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The Effect of Inflammation on the Development of Immunity to Anthrax in Guinea Pigs.

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The effect of inflammation on the virulence of anthrax in guinea pigs was discussed in the preceding paper. The effects on immunity, which are of more immediate practical importance, will be considered here. Most of this type of work on anthrax has been done in connection with the saponin vaccines of Mazzucchi (1929) and Hruska (1931). These workers used anthrax strains suspended in saponin solution as vaccines, and showed that the intense inflammation which followed inoculation resulted in a decrease in virulence and in an increased production of immunity. Some years ago [Sterne (1939)], I reviewed in detail the conflicting reports on Mazzucchi's vaccine, and confirmed, in the accompanying paper, that a saponin excipient increased the immunizing power of anthrax vaccine.

The work reported on here makes clearer the circumstances in which this increased immunizing power occurs.

Experiments.

The " vaccine " used in these experiments was a sporulated culture of an immunizing, avirulent, uncapsulated variant of a virulent anthrax strain. [Sterne (1945)]. The dose used to test immunity was approximately 100 lethal doses of a guinea-pig virulent strain.

1, *The general Effect of acute Inflammation on the Production of Immunity to Anthrax in Guinea Pigs.*

It was shown in the preceding paper that an acute inflammation anywhere in the body retarded the development of a killing strain of anthrax. It should, therefore, also retard the development of a vaccine strain and so lower the degree of immunity produced. Preliminary experiments indicated that this was the case, and, accordingly, the following experiment —summarized in table 1—was arranged to illustrate this point. One lot of guinea pigs received 0·5 c.c. of a $\frac{1}{4}$ per cent. saponin solution subcutaneously on the abdomen, followed 24 hours later by 0·1 c.c. of a sub-immunizing dose of vaccine subcutaneously in a hind-limb. Another lot of guinea pigs received vaccine without any preliminary treatment with saponin. Four weeks later, the guinea pigs in both groups were inoculated with about a 100 lethal doses of the virulent test strain. The results (which conformed to the results with similar tests on killing strains) showed that significantly fewer guinea pigs were immunized in the saponin-prepared group.

Thus the development of anthrax is retarded by an acute inflammation situated at a distance from the site of infection. The invasiveness of virulent strains and the immunizing power of avirulent strains are reduced.

TABLE 1.

The effect of inflammation caused by saponin on the immunizing power of anthrax injected at a different site.

No. of Guinea Pigs.	Inflammation provoked by	Immunized with	Tested 4 weeks later with	Lived.	
				No.	Percentage.
50	0·5cc. 1/8th per cent. saponin in fore-leg	Small dose avirulent spores in hind-leg	100 m.l.d. spores	13	26
50	No inflammation	Small dose avirulent spores in hind-leg	100 m.l.d. spores	33	66
10	No inflammation	Not immunized	100 m.l.d. spores	0	0

2. *The Effects of different Excipients on the Immunizing Power of avirulent Vaccine Strains.*

From the results with virulent strains in the preceding paper, it would be expected that the excipient would have little effect on the immunizing power of large doses of vaccine, but would increase materially the immunity elicited by small doses. This was found to be the case. Large doses immunized so well, with or without any special excipient, that significant differences between groups were not perceptible. When small immunizing doses, which did not elicit a solid immunity to the challenging dose, were used all the irritant excipients tested enhanced the immunizing power.

The effects of inoculating guinea pigs with sub-immunizing amounts of vaccine suspended in different excipients are summarized in table 2. The results paralleled those obtained with small doses of virulent spores in various excipients. All the irritants favoured the multiplication of the vaccine (as could be tested by puncturing and examining the lesion), and consequently increased the immunizing power. This improvement was proportional to the amount of reaction produced by the irritant. Saponin, $CaCl_2$, 20 per cent. Na_2SO_4, 20 per cent. NaCl, had the greatest effect in increasing the immunizing power, 50 per cent. glycerine had a noticeable effect, alum was less effective, and agar had no demonstrable effect, in the concentrations used.

DISCUSSION.

The work reported in this and in the preceding paper shows that several factors must be considered when discussing the effects of inflammation on immunity in anthrax.

An acute inflammation provoked anywhere in the body has the same general retarding effect on the development of a vaccine strain that it has on the development of a virulent strain. In the first case the result is a diminished immunity and in the second a diminished virulence. To this

TABLE 2.

Effect of various excipients on the immunizing power of small doses of avirulent anthrax vaccine.

No. of Guinea Pigs.	Immunized with	Vaccine suspended in	Immunity tested 4 weeks later with	Lived.	
				No.	Percentage.
20	Small dose avirulent spores	2 per cent. saponin	100 m.l.d.	19	95
20	Small dose avirulent spores	1 per cent. saponin	100 m.l.d.	20	100
20	Small dose avirulent spores	$\frac{1}{2}$ per cent. saponin	100 m.l.d.	20	100
54	Small dose avirulent spores	$\frac{1}{4}$ per cent. saponin	100 m.l.d.	52	96
28	Small dose avirulent spores	$\frac{1}{8}$ per cent. saponin	100 m.l.d.	23	82
20	Small dose avirulent spores	$\frac{1}{16}$ per cent. saponin	100 m.l.d.	13	65
62	Small dose avirulent spores	0·85 per cent. NaCl	100 m.l.d.	15	24
52	Not immunized	—	100 m.l.d.	0	0
6	Small dose avirulent spores	30 per cent. NaCl	100 m.l.d.	6	100
20	Small dose avirulent spores	20 per cent. NaCl	100 m.l.d.	19	95
24	Small dose avirulent spores	10 per cent. NaCl	100 m.l.d.	13	54
20	Small dose avirulent spores	5 per cent. NaCl	100 m.l.d.	1	5
32	Small dose avirulent spores	0·85 per cent. NaCl	100 m.l.d.	4	13
32	Not immunized	—	100 m.l.d.	0	0
46	Small dose avirulent spores	50 per cent. glycerine	100 m.l.d.	30	65
20	Small dose avirulent spores	25 per cent. glycerine	100 m.l.d.	3	15
20	Small dose avirulent spores	10 per cent. glycerine	100 m.l.d.	2	10
48	Small dose avirulent spores	0·85 per cent. NaCl	100 m.l.d.	9	19
48	Not immunized	—	100 m.l.d.	0	0
24	Small dose avirulent spores	2·5 per cent. $CaCl_2$	100 m.l.d.	18	75
20	Small dose avirulent spores	20 per cent. Na_2SO_4	100 m.l.d.	19	95
20	Small dose avirulent spores	50 per cent. glycerine + 10 per cent. NaCl	100 m.l.d.	11	55
13	Small dose avirulent spores	$\frac{1}{2}$ per cent. alum	100 m.l.d.	6	46
17	Small dose avirulent spores	0·1 per cent. agar	100 m.l.d.	2	12
96	Small dose avirulent spores	0·85 per cent. NaCl	100 m.l.d.	30	31
75	Not immunized	—	100 m.l.d.	0	0

extent, the use of an irritant excipient which causes a marked oedematous reaction lowers the immunizing power of a vaccine. However, the effect is counterbalanced, and in appropriate circumstances more than counterbalanced, by the increased rate of multiplication of spores or bacilli in damaged tissue.

This stimulation is best demonstrated by injecting subliminal immunizing doses of spores made up in an irritant excipient. The increase in immunizing power is considerable; but, it must be emphasized, this is only evident when small doses of vaccine are used. The process is analogous to that seen with small doses of virulent spores. The subliminal immunizing dose multiplies rapidly in the area affected by the irritant and attains the threshhold necessary for immunization before the defence mechanism is fully mobilized. This increment is readily perceptible, far more so than a similar increment to a large immunizing dose, which immunizes solidly, irrespective of the excipient used.

The antagonistic action between early stimulation due to tissue destruction and later inhibition due to the development of acute inflammation is the key to the interpretation of the effects of inflammation on immunity in anthrax. With an irritant such as saponin, which causes extensive necrosis accompanied by oedema, both factors operate. However, when small doses of immunizing spores are used, the early stimulus, which converts a fraction of an immuizing dose into a full immunizing dose, outweighs the general inhibition developing later. When large doses of spores are used, the inhibitory effect becomes the more prominent. It is not unreasonable to draw analogy between these experiments and those done previously with virulent strains, and to deduce that the secondary inhibition is not induced by irritants such as concentrated NaCl, which do not provoke oedema formation.

Saponin is widely used in practice as an excipient for anthrax vaccines. Mazzuchi claimed success for the use of virulent strains suspended in 20 per cent. saponin. The very violent reaction this caused greatly limited virulence. Had Mazzucchi used attenuated strains he would have found, as others found, that immunizing power was also decreased. He happened to strike a precarious balance between the high immunizing power of a virulent strain and the very marked inhibitory effect of the reaction caused by the high concentration of saponin used. Staub (1932), and Ramon and Staub (1936), who suspended attenuated strains in concentrated saponin obtained poor immunity. They were working in the inhibiting range of saponin activity without the compensation of using a virulent strain. Workers who used lower concentrations of saponin with substantial numbers of spores were unable to detect any advantage, as they were using spore doses too large to be appreciably benefitted by the saponin. Nevertheless, the use of saponin spread, because the concentration of saponin that gave tolerable reactions in practice, and the dose of spores usually included in vaccines were in the range within which stimulation occurs.

Ramon and Staub (1935, 1936) strongly recommended excipients of lanolin and olive oil, or of a mixture of alum and agar. Ramon considered the action analogous to that of alum on formol-toxoids—slow absorption resulting in a prolonged antigenic stimulus. I have been unable to find any evidence for this contention. The increased immunity is due to the rapid increase of the inoculum in damaged tissue, and is elicited by any sufficiently irritant excipient.

It is worth noting that 50 per cent. glycerine (used in the Onderstepoort vaccine as a preservative) has a stimulating action on immunity, although this is less marked than that of saponin or 20 per cent. NaCl. The simplicity and cheapness of the 20 per cent. NaCl excipient have encouraged us to start field trials. It has advantages over saponin in that it does not foam and also acts as a preservative. Its lack of inhibitory action might be a disadvantage with Pasteur type vaccine, but is probably not important with the avirulent strains used at Onderstepoort.

There was no evidence that the beneficial action of saponin was due to any effect on the bacilli. [Homotov (1936)]. The action on the tissues is sufficient to explain the main findings. The dose of spores usually included in vaccines falls in the range appreciably benefitted by an irritant excipient.

Summary.

1. An acute inflammation has the same general inhibitory effect on immunizing power that it has on the virulence of anthrax strains.

2. Irritant excipients have the same local stimulating effect on small immunizing doses of anthrax that they have on small virulent doses.

3. The apparent difference in the effect of irritants on small and on large immunizing doses of spores is because the raising of a sub-immunizing dose to a full immunizing is far more perceptible than the same order of increase of a large dose.

4. The probable advantages of using 20 per cent. NaCl solution as an excipient are pointed out.

The bearing of these findings on earlier reports is discussed.

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