rocytes was compared with that of the enzyme extract. Both determinations were conducted in the presence of 50 m*M* K^+. The extract contained ⅕ of the total deaminase activity of the cells and 1/40 of the total hemoglobin content. The deaminase has a broad *p*H optimum at 7.1. The enzyme does not deaminate adenine, adenosine, adenosine diphosphate or adenosine triphosphate. That the ion activation of the enzyme is not due to a specific effect of tris (since this was present in the enzyme preparation) was evident from: (i) the fact that the enzyme could be extracted from the precipitate by salt solutions other than tris and (ii) the variation of tris concentrations over a wide range did not influence the effect of activating ions. When the enzyme solution, or various water dilutions of it, was centrifuged at 115,000*g* for ½ hour, no precipitate was observed, and samples taken from the top and the bottom portions of the centrifuged solution had equal activity. Although some of the enzyme was extracted readily from the precipitate containing the erythrocyte membranes, even repeated extraction did not result in a membrane fraction free from deaminase. In fact membranes prepared by several other methods (*2, 4, 5*) also contained the deaminase activity with properties similar to those of the soluble enzyme.

Figure 1 shows the effect of varying concentrations of the activating ions on the rate of deamination. The reaction conditions were the same as described for Table 1. The reaction time was 20 minutes. During this period the rate of reaction was a linear function of time. The *Km* values for K^+ and Na^+ were 3 and 8. These values are the same as those for adenosine triphosphatase (*5*) and adenylic kinase (*6*).

An unusual feature of the erythrocyte 5′-adenylic acid deaminase is its activation by ammonia, the product of the reaction it catalyzes. Conway and Cook (*7*), studying the deamination of 5′-adenylic acid by erythrocyte hemolyzates at high substrate concentrations (2 to 60 m*M*), found an unusual increase in the rate of reaction with increase of substrate concentration over 6 m*M*. Their results could well be explained by the above-mentioned autocatalytic nature of the 5′-adenylic acid deaminase (*9*).

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"Apocatalase" of Catalase-Negative Staphylococci

Abstract. *Crystalline catalase of high purity and activity was prepared from a heme-requiring* Staphylococcus aureus *mutant, JT/52, grown in the presence of heme. A crystalline protein was obtained from the same cells grown without heme. This enzymatically inactive protein and the catalase cross-react strongly when tested by a variety of immunochemical tests.*

Staphylococcus JT/52 requires a heme source for the synthesis of catalase (*1*). Cells of this mutant strain grown in the absence of heme, washed free of nutrients, and resuspended in a buffer solution become catalase positive upon addition of hemin (*2*). Maximum catalase activity (similar to that of the heme-independent parent strain SG 511) is obtained within 30 minutes (37°C) after hemin is added to the resting cells. This rapid formation of catalase, which, depending on the condition of the cells, requires the presence of coenzyme A and glucose (*3*), was taken as an indication for the presence of both preformed apocatalase and a synthesizing system that combines the apoenzyme with the prosthetic group.

Numerous futile attempts have been made to demonstrate this "terminal synthesis" in a cell-free system (*4*). Since no trace of activity can be observed in such experiments, the question was raised whether apocatalase is indeed formed independently in the absence of heme. This would mean, then, that free heme had a profound influence on the protein synthesis of hematin enzymes. On the other hand, the synthetic deficiency of the cell-free system could well be due to the absence of essential structural elements, the liberation of inhibitors, or other factors.

This report contains the main results of an investigation undertaken to decide this question of long standing and some consequence. Our experimental approach was based on the assumption that apocatalase would cross-react immunologically with catalase. Antibody against pure bacterial catalase could then be used as a guide for the detection of the enzyme's protein moiety.

The catalase to be used as antigen was prepared by a modification of the method of Herbert and Pinsent (*5*) from JT/52 cells grown on the surface of nutrient agar containing 3.0 μg of hemin (Pentex) per milliliter. The catalase activity of such cells could not be enhanced by further addition of hemin. The final preparation of the crystalline enzyme was of high purity. It had the following characteristics: The Kat. f. (Katalasefähigkeit or catalase ability), calculated from velocity constants (k) obtained spectrophotometrically at 240 mμ (*6*), was 124,000. Assuming a molecular weight of 232,000 (*5, 7*), the specific activity was calculated as $k'_1 = 5.3 \times 10^7\ M^{-1}\ sec^{-1}$, and the molar extinction coefficient as ε 406 (max) = 420 $mM^{-1}\ cm^{-1}$. The ratio OD_{406}: $OD_{276.5\ (max)}$ was 1.05. The sedimentation pattern in the ultracentrifuge was that of a single component with a sedimentation constant $s_{20,b} = 10.5 \times 10^{-13}$ sec. In double diffusion plates the catalase formed a single, sharp band with its corresponding antibody. Immunoelectropherograms showed a single arc. The enzymatic activity was significantly and specifically inhibited by anticatalase (80 percent inhibition with excess antibody).

The JT/52 cells grown in the absence of heme were submitted to a similar extraction procedure. Guided by double-

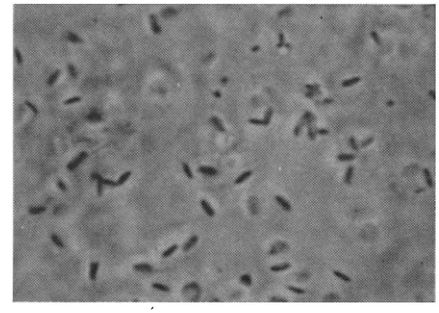

Fig. 1. Crystalline "apocatalase" from the catalase negative mutant (about × 1300).

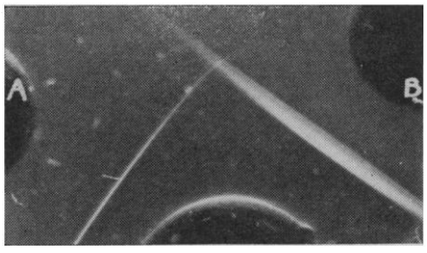

Fig. 2. Double diffusion in agar with anticatalase in center well, "apocatalase" in well *A*, and catalase in well *B*.

diffusion assays with bacterial anticatalase as antibody, the final "apocatalase" preparation was obtained as a colorless precipitate from 38 to 40 percent $(NH_4)_2SO_4$. Redissolved in water and adjusted to *p*H 8.5, the faintly yellow, enzymatically completely inactive solution crystallized as minute plates upon slow evaporation (Fig. 1).

Ultracentrifugation of the "apocatalase" revealed the presence of two components with distinctly different sedimentation constants, a fast moving ($s_{20,b} = 5.3 \times 10^{-13}$ sec) and a very slowly moving one ($s_{20,b} = 0.84 \times 10^{-13}$ sec). In spite of this heterogeneity, single, sharp precipitation bands formed with the homologous antibody and also with anticatalase in double-diffusion plates. Although "antiapocatalase" precipitates the catalase, it does not inhibit its enzymatic activity.

Figures 2 and 3, in which catalase and "apocatalase" diffuse from adjacent wells toward anticatalase and antiapocatalase respectively, show strikingly different patterns of double diffusion. Though puzzling at first glance, these patterns reveal some of the reactant's characteristics and allow certain conclusions. In Fig. 2 the catalase-anticatalase band crosses, apparently undisturbed, the band formed by apocatalase and anticatalase. The latter, however, forms a typical weak "spur" that curves toward the former. Figure 3 on the other hand, shows an unmistakable pattern of fusion or "identity."

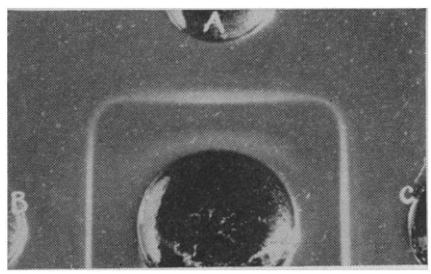

Fig. 3. Double diffusion in agar with "antiapocatalase" in center well, catalase in well *A*, and "apocatalase" in wells *B* and *C*.

In consideration of the notorious heterogeneity of even the most specific antibodies, these immunodiffusion patterns suggest the following interpretation. Figure 2 reflects the presence of antibody molecules (anticatalase) that differ in their specificity for the whole antigen and for smaller and larger parts of it (subunits?). Their different relative concentrations are responsible for the different quality of the "spurs." However, the anti-apocatalase (Fig. 3), produced in response to an antigen that is presumably a mixture of antigenically similar subunits (none larger, but many smaller than half the size of the catalase molecule), cannot contain antibody molecules that are highly specific only for the whole catalase molecule or major parts of it. Thus, a pattern of identity is to be expected.

In conclusion, the presence of at least large parts of the protein moiety of catalase, a classical hematin enzyme, has been demonstrated in catalase negative cells. The apoenzyme is apparently synthesized before the incorporation of the prosthetic group and independently of its presence. Whether our crystalline "apocatalase" represents a mixture of antigenically similar, true precursors of catalase, or whether extraction and purification procedures gave rise to "subunits" originating from a fully synthesized, native apocatalase cannot be decided at present (*8*).

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Ruby Laser as a Microsurgical Instrument

Abstract. *Morphological changes at the cell level have been produced experimentally by a pulsed ruby laser (optical maser). A pulse duration of approximately 500 microseconds caused discrete damage to structures in the cell without irreversible damage to the surrounding area.*

By the use of a focused ruby laser (*1*), openings up to 25 μ in diameter have been produced in the cell walls of the alga, *Spirogyra*, without causing irreversible damage. The relative transparency of the cell wall to laser beams permitted microsurgery to be performed on the internal structures of the algal cell without damage to the exterior cell wall. It was possible to sever the chloroplast helix, disrupt crystals, and produce localized coagulation of the chloroplasts and cytoplasm. Focusing the laser beam on the cell nucleus caused coagulation and a reduction in nuclear volume. Ultraviolet irradiation of the nucleus of a cell also caused a slight reduction in volume and a condensation of ultraviolet-absorbing material at the nuclear membrane (*2*).

Instrumentation for the experiments consisted of a low-power ruby laser (*3*) incorporated and integrally mounted with a triocular biological microscope. This permitted precise positioning of the laser beam on the specimen. The laser has a maximum output of 20 mj per pulse. The pulse duration is 500 μsec. It is possible to view the specimen immediately after irradiation with the beam. Cross lines in the ocular permit fast and accurate positioning of the target, since the laser beam is coincident with the optical path of the microscope.

Laser spot size incident upon the specimen is varied by changing the power of the microscope objectives. The greater the magnification, the smaller the focused spot becomes. The energy which is incident upon the specimen may be measured with the use of a thermopile (*4*). Irradiating energy is controlled via metallized neutral density filters of known transmittances which are interposed between the laser and the focusing objectives.

The experiments indicated that, unless relatively high beam energies were used, the laser beam passed through the cell wall without causing any visible effect on it. Methylene blue chloride was applied to the cell wall, since the laser emission is in the deep red part