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Catalase Activity in *Anaplasma marginale*

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ABSTRACT

WALLACE, W. R. (Louisiana State University, Baton Rouge), AND G. T. DIMOPOULLOS. Catalase activity in *Anaplasma marginale*. J. Bacteriol. **90**:309-311. 1965.—Extracts of erythrocytes infected with *Anaplasma marginale* were found to contain more catalase activity than normal erythrocytic preparations. The increase in catalase activity appeared concurrently with increases in the number of erythrocytes containing *Anaplasma* bodies. Antisera against normal bovine erythrocytes and *Anaplasma*-infected erythrocytes were prepared in rabbits to test the source of increased catalase activity during anaplasmosis. Antiserum against normal erythrocytes decreased the catalase activity of extracts of normal erythrocytes by 271 units per ml and of partially purified *Anaplasma* bodies by only 120 units. Rabbit antiserum against *Anaplasma*-infected bovine erythrocytes removed only 73 units of activity from normal erythrocytes, but decreased the activity of the partially purified *Anaplasma* bodies by 260 units, indicating the association of catalase with the marginal body.

Information on the biochemical characteristics of *Anaplasma marginale* is scanty. Attempts to grow the blood parasite in vitro have not resulted in the harvesting of sufficient cell crops for chemical analyses (*Bergey's Manual*). Histochemical analyses have shown peroxidase, dehydrogenase, and alkaline phosphatase to be absent in the marginal body (Moulton and Christensen, 1955). In the investigations reported here, high levels of catalase activity were demonstrated in partially purified *Anaplasma* bodies without obtaining stroma-free preparations.

MATERIALS AND METHODS

Preparation of enzyme. The methods of maintaining, inoculating, and observing calves during anaplasmosis were described previously (Dimopoullos and Bedell, 1962). Catalase was extracted from erythrocytes and partially purified *Anaplasma* bodies or antigen (Rogers, Hidalgo, and Dimopoullos, 1964) by the method previously described for obtaining lactic dehydrogenase from duck erythrocytes (Sherman, 1961), with the following modifications. Erythrocytes, freed from plasma and white blood cells by washing three times in physiological saline (0.85%) and centrifugation at 800 × *g* for 15 min, and partially purified *Anaplasma* bodies were diluted 1:1 with 0.067 M phosphate buffer at *p*H 7.0. The mixture was then frozen (in a Dry Ice-alcohol mixture at −70 C) and thawed three times and centrifuged at 4 C for 20 min at 12,000 × *g*. The supernatant fluid was used in 0.01- to 0.04-ml samples as enzyme source, depending on its relative activity.

Experimental. Preliminary studies were carried out on separate pools of normal and infected erythrocytes from four calves in each group. Hematological examinations of the erythrocytes of two animals were conducted periodically throughout infection with *A. marginale*, and were related to the catalase activity of the preparations.

Rabbit antisera were prepared against normal bovine erythrocytes and against *Anaplasma*-infected bovine erythrocytes to determine the source of increased catalase activity in partially purified *Anaplasma* body preparations as compared with normal red blood cells. These antisera were diluted 1:5 either in extracts of partially purified *Anaplasma* bodies or in normal erythrocytic extracts, incubated for 1 hr at 20 C, and then assayed for catalase activity. Control samples of both erythrocytes and partially purified *Anaplasma* bodies or antigen were diluted in normal rabbit serum.

Determination of activity. Analyses for catalase activity were carried out by a spectrophotometric method previously described (Bergmeyer, 1963). A unit of catalase is defined as that amount which will liberate one-half of the peroxide oxygen from a hydrogen peroxide solution of any concentration in 100 sec at 25 C (Bergmeyer, 1955).

RESULTS AND DISCUSSION

Preliminary studies revealed higher catalase activity in the infected pools than in the normal pooled erythrocytes. The average activity in the normal pool was 534 units per ml of packed red blood cells, whereas in the infected pool the average was 692 units per ml.

To determine the time during infection when the increase occurred as related to degree of infection, erythrocytes were assayed for catalase activity throughout the course of disease. As the percentage of infected erythrocytes increased during the disease, the catalase activity also increased (Table 1). Since marginal bodies were present in the erythrocytes during increased activity, the indication was that the activity was associated with marginal bodies. The fluctuation in catalase activity and *Anaplasma* body counts was possibly due to immature red blood cells. During anaplasmosis anemia, immature cells appear in great numbers, and they have been shown to be carriers of higher catalase activity than older cells (Allison and Burns, 1955; Bishop and Surgenor, 1964; Westerman, Pierce, and Jensen, 1963).

TABLE 1. *Changes in catalase activity and Anaplasma body counts in erythrocytes throughout anaplasmosis infections*

Day*	Calf 1		Calf 2	
	Catalase activity (units/ml)	ABC†	Catalase activity (units/ml)	ABC
−2	690	—	483	—
0	666	—	463	—
2	700	1.0	603	1.0
4	680	1.0	723	1.0
6	723	1.5	747	2.5
9	738	9.0	817	30.5
11	755	55.5	956	24.5
13	785	62.5	865	76.0
16	Died			4.0
18			780	2.0
20			825	1.5

* Day relative to inoculation date.

† ABC = *Anaplasma* body count in per cent erythrocytes showing bodies.

TABLE 2. *Changes in catalase activity of normal erythrocytes and partially purified Anaplasma bodies when treated with various rabbit antisera*

Cells	Catalase (units/ml)	Decrease (units/ml)	Per cent decrease
Normal erythrocytes	758	—	—
Normal erythrocytes + RAS-NRBC*	487	271	35.8
Normal erythrocytes + RAS-ARBC†	685	73	9.6
Partially purified *Anaplasma* bodies	410	—	—
Partially purified *Anaplasma* bodies + RAS-NRBC	290	120	29.3
Partially purified *Anaplasma* bodies + RAS-ARBC	150	260	63.4

* RAS-NRBC = rabbit antiserum against normal bovine red blood cells.

† RAS-ARBC = rabbit antiserum against infected bovine red blood cells.

The antigen or partially purified marginal bodies were then assayed for catalase activity, and concentrations of 400 to 775 units per g (wet weight) were obtained in various preparations.

To further identify the marginal body as the source of increased catalase activity, rabbit antisera were prepared against normal bovine red blood cells and *Anaplasma*-infected erythrocytes. These antisera were incubated with extracts of normal bovine erythrocytes and partially purified *Anaplasma* bodies along with controls containing normal rabbit serum. Rabbit antiserum prepared against normal erythrocytes reduced catalase activity in normal erythrocytic extracts by 271 units, whereas only 73 units of the activity of normal erythrocytes were removed by incubation with rabbit antiserum against *Anaplasma*-infected erythrocytes (Table 2). Partially purified *Anaplasma* bodies lost 120 units of their activity when incubated with rabbit antiserum against normal erythrocytes. This observation demonstrated either the presence of stromatal material attached to the bodies or a similar protein moiety of at least part of the catalase of the body to that of the cell. The mixing of rabbit antiserum prepared against *Anaplasma*-infected erythrocytes with partially purified *Anaplasma* bodies decreased activity in the body preparation by 260 units. These data suggest that the increase in catalase during anaplasmosis comes from the marginal body rather than the erythrocyte.

The exact mechanism and purpose of catalase activity in the parasitized cells is unknown, but this activity may aid in the decomposition of peroxide or in catalysis of peroxidation reactions. This mechanism is likely to remain obscure until the organisms can be grown free from host cells or purified to the extent that they are free from erythrocytic material.

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