THE ACTIVITY OF CATALASE IN PASTEURELLA TULARENSIS

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In an investigation (Sherstinsky, 1951; Yaniv, 1951) of the factors governing the virulence of *Pasteurella tularensis* (*Bacterium tularense*), the correlation between the activity of catalase and virulence was studied. This line of approach was suggested by the fact that in the *Brucella* group (Huddleson and Stahl, 1943) and in *Pasteurella pestis* (Rockenmacher, 1949) high virulence appears to be associated with high catalase activity.

Strains of *P. tularensis* have been compared, which were derived from the same parent strain, but were characterized by varying degrees of virulence. No relation between catalase activity and virulence could be found. A number of observations are reported which have a bearing on the general picture of the enzyme and the mechanism of its action

MATERIALS AND METHODS

The strains used were: Vir, SMR I, AS, 176, and BO. All were derived from a common parent strain (virulence in mice LD_{50} $10^{-7.5}$). The strain Vir (LD_{50} 10^{-10}) was obtained after several passages through mice; strain SMR I (LD_{50} 10^{-2}) was a streptomycin-resistant strain (Yaniv, 1951). Strain AS (LD_{50} $10^{-2.5}$) resulted from growing the parent strain in the presence of antitularemic serum. After one hundred rapid transfers on glucose-cysteine-blood-agar slopes, the parent strain became greatly reduced in virulence, giving strain 176 (LD_{50} 10^{-1}). The BO strain was a virulent mutant (LD_{50} 10^{-9}), which had been adapted to glucose-cysteine-agar without an addition of blood or yolk. If not stated otherwise, the Vir strain was used for the experiments.

The microorganisms were cultivated on glucose-cysteine-yolk-(5 to 10 per cent) agar slopes. The usual medium containing blood was avoided because of the danger of interference of the blood catalase.

The slopes were washed with saline, usually after 48 hr incubation at 37 C. Further washing of the bacteria proved unnecessary since bacteria three times washed showed the same catalase activity as unwashed ones. Moreover, the first washings of the culture medium had no influence on liver catalase, prepared according to Keilin and Hartree (1945).

The final concentration of bacteria used (5×10^{10} per ml) corresponds to 40 per cent transmission at 600 mμ in the Coleman Jr. spectrophotometer. The saline washings of an unseeded slope showed practically no catalase activity (initial activity $Ko < 1 \times 10^{-3}$) and were used as blank (100 per cent transmission).

The catalase activity was determined by the titrimetric method of Jolles as modified by Sumner (1941). A concentration of 10^{10} microorganisms per ml

was used. The rate of decomposition of hydrogen peroxide was measured every three minutes, and Ko calculated from the experimental K values using the formula (Lemberg and Legge, 1943):

$$Ko = \text{antilog} \frac{\frac{1}{2}(t_1 + t_2) \log K_{t_1-t_2} - \frac{1}{2}(t_2 + t_3) \log K_{t_2-t_3}}{\frac{1}{2}(t_3 - t_1)}$$

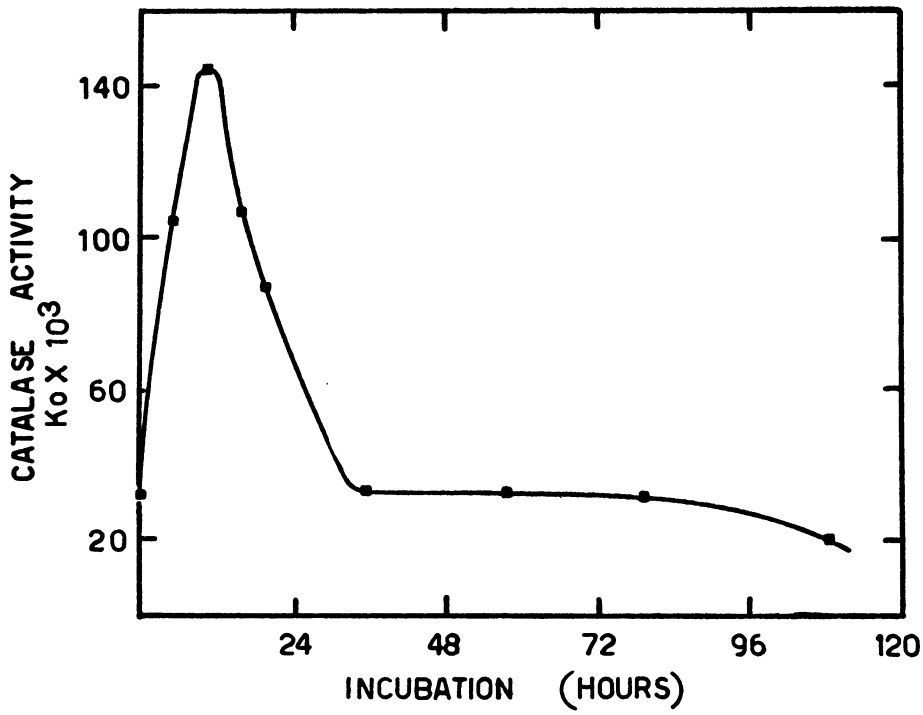

Figure 1. Influence of the culture age on the activity of catalase (Vir strain; turbidity = 40 per cent transmission).

RESULTS

Concentration of bacteria and catalase activity. A series of dilutions of a thick bacterial suspension (23 per cent transmission) was made, and their Ko values were determined using 0.006 N H_2O_2. Up to a twentyfold dilution of the original suspension, the catalase activity was directly proportional to the number of organisms.

Influence of age of culture on catalase activity. The catalase activity was tested after various periods of incubation at 37 C and on suspensions of the same standard turbidity (40 per cent transmission). The highest activity was found for a 10 to 12 hr culture, whereas maximum growth was obtained only after 48 hr. Cultures older than 10 to 12 hr showed a rapid decrease in activity which reached a constant level after 48 hr.

In the early phases of growth the absolute values of the catalase activity

differed considerably from experiment to experiment, but the slope of the activity-*vs*-time curve was always the same. Figure 1 gives a typical example.

The effect of substrate concentration on the activity of catalase. At the optimum concentration found by Rockenmacher (1949) for *P. pestis* (0.5 N H_2O_2), immediate formation of oxygen bubbles is observed, but the catalase is inactivated very rapidly, while for 0.07 N H_2O_2, the optimum concentration reported for purified liver catalase (George, 1949), too small a value of Ko was obtained.

The dependence of Ko on the substrate concentration was, therefore, determined for *P. tularensis*. Figure 2 shows that Ko decreases with increasing concentration of hydrogen peroxide. As during the reaction the concentration of

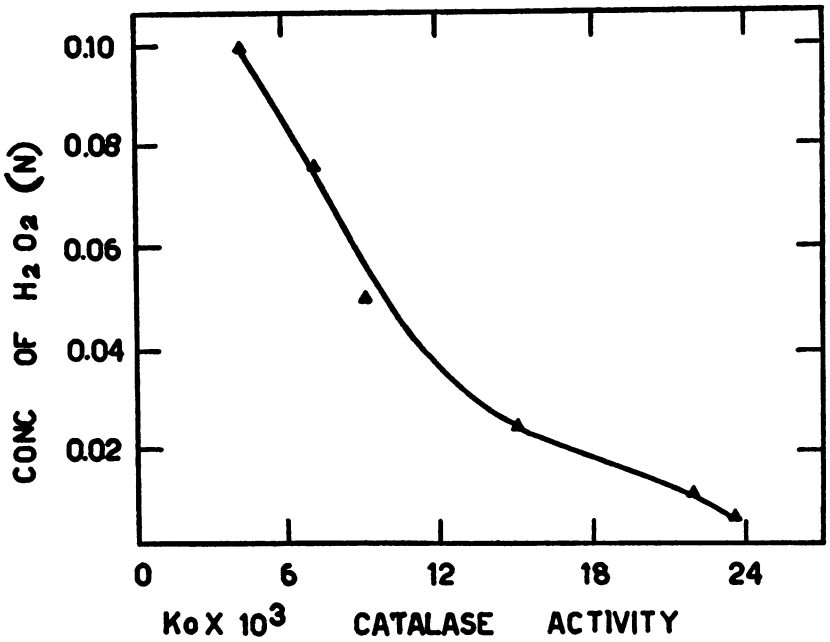

Figure 2. Influence of substrate concentration on the activity of catalase (Vir strain).

hydrogen peroxide decreases continuously with time, one should expect a continuous rise in the reaction velocity, provided the enzyme is not appreciably destroyed during the reaction.

The influence of the reaction time on the K values at 0.1 N and 0.01 N H_2O_2 was investigated. At the lower concentration the K values increase with time; at the higher one they decrease with time (figure 3).

Reversibility of the depression of Ko by high H_2O_2 concentration. The microorganisms were exposed to 0.1 N H_2O_2. After a contact of three min the mixture was diluted fivefold with 0.01 N phosphate buffer (pH 6.8). The H_2O_2 concentration was now 0.0175 N, due to the combined effect of enzymatic decomposition during 3 min and of dilution. The rate of decomposition was then determined, and the Ko values were calculated. In another experiment, the initial contact

with 0.1 N H_2O_2 was maintained for 30 min. For comparison, part of the same bacterial suspension was exposed to 0.0175 N H_2O_2 without previous contact with the higher concentrations. The results are summarized in table 1.

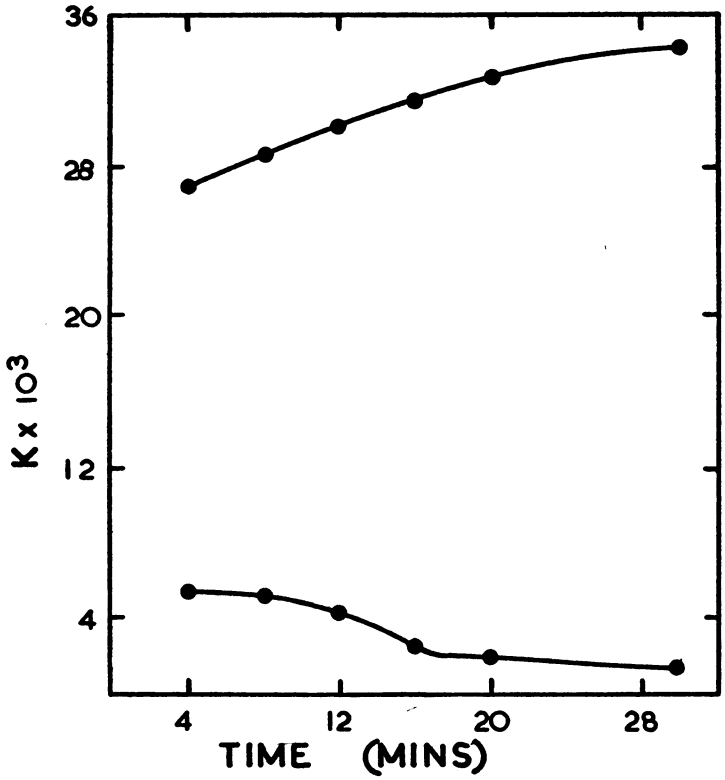

Figure 3. Change of the monomolecular reaction constant (K) with time of reaction. Upper curve: 0.01 N H_2O_2. Lower curve: 0.10 N H_2O_2.

TABLE 1

Influence of concentration of H_2O_2 on the activity of catalase

	EXPERIMENTAL CONDITIONS	Ko X 10^3
A	Action on 0.1 N H_2O_2	3.2
B	Action on 0.0175 N H_2O_2	25.7
C	Contact for 3 min with 0.1 N H_2O_2, then dilution (final concentration 0.0175 N)	24.0
D	Contact for 30 min with 0.1 N H_2O_2, then dilution (final concentration 0.0175 N)	2.5

The inhibition caused by high substrate concentration is reversible when the time of contact is short (3 min). After longer contact (30 min), an irreversible inhibition has occurred.

The activity of catalase in different strains. In view of the results reported in

the preceding paragraph, the virulent and avirulent strains were compared both at 0.1 N and at 0.01 N H_2O_2. Tables 2 and 3 summarize the results. Their statistical evaluation shows no significant differences of the five strains in their catalase activity.

TABLE 2

Activity of catalase from different strains of Pasteurella tularensis (substrate 0.01 N H_2O_2). Age of cultures: 48 hr

STRAINS	VIRULENCE LD_{50}	Ko × 10^3				
		Experiment no.				
		1	2	3	4	5
Vir	10^{-10}	21.0	24.0	29.2	25.4	26.0
SMR I	10^{-2}	33.2	18.0	30.2	25.0	25.6
AS	$10^{-2.5}$	19.5	26.0	25.5	26.0	25.3
176	10^{-1}	19.3	32.5	29.2	35.2	31.2
BO	10^{-9}	32.0	28.0	—	—	—

TABLE 3

Activity of catalase from different strains of Pasteurella tularensis (substrate 0.1 N H_2O_2). Age of cultures: 48 hr

STRAINS	Ko × 10^3				
	Experiment no.				
	1	2	3	4	5
Vir	4.1	4.7	4.6	6.7	7.4
SMR I	8.4	3.0	4.2	6.4	5.8
AS	5.4	6.3	5.7	5.2	6.3
176	3.7	6.4	6.4	5.5	5.5

TABLE 4

Activity of catalase from different strains of Pasteurella tularensis (substrate 0.01 N H_2O_2). Age of culture: 12 hr

STRAIN	Vir	SMR I	AS	176	BO
Ko × 10^3	117.4	100.0	106.7	143.8	112.3

The experiments were conducted after incubation of 48 hr when, as shown previously, the catalase activity has become stationary. A number of experiments conducted after 12 hr of incubation revealed no differences between the various strains (table 4).

DISCUSSION

Three conclusions emerge from the results reported here:

a. The catalase activity of *P. tularensis* strains is not correlated with their

virulence. This is emphasized by the fact that the strains tested were derived in different ways from the same parent strain.

b. In *P. pestis* and other microorganisms (Virtanen and Karstrom, 1925) the catalase activity remains constant for incubation times between 24 and 72 hr or more, but the catalase activity in *Micrococcus lysodeikticus* increases steadily even after full growth is reached (Herbert and Pinset, 1948). The catalase activity of *P. tularensis* is highest after 10 to 12 hr of incubation and then decreases rapidly until a steady level is reached after 48 hr. A similar observation has been made by Wooldridge and Glass (1937) regarding the glucose dehydrase and amino acid dehydrase of *Escherichia coli*. One is tempted to seek a correlation between the two observations as the two enzymes produce the hydrogen peroxide which is destroyed by catalase. The fact that the activity of catalase in *P. tularensis* is highest in the logarithmic phase of growth would then point to an important role of the enzyme in the metabolism of the bacterium.

c. The decomposition of hydrogen peroxide by catalase (purified enzyme from liver) follows a monomolecular course up to 0.2 N substrate concentration (George, 1949). Virtanen and Karstrom (1925) found this independence (up to 0.1 N H_2O_2 concentration) for the catalase of a variety of microorganisms. For *P. tularensis*, on the other hand, the optimum value of the substrate concentration is much lower (below 0.01 N), and the Ko values decrease with increasing hydrogen peroxide concentration (figure 2). This inactivation is reversible and, therefore, differs from the irreversible inhibition caused by prolonged contact of the enzyme with high (0.1 N) concentrations of substrate.

This observation appears to dispose of an old controversy (Zeile, 1934). The inhibition of catalase by high concentrations of ethyl hydrogen peroxide has been observed previously (Haurowitz, 1937; Keilin and Hartree, 1935; Stern and Dubois, 1937) and has been explained by Oppenheimer (1938) by the assumption that hydrogen peroxide must attach itself to two active centers on the enzyme surface. This is prevented by the high concentration of substrate at which each substrate molecule attaches itself to only one of the active centers. This one centered attachment is relatively loose and is reversed by dilution. Such a picture of the initial mechanism of catalase action is in accord with recent general theories of enzyme action in which two sites are postulated to explain the observed effects.

At lower H_2O_2 concentrations (0.01 N) at which no irreversible inhibition yet occurs, the K values increase with time (i.e., with decreasing substrate concentrations) because of the gradual disappearance of the effect of reversible inhibition. At high H_2O_2 concentrations, however, the K values decrease with time, due to the irreversible inactivation of the enzyme (figure 3).

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SUMMARY

In *Pasteurella tularensis* the content of catalase is highest after an incubation of 12 hours, and then decreases rapidly until after 48 hours a steady level is reached.

There is no correlation between catalase activity and virulence.

The optimum substrate concentration for *P. tularensis* is below 0.01 N.

A reversible inhibition and an irreversible inhibition (destruction of the enzyme) have been observed, the former reversed by simple dilution. This observation is discussed in terms of the two center theory of enzyme action.

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