *Z. Naturforsch.*, **16b**, 461 (1961); **18b**, 516 (1963); (c) H. Z. Levinson, *Rio. Parassitol.*, **27**, 47 (1966); (d) A. S. Meyer, H. A. Schneiderman, and L. J. Gilbert, *Nature*, **206**, 272 (1965).