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The Immunization of Laboratory Animals against Anthrax.

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GUINEA-PIGS and rabbits can be immunized against anthrax without difficulty; mice as a rule cannot. The very interesting work of Tomcsik and Bodon (1934) and Tomcsik and Iványovics (1938) on the passive immunization of mice supports Iványovic's (1938) suggestion that mice have a unique and peculiar immunity mechanism against anthrax.

The following experiments were done to see whether mice could be actively immunized with an uncapsulated avirulent anthrax variant, and to compare their reactions with those of guinea-pigs. Uncapsulated variants have been found to immunize guinea-pigs, rabbits, goats, sheep, cattle, and horses; Stamatin and Stamatin (1936), Stamatin (1937), Sterne (1937a, 1937b, 1940).

EXPERIMENTS.

1. *The Active Immunization of Guinea-pigs and Mice against Anthrax.*

Strains used:—

(1) $34F_2$; a rough, uncapsulated, avirulent, immunizing variant isolated on 12.8.36 from a virulent strain grown on serum agar in carbon dioxide.

(2) Boiled $34F_2$; a dense suspension of (1) killed by boiling.

(3) 9Ba; a rough, uncapsulated, avirulent, non-immunizing variant isolated on 30.3.35 from an avirulent, continuously dissociating, smooth mucoid strain.

Guinea-pigs and mice were immunized as shown in Table I. Their immunity was tested with 0·1 c.c. (±100 guinea-pig M.L.D.) of a glycerine-saline spore suspension of a Pasteur II strain.

TABLE I.

Immunization of Guinea-pigs and Mice against Anthrax.

Results of Tests on.	All animals tested with 100 M.L.D. (Guinea-pig) Pasteur II, strain two weeks after immunization with.				
	One Dose $34F_2$.	One Dose $34F_2$ (Boiled).	One Dose 9 Ba.	Several Doses 9 Ba.	Nil (Controls).
Guinea-pigs.....	59/64 (92 per cent.)	—	0/14 (0 per cent.)	0/12 (0 per cent.)	1/133 (0·8 per cent.)
Mice.....	88/160 (55 per cent.)	13/71 (23·9 per cent.)	42/147 (28·6 per cent.)	—	13/155 (8·4 per cent.)

59/64 = 59 survivors out of 64 tested.
92 % = percentage survivors.

Thus guinea-pigs immunized with $34F_2$ were almost completely resistant to the test dose, while those immunized, or hyperimmunized, with 9Ba were fully susceptible. The results got with mice were less clear. Mice immunized with $34F_2$ were significantly more resistant ($P < \cdot 01$, Fisher's χ^2 method) than those treated with either 9Ba or boiled $34F_2$. The difference between the effects of 9Ba and boiled $34F_2$ was not significant, while mice treated with either of these were significantly ($P < \cdot 01$) more resistant that the controls.

It might be argued that the more feeble response of the mouse to immunization was due to its greater susceptibility. This is not borne out by the fate of the controls. Three experiments to test the relative susceptibility to anthrax of mice and guinea-pigs are summarized below. In each experiment the mice and the guinea-pigs were inoculated with the same size dose of a Pasteur II strain.

TABLE II.

Relative Susceptibility to Anthrax of Mice and Guinea-pigs.

Experiment.	Animals Inoculated.	Results.	Mean Survival time (days) of animals that died.	Significance of difference in survival time (Fisher's t Test).
1	Guinea-pigs.....	1/67	$2\cdot9 \pm 0\cdot14$	$P < \cdot 01$
	Mice.....	11/122	$5\cdot2 \pm 0\cdot23$	
2	Guinea-pigs.....	0/10	$2\cdot3 \pm 0\cdot21$	$P < \cdot 01$
	Mice.....	0/42	$4\cdot5 \pm 0\cdot34$	
3	Guinea-pigs.....	0/9	$2\cdot6 \pm 0\cdot20$	$P < \cdot 01$
	Mice.....	5/41	$5\cdot1 \pm 0\cdot47$	

1/67 = one animal survived of 67 inoculated.

Thus in every experiment the mice proved significantly less susceptible than guinea-pigs.

2. *The Rate of Development of Anthrax Immunity in Guinea-pigs.*

A number of guinea-pigs were inoculated with a large dose of the immunizing strain $34F_2$. Another lot received a similar dose of the non-immunizing strain 9Ba. At intervals thereafter the guinea-pigs were tested as shown in Tables III and IV. The vaccine was injected into a hind limb; the test dose into a fore limb.

TABLE III.

Rate of Development of Immunity in Guinea-pigs.

No. of Guinea-pigs.	Immunized with	Tested with 500 M.L.D. Pasteur II after	Results.
3	$34F_2$.....	1 day.....	† (3), † (3), † (3).
3	9Ba.....	1 day.....	† (3), † (3), † (3).
6	—	1 day.....	† (3), † (3), † (3), † (3), † (3), † (3).
3	$34F_2$.....	2 days.....	† (2), † (2), † (3).
3	9Ba.....	2 days.....	† (2), † (2), † (3).
6	—	2 days.....	† (2), † (2), † (2), † (2), † (2), † (3).
3	$34F_2$.....	4 days.....	† (3), † (4), L.
3	9Ba.....	4 days.....	† (2), † (3), † (3).
6	—	4 days.....	† (2), † (2), † (2), † (2), † (3), † (3).
3	$34F_2$.....	5 days.....	L, L, L.
3	9Ba.....	5 days.....	† (2), † (3), † (3).
6	—	5 days.....	† (2), † (2), † (2), † (2), † (3), † (3).
2	$34F_2$.....	8 days.....	L, L.
3	9Ba.....	8 days.....	† (3), † (3), † (3).
3	—	8 days.....	† (2), † (2), † (3).
3	$34F_2$.....	18 days.....	L, L, L.
2	9Ba.....	18 days.....	† (2), † (3).
6	—	18 days.....	† (2), † (2), † (2), † (3), † (3), † (3).
3	$34F_2$.....	24 days.....	L, L, L.
3	9Ba.....	24 days.....	† (3), † (3), † (4).
3	—	24 days.....	† (2), † (2), † (3).

L = lived. † (2) = Died in two days.
— = non-immunized controls.

Thus immunity was shown by guinea-pigs inoculated with $34F_2$ from the 4th day, and was solid from the 5th. No immunity was ever elicited by 9Ba.

Another experiment (Table IV) was carried out to see whether immunity could be detected 24 hours after vaccination. The test dose was reduced to 100 M.L.D. Twice as much 9Ba was given as $34F_2$, as an additional control on possible non-specific effects.

TABLE IV.

Immunity of Guinea-pigs 24 Hours after Vaccination.

No. of guinea-pigs.	Immunized with	Tested with 100 M.L.D. Pasteur II strain after	Results.	Mean survival time in days of guinea-pigs that died.
39.....	$34F_2$.....	24 hours.....	9/39	$4{\cdot}5 \pm 0{\cdot}55$
24.....	9Ba.....	24 hours.....	0/24	$3{\cdot}2 \pm 0{\cdot}85$
26.....	Controls..... (non-immunized).		0/26	$2{\cdot}6 \pm 0{\cdot}66$

There was a significantly greater number of survivors in the group immunized with strain $34F_2$. Moreover the mean survival time of the guinea-pigs that died in this group was significantly longer ($P < {\cdot}01$, Fisher's t test) than that of the guinea-pigs in the other groups. Group 9Ba did not show a significantly longer mean survival time than that of the controls.

One may conclude that guinea-pigs inoculated with strain $34F_2$ showed a distinct and specific increase in resistance to anthrax, 24 hours after vaccination.

DISCUSSION.

Guinea-pigs and mice differed markedly in their reactions to active immunization against anthrax. The former rapidly developed a strong immunity when inoculated with the avirulent, immunizing strain $34F_2$. Some immunity was demonstrable as early as 24 hours after vaccination. This was almost certainly specific as guinea-pigs immunized or hyperimmunized with variant 9Ba showed no immunity whatever.

Mice inoculated with $34F_2$ showed a significant and considerable increase in resistance to anthrax. This, however, was not nearly so marked as in guinea-pigs. Mice inoculated with the non-immunizing strain 9Ba, or with killed suspensions of $34F_2$ also showed a significant increase in resistance. Although this increase was not as great as with $34F_2$, it was large enough to make an interpretation of the results difficult. It showed that much of the apparently specific increase in resistance produced by $34F_2$ could be non-specific. As the mice inoculated with $34F_2$ reacted more severely than those inoculated with the other strains, the influence of non-specific factors, such as inflammation, might have been correspondingly greater.

Clearly, mice are not nearly as easy to immunize actively as guinea-pigs. It may be that they never develop more than a low grade specific immunity. On the other hand mice appear more prone than guinea-pigs to develop considerable non-specific resistance. These findings could not be ascribed to the mouse's great susceptibility to anthrax, for repeated tests proved them less susceptible than guinea-pigs.

The mouse appeared to differ from other animals in its ability to develop active immunity to anthrax after inoculation with uncapsulated, immunizing variants.

Conclusions.

(1) Guinea-pigs inoculated with an avirulent, uncapsulated variant from a virulent anthrax strain rapidly developed immunity to anthrax. A significant, specific, increased resistance was detectable 24 hours after vaccination.

(2) Mice could not be immunized as easily as guinea-pigs; but showed more tendency than guinea-pigs to the production of an increased non-specific resistance.

(3) Repeated tests showed mice to be less susceptible than guinea-pigs to a test dose of approximately 100 guinea-pig M.L.D. of a Pasteur II strain.

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