

PLAGUE POISONS AND VIRULENCE *

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The brief note here given represents a few experiments which were made incidental to a study aiming at immunity to septicemic and pneumonic plague.

Friedberger¹ discovered that powerful poisons could be produced if complement, as present in normal guinea-pig serum, was allowed to act on suspensions of bacteria. These poisons, or "anaphylatoxins," as he named them, give acute shock on injection and cause the death of the animal. Recently Zinsser² showed that animals may acquire distinct tolerance of such poisons and survive large doses of the "proteotoxins" without evidencing any noticeable shock after the preliminary dose, which is so measured as to give a slight shock to the animal. It was also demonstrated that these products apparently possess aggressin-like properties, which, if injected in combination with sublethal doses of bacilli, such as typhoid bacilli, cause death through a resulting bacteriemia.

In these experiments I have tried to obtain Zinsser's proteotoxins from plague bacilli in an attempt to immunize animals with this poison. Contrary to results obtained with other organisms, it was impossible to develop a poison which would give the slightest shock when injected intravenously. Curiously enough, all the animals so treated died of acute plague after several days. Postmortem examination revealed the presence of plague bacilli in great numbers in the blood and organs. Evidently the few bacilli which remained in the supernatant fluid after prolonged centrifugation of the serum plus the organisms, had become more virulent and an aggressive action, as noted by Zinsser, had occurred.

The culture used in these experiments was an avirulent Shanghai strain of *B. pestis* that had been growing on artificial media in this laboratory for 1½ years. The method of procedure was, in general, as follows: An 18- to 24-hour-old culture, grown on agar, was washed off with 1 c.c. of salt solution

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¹ Berl. klin. Wechnschr., 1910, 47, pp. 1490, 1922.

² Jour. Exper. Med., 1914, 20, p. 387.

and incubated with normal guinea-pig serum for various periods at 37 C. At the end of these periods the emulsions were centrifugated at high speed for 1 hour or more, and the supernatant fluids injected intravenously into guinea-pigs.

Experiment 1.—A 24-hour-old culture was incubated with guinea-pig serum (10 c.c.) for 3½ hours.

Guinea-Pig	Weight	Dose	Result
1	432 gm.	1.0 c.c.	No shock. Died after 7 days. Organs and blood full of plague bacilli.
2	411 gm.	0.5 c.c.	No shock. Lived.
3	423 gm.	2.0 c.c.	No shock. Died after 6 days. Same as 1.

In this experiment no shock was produced with doses varying from 0.5 to 2 c.c. Since the incubation period used here seemed rather short, Experiment 2 was made.

Experiment 2.—An 18-hour-old culture of *B. pestis* was washed off with 1 c.c. of salt solution and incubated with 6.5 c.c. of normal guinea-pig serum for 5½ hours.

Guinea-Pig	Weight	Dose	Result
1	310 gm.	2.0 c.c.	No shock. Died after 4 days with acute plague. All organs and blood contained enormous numbers of bacilli.
2	365 gm.	2.5 c.c.	No shock. Died after 5 days. Plague bacilli in all organs and blood.
3	295 gm.	1.5 c.c.	No shock. Died after 5 days. Acute plague as in 1 and 2.

A longer incubation period had no effect on the production of bacillary poison. In Experiment 3 the amount of culture was increased.

Experiment 3.—Two agar cultures, 24 hours old, were washed off with 0.5 c.c. of salt solution, respectively, and incubated with 6 c.c. of guinea-pig serum for 5½ hours.

Guinea-Pig	Weight	Dose	Result
1	196 gm.	1.5 c.c.	No shock. Died after 1 day. No plague bacilli found in smears or in cultures.
2	227 gm.	2.0 c.c.	No shock. Died after 3 days. Typical plague infection. All organs and blood contained great numbers of <i>B. pestis</i> .
3	276 gm.	2.5 c.c.	No shock. Died after 4 days. Same as 2.

An increased amount of culture had no effect on the action of complement so far as producing a visible shock was concerned. In this experiment, as in the preceding ones, some of the animals showed symptoms of discomfort and illness. If we remark the accelerated death in this series of guinea-pigs, it seems very likely that the poison was more marked. When we say "no shock," we wish to imply that the animal did not show the usual symptoms attending anaphylactic or anaphylatoxic poisoning, altho in a few instances very slight tokens of illness could be detected. In Experiment 4 a greatly prolonged period of incubation was tried.

Experiment 4.—A 24-hour-old culture was washed off with 1 c.c. of salt solution and incubated with 9 c.c. of guinea-pig serum for 16 hours at 37 C. The mixture was centrifugated at high speed for 2 hours and then injected in the same manner as before. A control was incubated with salt solution for the same period and likewise injected.

Guinea-Pig	Weight	Dose	Result
1	243 gm.	3.0 c.c.	No shock. Appeared sick. Died after 36 hours. The subcutaneous vessels engorged. Glands inflamed and enlarged. Spleen enlarged and slightly necrosed. Edema marked. No plague bacilli demonstrated in the blood or organs.
2	237 gm.	2.5 c.c.	No shock. Appeared sick. Died within 36 hours. Postmortem findings same as in 1.
3*	267 gm.	3.0 c.c.	No shock. Well. Died after 36 hours. Postmortem findings same as in 1 and 2.

* Control.

Here there is evidence of poisoning. The animals showed all signs of a toxemia and no plague bacilli were found in any of the organs or in the blood. It will be seen, however, that the poison produced did not give any actual shock and that the salt-solution control, moreover, acted exactly like the serum from the treated cultures. These results show, therefore, that in this instance a prolonged incubation period effected autolysis of the bacteria and liberated the endotoxins.

The marked absence of shock in the foregoing experiments did not warrant the supposition that a poison, if at all produced by the action of complement, could by itself exert such strong aggressive action as to kill animals which were injected with exceedingly minute amounts of plague bacilli in themselves not virulent. The few organisms which were not removed by centrifugation could not, by any stretch of the imagination, be held responsible for the death of the animals. In Experiment 5 there is decided evidence that contact with normal guinea-pig serum enhances the virulence of the plague bacilli.

Experiment 5.—A 24-hour-old culture of the avirulent strain used in the preceding experiments was washed off with 1 c.c. of salt solution and incubated with normal guinea-pig serum in the proportions of 0.2 c.c. of the bacterial suspension to each 2 c.c. of the serum. At the end of each incubation period the emulsions were centrifugated, the sediment carefully washed in order to remove all traces of serum, and the bacteria resuspended in salt solution. Injections were then given intraperitoneally with graded doses of the organisms.

Guinea-Pig	Weight	Dose	Incubation	Result
1	342 gm.	0.2 c.c.	5 hr.	Died within 30 hours of acute plague. All organs and blood contained enormous numbers of bacilli
2	305 gm.	0.2 c.c.	22 hr.	Died after 28 hours. Same findings as in 1
3	510 gm.	0.1 c.c.	5½ hr.	Died after 44 hours. Acute plague. Postmortem findings same as in 1
4	576 gm.	0.1 c.c.	5½ hr.	Died after 72 hours. Same as preceding
Controls				
1	344 gm.	0.1 c.c.	Lived. Well
2	250 gm.	0.1 c.c.	Lived. Well
3	348 gm.	0.2 c.c.	Lived. Well
4	372 gm.	0.2 c.c.	Lived. Well
5	380 gm.	0.3 c.c.	Died after 5 days
6	305 gm.	0.4 c.c.	Died after 3 days

In this experiment there is conclusive proof that contact with the serum has enhanced the virulence of the culture. If the control animals are compared with the heaviest test animal, it will be noted that the latter succumbed to at least one-seventh the dose required to kill the former in approximately the same time. This increase in virulence by itself, however, cannot account for the death of the animals treated with plague proteotoxin containing a few bacteria. It is evident that the aggressive action of the poison goes hand in hand with the increase of virulence to bring about the results noted.

These experiments repeated with a virulent strain of plague bacteria, gave identical results except that the animals died sooner after receiving the dose of proteotoxin. Naturally this was to be expected, because the culture was far more virulent and the few remaining bacilli in the guinea-pig serum, when further increased in virulence, brought about an overwhelming septicemia.

Whether or not the same results obtain when sensitized cultures are used, remains to be seen. This phase of the problem is being studied and will make the subject of a separate report.

The advantages possessed by such a poison for immunization purposes become very evident when we consider that we are likely to

obtain the active principle of the organism by resorting to such a method. From previous work done in immunity to bubonic plague, we have all reasons to believe that the more nearly we can approximate a virulent, or at least, a living culture for purposes of immunization, the more hopeful will be the results. Strong's method of inoculating with a living avirulent strain demonstrates this point but is open to the objection that a living culture is uncertain, if not dangerous, since one is ignorant as to the fate of such bacilli after they are injected into the human body. The experiments here reported confirm the likelihood of just such an unfavorable outcome. Assuming that contact with a normal serum, under body conditions, simulates, in a general way, what might occur in the human body, we should not hazard such a method very freely for large-scale immunization. Lustig and Galeotti,³ and more recently Rowland,⁴ have demonstrated the value of active bacillary substances for immunization in bubonic plague. These authors obtained excellent results with their so-called "nucleoproteins" of the plague organism.

The value of plague proteotoxins in pneumonic and septicemic plague is now being studied, and we hope to report on this work in a future communication.

³ Report of the International Plague Conference, Mukden, 1911.

⁴ Jour. Hyg., 1912, 12, p. 344.