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The Effect of Inflammation on the Survival of Guinea Pigs infected with Anthrax.

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THE extensive literature on non-specific resistance to bacterial infections will not be reviewed in detail, as good surveys were given by Philipson (1937) and Boquet and Stamatin (1939). Most workers in this field have injected bacteria, bacterial products or extracts, or simpler organic or inorganic substances into animals and then shown that these animals were more resistant than controls to the injection of living heterologous bacteria into the prepared area. One might be more general and say that the development of bacteria injected into an inflamed area is retarded to a lesser or greater degree. Boquet and Stamatin emphasized that the inhibition was strictly local. However, Klein (1893), Issaeff (1894), Sobernheim (1895), Bechhold (1922), Kepinov (1924), and other workers, have shown that non-specific procedures such as those indicated above raised the general resistance to infection.

The common factor underlying the different procedures appears to be that they all provoke inflammatory changes, either by the direct injection of irritant chemicals or killed micro-organisms into the skin or the peritoneal cavity, or by the injection of antigen into previously sensitized animals [Philipson (1937)] or by the production of an unrelated disease [Pullinger (1938)]. The work of Hruska (1931), and Mazzucchi (1929) on the effect of adding saponin to anthrax vaccines is an important application in practice of the effects of inflammation on virulence and immunity.

Many workers have demonstrated direct antagonism *in vivo* between different micro-organisms. However, such phenomena as the destruction of *B. anthracis* by *Ps. pyocyaneous*, or by sporulating aerobes, or by moulds, fall outside the scope of this paper.

EXPERIMENTAL.

This work was done on anthrax in guinea pigs. The test spore suspensions were prepared from Pasteur II strains passaged for several generations in guinea pigs to exalt their virulence. These suspensions kept for several months with little deterioration.

1. *The General Effect of Acute Inflammation on the Resistance of Guinea pigs to Anthrax.*

The usual procedure was to inject an irritant into a fore-limb or intraperitoneally and a test dose of anthrax spores (50 to 100 lethal doses) into a hind-limb. Table 1 shows the effect of injecting the irritant 24

hours before the anthrax spores. The disease was appreciably retarded with irritants such as turpentine, saponin, and calcium chloride, while irritants such as lactic acid, concentrated sodium chloride, and concentrated sodium sulphate, had little effect. All these irritants caused extensive necrosis, but only the first group provoked the formation of any considerable oedema. Generally speaking, the inhibitory effect occurred with irritants which provoked oedema formation in addition to necrosis, and the magnitude of the inhibition was very roughly proportional to the extent of the oedema. The local anthrax lesion was appreciably smaller in the animals in which an inflammation had been caused than in the controls.

A large number of experiments were also carried out in which the interval between the injection of the irritant and the spores was varied. The inhibitory effect was found when the irritant was injected from four days before, to eight hours after the anthrax.

Other methods of producing large oedemas were tried, and all retarded the development of anthrax. The general inhibitory effect was not related to any increase or decrease of circulating leucocytes.

2. *The Effect of Injecting Anthrax Spores into an Inflamed Area.*

The majority of the experiments done by Boquet and Stamatin (1939) were of this type and they showed a marked inhibition of the severity of anthrax injected into the inflamed areas. In table 2 are shown the effects of injecting an irritant subcutaneously and following this up with a large test dose of spores (50 to 100 m.l.d.) into the same area after various intervals. There was inhibition of a fairly high order, considerably higher than shown in the first series of experiments. This was shown by inflammations from 8 to 120 hours old, but was at its maximum with inflammations 24 to 48 hours old. The inhibitory effect of inflammations younger than 8 hours was of the same order as the general effect noted previously. Again the irritants that caused necrosis without marked oedema had little effect on virulence (table 2A).

Up to this point, the results agreed with those of Boquet and Stamatin. When, however, the test dose was reduced to 1/500th of that previously given, the results obtained were very different. In the experiment set out in table 2B, this small dose was injected into a saponin inflamed area at 0 to 480 minutes after the saponin injection. The zero time represents saponin and spores given simultaneously. The control group received the spores in a normal site. It is clear, from the table, that the deaths still occurred later in the saponin groups; but the proportion of deaths in these groups was considerably higher than in the controls. This complete reversal of the findings with larger doses of spores is far more clearly shown in the series of experiments summarised in table 3.

3. *The Effect of Mixing Anthrax Spores with Excipients Causing Necrosis and Inflammation.*

The effect of various excipients on the virulence of micro-organisms is of great practical importance. Hruska (1931) and Mazzucchi (1929) and others claimed that anthrax spores in saponin were considerably reduced in virulence. There has been a lack of unanimity on this point. In table 3 are set out the results of using different excipients with doses of anthrax spores varying from approximately 100 lethal doses to a five-hundredth of this amount.

TABLE 1.

Effect of Inflammation Provoked by Different Chemicals on 50 to 100 Lethal Doses Anthrax Spores Injected 24 Hours Later at Another Site.

No. of Guinea Pigs.	0·5c.c. of following chemicals subcut. in fore-limb.	24 hours later 0·1c.c. spores in hind-limb.	RESULTS.						
			Dead by						Lived.
			2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	
20.....	2 per cent. saponin	50–100 m.l.d.			5	11	4		0
20.....	1 per cent. saponin	50–100 m.l.d.			10	7	3		0
20.....	½ per cent. saponin	50–100 m.l.d.		4	12	2	2		0
70.....	¼ per cent. saponin	50–100 m.l.d.	1	33	32	4			0
20.....	⅛ per cent. saponin	50–100 m.l.d.		12	5	2		1	0
20.....	$^1/_{16}$ per cent. saponin	50–100 m.l.d.	1	11	6	1	1		0
20.....	$^1/_{32}$ per cent. saponin	50–100 m.l.d.	6	9	5				0
35.....	10 per cent. $CaCl_2$	50–100 m.l.d.	5	17	10	2	1		0
45.....	Turpentine	50–100 m.l.d.	4	19	18	2	1		1
20.....	20 per cent Na_2SO_4	50–100 m.l.d.	20						0
20.....	20 per cent. NaCl	50–100 m.l.d.	19		1				0
10.....	10 per cent. lactic acid	50–100 m.l.d.	10						0
240.....	Nil	50–100 m.l.d.	188	46	6				0

TABLE 2.

Effect of Injecting 50 to 100 Lethal Doses Anthrax Spores into Inflammations of Different Ages.

No. of Guinea Pigs.	Inflammation provoked by 0·5cc. subcut. of	50–100 m.l.d. spores into inflamed area after	Results.									
			Dead by								Lived	
			2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	*nth day.	No.	Percentage.
25	¼ per cent. saponin	120 hours		9	10	3				1	2	8
36	¼ per cent. saponin	72 hours		5	10	3	1	1		1	15	42
25	¼ per cent. saponin	48 hours		3	1	6	1	1		3	10	40
21	¼ per cent. saponin	32 hours				2	2	2			15	71
105	¼ per cent. saponin	24 hours	1		2	7	13	9	5	4	64	61
41	¼ per cent. saponin	8 hours		1	4	11	3	1	4	5	12	29
20	¼ per cent. saponin	2 hours		9	7	3			1		0	0
83	Nil (controls)	spores only	60	22	1						0	0

* = 9th and following days.

TABLE 2A.

Comparison of Effects of Injecting 50-100 m.l.d. Anthrax Spores into 24 Hours' Old Necroses Accompanied by Oedema (A), and Unaccompanied by Oedema (B).

No. of Guinea Pigs.		Inflammation provoked by 0·5cc. subcut. of	50–100 m.l.d. in necrosed area after	Results.									
				Dead by								Lived.	
				2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	*nth day.	No.	Percentage.
105	** A	¼ per cent. saponin	24 hours	1		2	7	13	9	5	4	64	61
40	A	10 per cent. $CaCl_2$	24 hours	3	5	7	3	5	5		5	7	18
40	A	Turpentine	24 hours	1	3	6	7	6	3	7	3	4	10
40	B	30 per cent. NaCl	24 hours	13	18	5	2	2				0	0
10	B	20 per cent. Na_2SO_4	24 hours	7	3							0	0
78		Nil (controls)	Spores only	57	20	1						0	0

*nth = 9th and following days. **A = oedema. **B = no oedema.

TABLE 2B.

The Effect of Injecting a Small Dose of Anthrax Spores (Circa 1/10 of a Lethal Dose) into Sites prepared with Saponin.

No. of Guinea Pigs.	Inflammation provoked by 0·1cc. of	$^1/_{10}$th m.l.d. spores injected into inflamed area after	Results.									
			Dead by								Lived	
			3rd day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	nth day.	No.	Percentage.
40	½ per cent. saponin	480 min.					2	3	5	11	19	48
40	½ per cent. saponin	320 min.				2	8	11	4	8	7	18
40	½ per cent. saponin	240 min.			1			2	5	7	25	63
40	½ per cent. saponin	160 min.		6	2	3		2	1	8	18	45
40	½ per cent. saponin	120 min.		1	7	3	3	3	3		20	50
40	½ per cent. saponin	80 min.		11	9	1		2			17	43
40	½ per cent. saponin	60 min.		3	14	7	1		1		14	35
40	½ per cent. saponin	40 min.		17	8	2	1	1		1	10	25
40	½ per cent. saponin	20 min.	2	18	10	3		1		1	5	13
40	½ per cent. saponin	10 min.		13	16	2	2	1	3		3	8
40	½ per cent. saponin	5 min.		17	10	4	1	1			7	18
40	½ per cent. saponin	0 min.		22	13	2					3	8
80	Nil (spores only).		11	7	1	5		3		1	52	65

nth = 10th and following days.

The development of the larger doses was markedly retarded by excipients that elicited oedema, but not by excipients causing necrosis with little oedema. The retarding effect of the saponin is clearly evident. When the dose of spores was reduced to a half and to a tenth of a lethal dose an entirely different result appeared. All the excipients now used markedly increased the virulence of the spores. Although saponin and calcium chloride, which provoke an oedema response, delayed the onset of death, the end result was far more deaths in the groups where an irritant excipient was employed than in the controls. A striking result was obtained when small doses of vegetative bacilli were used instead of spores. An excipient containing a small amount of histamine also increased the virulence of small doses of spores.

The points emphasized are that excipients such as saponin and calcium chloride, which cause necrosis and oedema, slow down the development of large anthrax inocula, while irritants such as concentrated salts, lactic acid, etc., which do not elicit an oedema have little effect. However, all these irritants, whether they retard the development of large inocula or not, markedly increase the killing power of small inocula. Even here the irritants that provoke an oedema delay the onset of deaths.

Discussion.

The general action of inflammation accompanied by considerable oedema on the virulence of anthrax is interesting; but the discussion of this and related phenomena will be left for a separate paper.

The effects of injecting anthrax spores into an inflamed area, or of injecting spores or bacilli with irritant excipients, depend on the nature of the irritant and on the dose of spores or bacilli used. The apparently contradictory observations made here that excipients such as saponin increase the virulence of small inocula and decrease that of large inocula is one of the reasons for the conflicting reports on the action of saponin found in the literature. The effects of local and general factors evoked by inflammation vary with the size of the anthrax inoculum. The initial tissue destruction caused by any irritant, favours the multiplication of small inocula of about $^1/_{10}$th to $\frac{1}{2}$ a lethal dose, so that the effective value of the inoculum is increased from a fraction to a full lethal dose. Such an increase is very striking, far more so than a similar increase imparted to say a 100 lethal doses, which already excercise a maximum effect. Although the irritant acts in the same way on both small and large inocula, only the effect on the former is evident. The general retarding effect of inflammation also acts on both small and large inocula. In the former, the general retarding effect, although shown by the delayed onset of deaths, is more than counterbalanced by the increased rate at which the bacilli multiply in the injured tissue. This stimulation is not so obvious when large inocula are used; when the general retarding effect of inflammation on virulence becomes more prominent. All the irritants tested increased the killing power of small inocula. Those irritants that also provoked oedema formation caused a delay in the onset of deaths, even where the final tally showed that the killing power of the inoculum was enhanced. Only those irritants evoking oedema formation excercised an inhibitory effect on large inocula.

TABLE 3

Effect of Different Excipients on Various Anthrax Inocula.

No. of Guinea Pigs.	Excipient.	Dose spores.	Results.								
			Dead by							Lived	
			2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.	nth day.	No.	Percentage.
20	0·2cc. 1½ per cent. saponin	50 m.l.d.				1	8	5	2	4	20
40	0·2cc. ½ per cent. saponin	50 m.l.d.			5	16	8	6	4	1	2½
20	0·2cc. ¼ per cent. saponin	50 m.l.d.		2	7	4	3	1	1	2	10
20	0·2cc. ⅛ per cent. saponin	50 m.l.d.		9	9		1		1	0	0
20	0·4cc. 20 per cent. Na_2SO_4	50 m.l.d.	18	2						0	0
15	0·4cc. 30 per cent. NaCl	50 m.l.d.	14	1						0	0
65	0·4cc. 0·85 per cent.. NaCl.	50 m.l.d.	46	13	3	3				0	0
20	0·1cc. 2½ per cent. saponin	5 m.l.d.					9	5	3	3	15
20	0·1cc. 1 per cent. saponin	5 m.l.d.			1	2	11	2	1	3	15
20	0·1cc. ½ per cent. saponin	5 m.l.d.		1	8	5	2	2	1	1	5
20	0·1cc. ¼ per cent. saponin	5 m.l.d.		6	10	3	1			0	0
40	0·1cc. 30 per cent. NaCl	5 m.l.d.	8	16	5	5	2			4	10
20	0·1cc. 15 per cent. NaCl	5 m.l.d.	9	5	5	1				0	0
20	0·1cc. 10 per cent. NaCl	5 m.l.d.	16	4						0	0
20	0·1cc. 5 per cent. NaCl	5 m.l.d.	5	11	4					0	0
20	0·1cc. 50 per cent. glycerine	5 m.l.d.	9	7	3	1				0	0
20	0·1cc. 25 per cent. glycerine	5 m.l.d.	6	10	4					0	0
20	0·1cc. 5 per cent. glycerine	5 m.l.d.	3	10	4	1	1			1	5
20	0·1cc. 2 per cent. lactic acid	5 m.l.d.	6	13	1					0	0
20	0·1cc. $^1/_{10}$ per cent. histamine	5 m.l.d.	7	8	3	1				1	5
90	0·1cc. 0·85 per cent. NaCl	5 m.l.d.	28	36	11	5	4	2		4	4½
50	0·1cc. ⅛ per cent. saponin	½ m.l.d.		4	33	5			1	7	14
50	0·1cc. 0·85 per cent. NaCl	½ m.l.d.	2	10	9	4	2	1	2	20	40
40	0·1cc. ½ per cent. saponin	$^1/_{10}$th m.l.d.			22	13	2			3	8
50	0·1cc. ⅛ per cent. saponin	$^1/_{10}$th m.l.d.		1	38	5	1		1	4	8
50	0·1cc. 8 per cent. $CaCl_2$	$^1/_{10}$th m.l.d.		8	37	2			2	1	2
50	0·1cc. 50 per cent. glycerine	$^1/_{10}$th m.l.d.		21	28	1				0	0
50	0·1cc. 20 per cent. NaCl	$^1/_{10}$th m.l.d.		40	10					0	0
20	0·1cc. $^1/_{100}$ per cent. histamine	$^1/_{10}$th m.l.d.		5	8	2			1	4	20
150	0·1cc. 0·85 per cent. NaCl	$^1/_{10}$th m.l.d.		15	16	8	5		6	100	67
50	0·1cc. 20 per cent. NaCl	$^1/_{50}$th m.l.d. (bacilli)	11	37	1	1				0	0
50	0·1cc. 0·85 per cent. NaCl	$^1/_{50}$th m.l.d. (bacilli)		1						49	98

nth = 8th and following days.

Summary and Conclusions.

(1) The development of anthrax in guinea pigs is slowed down if an acute inflammation accompanied by an oedema is provoked elsewhere in the body. Thus the injection of saponin into a fore-limb or into the peritoneal cavity retards an anthrax inoculum in the hind-limb. Tissue destruction with inconsiderable oedema, as caused by concentrated salt solution, does not have this effect.

(2) Large doses of anthrax injected into inflamed areas are retarded and often completely inhibited. However, the killing power of fractions of a lethal dose are enhanced if injected into early inflammatory, oedematous lesions, although the onset of deaths may be delayed. The effects of injecting large inocula into necrosed non-oedematous areas are neither increased nor diminished, while the killing power of small inocula is markedly increased.

(3) Excipients such as saponin which cause tissue destruction accompanied by considerable oedema slow down the development of large doses of anthrax. Small doses, fractions of a lethal dose, are, however, stimulated and their killing power greatly increased, although the onset of deaths is somewhat delayed. Excipients such as concentrated salt, which cause necrosis with little oedema, have no apparent effect on large anthrax inocula, but increase the killing power of fractions of a lethal dose, without delaying the onset of deaths.

(4) The apparently anomalous action of inflammation on small and on large inocula is explained by the fact that if stimulation raises say a fifth of a lethal dose to a full lethal dose, the effect is very obvious, whereas the raising of a hundred lethal doses an equivalent amount will be virtually undetectable.

REFFRENCES.

BECHHOLD, H. (1922). Tierexperimentelle Studien über Kolloidtherapie. *Münch. Med. Wochen*, Vol. 69, No. 41, pp. 1447–1449.

BOQUET, A. and STAMATIN, N. (1939). Action empêchante des foyers inflammatoires sur l'évolution du charbon bactéridien. *Ann. Inst. Past.*, Vol. 63, pp. 9–40.

HRUSKA, C. (1931). Vaccination contre le charbon bactéridien avec le virus non atténué. *Compt. Rend. Acad. Sc.*, Vol. 192, pp. 822–823.

ISSAEFF. (1894). Untersuchungen über die künstliche Immunität gegen Cholera. *Zeit. f. Hyg.*, Vol. 16, pp. 287–328.

KEPINOV, L. (1924). Etude sur l'immunité non spécifique. Action immunisante des filtrats bactérien non spécifique sur l'infection cholérique. *Comt. Rend. Soc. Biol.*, Vol. 91, pp. 244–246.

KLEIN, E. (1893). Die Anticholera Vaccination. *Cth. f. Bakt. und Parasitenkunde*, Vol. 13, pp. 426–432.

MAZZUCCHI, M. (1929). Risultati di un nuovo metodo di vaccinazione anticarbonchiosa con alte dosi di germi e di spore virulenti. *La Clin. Veter.*, Vol. 52, pp. 662–671.

PHILIPSON, J. (1937). Experimental studies on enhanced resistance to infection following non-specific procedures. *Act. Path. Scand. Supplement* 32.

PULLINGER, E. J. (1938). Induced tissue resistance to *Brucella abortus* infection. *Journal Path. and Bact.*, Vol. 47, No. 3., pp. 413–422.

SOBERNHEIM. (1895). Untersuchungen über die specifische Bedeutung der Choleraimmunität. *Zeit. fur Hyg. u. Inf. Krankheiten*, Vol. 20, pp. 438–488.