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TECHNICAL MANUSCRIPT 32

CHANGES IN BLOOD CONSTITUENTS
AND EVIDENCE OF
CENTRAL NERVOUS INVOLVEMENT
RESULTING FROM ANTHRAX TOXIN

NOVEMBER 1962

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
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ANIMAL RESEARCH

In conducting the research reported herein, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society of Medical Research.

U.S. ARMY CHEMICAL-BIOLOGICAL-RADIOLOGICAL AGENCY
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Lt. David F. Fitzpatrick
Lt. Jerry S. Walker
Morris A. Rhian
Frederick Klein
Ralph E. Lincoln

Process Development Division
DIRECTOR OF DEVELOPMENT

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ABSTRACT

After rabbits were challenged with sterile anthrax toxin, cholinesterase in the blood initially increased, then after 6 to 12 hours it began a gradual decrease to 40 to 50 per cent of that of the control. Cholinesterase inhibition also was observed in blood of monkeys infected with systemic anthrax. When Wistar rats were challenged with toxin, a marked increase of immature white blood cells was observed. Paralysis of the monkey and rabbit suggested central nervous system involvement. These observations on cholinesterase inhibition and/or shifts in white blood cells may be useful to detect establishment of systemic anthrax in cases of suspected exposures prior to the observance of the terminal septicemia.

Systemic anthrax of man or animals rarely is detected before the occurrence of the preterminal septicemia. A means of early detection, therefore, is needed in order to initiate treatment.

During our studies on pathogenesis of anthrax, studies were made of the effect of both the bacillus and of toxin alone on experimental hosts. Following challenge, inhibition of cholinesterase and a marked increase in immature granulocytic leucocytes was observed. These physiological reactions following challenge of experimental hosts with toxin or spores are discussed in this paper. Certain physiological effects purportedly due to toxin on the central nervous system are described. Since these tests are nonspecific, their limited medical value is recognized.

The quantitative procedure for determining red blood cell cholinesterase activity was that of Fleisher, Pope, and Spear,¹ which involves incubation of hemolyzed whole blood with acetylcholine and measurement of the color imparted by an indicator. The qualitative screening method of Limperos and Ranta² also was used in our initial work. This test indicates none, some, or major (not necessarily complete) inhibition of cholinesterase. White blood cell determinations were made using the methylene blue-phloxine in propylene glycol method of Randolph.³ Both total and differential counts were determined from the same wet preparation by this method.

The sterile anthrax toxin used in these studies was produced in vitro by the method of Thorne et al.⁴ For rabbits this toxin was concentrated by freeze drying to four times its original concentration and administered intravenously through the marginal or cephalic ear veins.

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Three groups of New Zealand white rabbits have been observed for physiological changes following challenge with from 25 to 65 milliliters of toxin. Ten rabbits were challenged. Various controls were run, including injection with saline, culture medium, antiserum, and toxin neutralized with heat or antiserum. Data for change in cholinesterase for one group of rabbits are given in Table I.

TABLE I. CHOLINESTERASE ACTIVITY IN RABBITS GIVEN IN VITRO TOXIN

Time, hours	Milliliters of 4 X Toxin Injected IV Into Each of Four Rabbits			
	25	35	50	65
0	1.70 ^a	2.22	1.91	2.00
24	1.80	2.36	1.32	1.90
48	1.40	2.22	1.40	1.62
60	1.34	1.80	1.64	1.48
72	1.30	Dead	1.14	1.40
100	0.88	--	1.02	Dead
102	Dead	--	Dead	--
Per Cent Decrease of Cholinesterase Activity	48	19	47	30

a. Micromoles of acetylcholine hydrolyzed.

An initial increase in cholinesterase activity from approximately 1.70 to 2.10 micromoles of acetylcholine hydrolyzed was observed at six hours, followed by a gradual decrease to 40 to 50 per cent of saline-injected control. Death occurred at three to five days. The animals that died showed maximum inhibition of cholinesterase activity at death. Survivors were equally inhibited but gradually regained activity following the critical period.

The cholinesterase change was followed in four rhesus monkeys challenged intradermally with 10^5 anthrax spores of the V1b strain. The qualitative test used indicated partial inhibition at 18 hours and "complete" inhibition at 24 hours after infection. The inhibition remained complete until death at 41 to 48 hours.

White blood cell counts were made of ten rabbits and eight Wistar rats challenged with toxin and two rhesus monkeys challenged with spores. After challenge with toxin, all species showed that total white blood cells increased several fold, accompanied by a marked increase in immature granulocytic cells. With the rabbit this change occurred at 12 hours and was accompanied by the observation of immature red blood cells, indicating a premature release of these cells from the bone marrow. The several kinds of controls showed little response or less extreme rise in white blood cells, and this rise was not accompanied by observation of immature red blood cells.

Our observations with toxin are parallel observations of Bloom et al on anthrax in rabbits to the extent that both groups observed a marked increase in immature granulocytic cells. Eight hours after challenge, a twofold increase in total count of white blood cells was observed for Wistar rats challenged with toxin. A reversal of the ratio of lymphocytes to granulocytes from a normal 80:15 to 40:60 was observed at seven to eight hours. This shift was a result of increased immature granulocytes. Lymphocytes remained normal. Increase in immature granulocytic cells was also observed with anthrax-infected monkeys. Immature red blood cells were observed only just before death, at which time the total white blood cell number was decreasing.

Paralysis has been most pronounced in rabbits challenged with toxin. In most rabbits a flaccid paralysis of the hind quarters was observed at 30 to 36 hours, which continued for 24 to 48 more hours. Initiation of paralysis was closely associated with the observation of cholinesterase inhibition. However, recovery from paralysis preceded significant rise in cholinesterase activity with moderate-to-complete control of the hind limbs being regained. Either full recovery occurred in rabbits challenged at the lowest dose or a spastic paralysis developed 10 to 15 minutes before death. At this time hypersensitivity to touch and sound occurred. The monkey challenged with toxin also showed spastic paralysis several hours before death. Rats showed marked hypersensitivity. In all animals, death occurred rapidly without a prolonged agonal period.

Our observations, which are preliminary and make use of unpurified toxin, show that cholinesterase inhibition is one of the physiological effects resulting from administration of anthrax toxin to experimental animals. There is uncertainty, however, as to whether this phenomenon can be attributed to the direct action of toxin or if it is the result of a "side reaction." Interference with protein metabolism has been suggested, because the decrease in activity gradually occurs over a period of time and never reaches complete inhibition. Inhibition also occurs during the course of disease. When challenge is with toxin alone, hypersensitivity is observed in each of the three species and paralysis in the monkey and rabbits.

The observations made during the course of disease and following administration of anthrax toxin, although preliminary, do indicate that there is involvement of the central nervous system, or, more specifically, a hypothalamic-hypophyseal-adrenal interaction (as in the Waterhouse-Friderichsen syndrome) that is responsible for the observed effects.

The two physiological responses followed in this work, although non-specific, may provide means of detecting anthrax prior to the septicemic stage in animals known to be exposed to anthrax. The tests have value because they forewarn the clinician as to what he might expect even though it does not provide him with a definite laboratory diagnosis. The viciousness of anthrax infection, its slight warnings of danger, followed by a precipitous course of rapid events leading to death, would make presumptive tests valuable additions to the armamentarium of the diagnostician.

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