core. The fact that leucine is incorporated in the core of the lens, a region where RNA synthesis is undetectable, implies that a relatively stable species of mRNA is present. Since the reservation must be made that very small amounts of RNA synthesis would not be detected by the autoradiographic technique used, the following experiment was performed to examine messenger stability more closely. Two groups of lenses were incubated, one in Charity Weymouth Medium and the other in the medium to which actinomycin D (30 μg/ml) was added for periods up to 10 hours. At intervals lenses were taken from each group and treated for 1 hour with C^{14}-leucine. Autoradiography of lenses treated with the antibiotic reveals that the heavy incorporation originally seen in the epithelium progressively disappears whereas the core remains unaffected. The control lenses incubated without the antibiotic showed no change.

After 8 hours in actinomycin D (Fig. 1*d*) the rate of incorporation in the cortex and part of the annulus had decreased to the level of that in the core with the exception of a small, highly radioactive ring around the equator. Virtually all incorporation had ceased in the epithelium. This pattern suggests that a stable mRNA exists throughout the body and part of the annulus whereas an unstable mRNA is present in the epithelium. Thus both stable and unstable mRNA's may coexist in cells of the cortex and part of the annulus. Observations on decrease of specific activities of electrophoretically separated proteins from various parts of the lens support these interpretations (*6*).

Our data indicate that the lens contains mRNA's of different half-lives, some relatively long and others short. Higher resolution and more quantitative autoradiography will be required to show whether both types of message coexist in some cells.

In control lenses there is almost no gradient of incorporation from the posterior surface of the lens to the core, and the C^{14}-leucine grains are evenly distributed after actinomycin treatment. This fact suggests that the pattern of C^{14}-leucine incorporation is not an artifact of diffusion. Since bisected lenses incorporated C^{14}-uridine in essentially the same pattern as whole ones, the pattern of RNA synthesis can also not be attributed to diffusion.

These data imply that lens cells pass through a definite sequence of macromolecular syntheses as they differentiate and migrate from one zone of the lens into another; DNA is synthesized exclusively in a region of the epithelium anterior to the annulus. In that zone, cells also make RNA and synthesize protein on the mRNA that appears to have a short half-life. In part of the annulus and throughout the entire body region, cells make protein on long-lived mRNA's. Possibly some cells of the cortex and annulus contain both short- and long-lived mRNA. The stable mRNA is present in greatest concentration in a ring of cells around the equator of the lens. RNA synthesis in the cortex decreases gradually toward the core, and its flow into the cytoplasm may be reduced. Cells of the core make little if any RNA but continue to synthesize protein for some time on stable mRNA.

A number of regulatory events must occur as the foregoing sequence is executed. (i) Genes concerned with DNA synthesis are repressed as cells move into the annulus. (ii) Either mRNA which was formerly unstable is stabilized, or genes concerned with the synthesis of stable mRNA's are activated when cells move through the annulus into the cortex. (iii) RNA synthesis is terminated in cells of the core.

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Catalase: Kinetics of Photooxidation

Abstract. *The kinetics of photooxidation of catalase is a four-step consecutive reaction, if each of the four porphyrin moieties acts independently. The rate of enzyme inactivation is a first-order reaction, resulting from destruction of a single porphyrin. The kinetics of absorbancy is more complex, depending upon the absorption probabilities of each of the microspecies. With equal probabilities, the log of the sum of the normalized absorptivities is a linear function of time.*

Catalase is a heme ezyme, believed to consist of four porphyrin moieties residing within identical or nearly identical subunits (*1*). The intact aggregate is required for enzyme activity; consequently, the destruction by photooxidation of only one of the porphyrins would suffice for complete inhibition, even though three-fourths of the absorbancy in the Soret band region (405 nm) remained. The kinetics of photooxidative enzyme loss will therefore be different from that of absorbancy loss (*2*).

The following model appears to provide the common kinetics for the two phenomena. If each of the porphyrin-containing monomers in the aggregate absorbs light and is destroyed independently, then at any time, t_i, after t_0, the population will contain four microspecies, with 4, 3, 2, or 1 heme residues per molecule (Fig. 1). The rate of the photochemical reaction will be a function of the concentration, C_i, of the absorbing species, of its absorptivity, k, of the light flux, and of the possible differential receptivity of each of the hemes, K_i, in the various monomers within the aggregate. For any given constant light flux, and in an optically thin solution, the set of differential equations describing the transformation of the porphotetramer into the porphotrimer, and so forth, is as follows, the group being designated Eq. 1.

$$\begin{aligned}
\frac{dC_4}{dt} &= -4\,kK_4C_4 \\
\frac{dC_3}{dt} &= 4\,kK_4C_4 - 3\,kK_3C_3 \\
\frac{dC_2}{dt} &= 3\,kK_3C_3 - 2\,kK_2C_2 \\
\frac{dC_1}{dt} &= 2\,kK_2C_2 - kK_1C_1
\end{aligned} \qquad (1)$$

The foregoing equations are of linear form and may be integrated by standard methods to yield the following set, which is designated Eq. 2 (p. 73).

The k's are a function of the wave length, λ. Since k is known for the enzyme, K_4 may be calculated from its kinetics. The absorbancy is the sum of contributions from each of the microspecies (Eq. 5, below), and it should be possible, in principle, to calculate the K_i's independently from the experimental data. However, for any given $k_\lambda = k$, if we assume the K_i's are identical, $kK = R$, where R is a constant. With these conditions and that of $C_0 = 1$, the above equa-

$$C_4 = C_0\, e^{-4\,kK_4 t}$$

$$C_3 = \frac{4\,C_0K_4}{(3\,K_3 - 4\,K_4)}\left[e^{-4\,kK_4 t} - e^{-3\,kK_3 t}\right]$$

$$C_2 = \frac{12\,C_0K_4K_3}{(3\,K_3 - 4\,K_4)}\left[\frac{e^{-4\,kK_4 t}}{(2\,K_2 - 4\,K_4)} - \frac{e^{-3\,kK_3 t}}{(2\,K_2 - 3\,K_3)} + \left(\frac{1}{(2\,K_2 - 3\,K_3)} - \frac{1}{(2\,K_2 - 4\,K_4)}\right)e^{-2\,kK_2 t}\right]$$

$$C_1 = \frac{24\,C_0K_4K_3K_2}{(3\,K_3 - 4\,K_4)}\left[\frac{e^{-4\,kK_4 t}}{(2\,K_4 - 4\,K_4)(K_1 - 4\,K_4)} - \frac{e^{-3\,kK_3 t}}{(2\,K_2 - 3\,K_3)(K_1 - 3\,K_3)} + \left(\frac{1}{(2\,K_2 - 3\,K_3)(K_1 - 2\,K_2)} - \frac{1}{(2\,K_2 - 4\,K_1)(K_1 - 2\,K_2)}\right)e^{-2\,kK_2 t} - \left(\frac{1}{(2\,K_2 - 3\,K_3)(K_1 - 2\,K_2)} - \frac{1}{(2\,K_2 - 4\,K_1)(K_1 - 2\,K_2)} + \frac{1}{(2\,K_2 - 4\,K_4)(K_1 - 4\,K_4)} - \frac{1}{(2\,K_2 - 3\,K_3)(K_1 - 3\,K_3)}\right)e^{-kK_1 t}\right] \quad (2)$$

tions simplify to the following set, designated Eq. 3.

$$\begin{aligned} C_4 &= e^{-4\,Rt} \\ C_3 &= -4\,e^{-4\,Rt} + 4\,e^{-3\,Rt} \\ C_2 &= 6\,e^{-4\,Rt} - 12\,e^{-3\,Rt} + 6\,e^{-2\,Rt} \\ C_1 &= -4\,e^{-4\,Rt} + 12\,e^{-3\,Rt} - 12\,e^{-2\,Rt} + 4\,e^{-Rt} \end{aligned} \quad (3)$$

The time course of each of the microspecies is shown in Fig. 2 where the value of R is obtained from the experimental data of the enzyme kinetics, described by $C_4 = e^{-4\,Rt}$. At $t = 30$ minutes, $C = 0.325$, and $R = 8.75 \times 10^{-3}$; this value of R may then be utilized to calculate the kinetics of the simplified system.

The sum of all the microspecies at any time t_i is C_i, which, by simple addition of the set of Eq. 3, is

$$\Sigma C_i = -e^{-4\,Rt} + 4\,e^{-3\,Rt} - 6\,e^{-2\,Rt} + 4\,e^{-Rt} \quad (4)$$

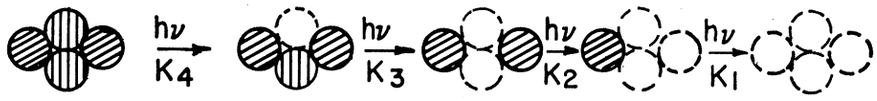

Fig. 1. Model for the photoinactivation of catalase. The enzymic activity is lost after the destruction of the first heme. However, three-fourths of the absorptivity remains. The necessity of four hemes for catalytic activity may reside in the requirement of four loci to orient two H—O—O—H's (for example, with one oxygen on each heme) in the most reactive positions. The loss of one locus, allowing virtually free rotation of one peroxide, is sufficient to destroy this unique configuration. Light is indicated by $h\nu$.

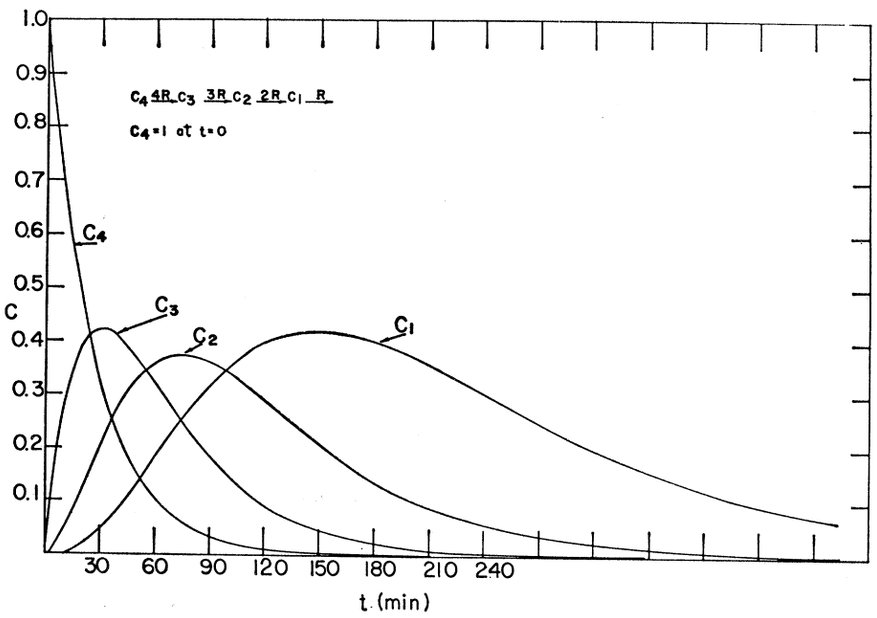

Fig. 2. Fractional values for C_i at times t. The value of $R = 8.75 \times 10^{-3}$ is derived from the enzymic experimental datum of $C_4 = e^{-4\,Rt} = 0.325$ at $t = 30$ min.

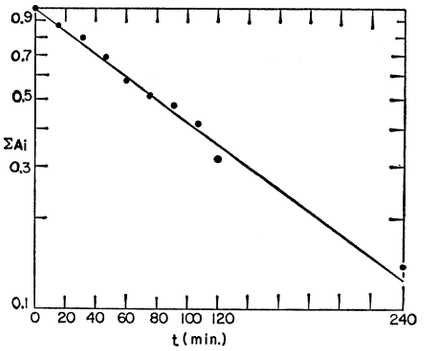

Fig. 3. Comparison of experimental values of absorptivity with those expected from the theory, which predicts a linear relation between the logarithm of the sum of the absorbancies of the microspecies plotted against time.

The absorbancy, A, at any time t is calculated from the sum of the contributions of each of the species:

$$A_{t,\lambda} = C_4 + (3\,C_3/4) + (2\,C_2/4) + (C_1/4) \quad (5)$$

The use of this condition in Eq. 3 yields:

$$A_{t,\lambda} = e^{-Rt} \quad (6)$$

that is, a curve of log A_i plotted against t is a straight line. This result may be compared with the experimental data if a limiting value is approximated from the shape of the photooxidation spectra. At an initial catalase concentration of 0.01 mg/liter, with an initial A at 405 nm of 0.670, the limit A (at $t = \infty$) appeared to have the value 0.380 (*3*). The difference, $A_0 - A_\infty = 0.290$, is the total span in absorptivity covered by the experiment. The absorbancies at intermediate times may be expressed as a fraction of this difference, that is $(A_{\text{expt.}} - A_\infty)/(A_0 - A_\infty)$. These are the points plotted in Fig. 3 and are in reasonable agreement with theory.

Interestingly enough, the initial absorbancy data may be put in linear form (to $t = 240$) if it is plotted as a simplified fourth order reaction, $1/A^3$ plotted against t, without any assumption as to a limiting value. However, there is no obvious theoretical basis for this relation, and it would obviously not hold for a system in which $A \neq 0$ at $t = \infty$.

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