

IMMUNIZATION WITH HEAT-KILLED MYCOBACTERIUM PARATUBERCULOSIS IN MINERAL OIL. II

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We reported recently some attempts to "immunize" sheep with heat-killed *Mycobacterium paratuberculosis* suspended in mineral oil (Sigurdsson and Tryggvadóttir, 1949). In that work we employed, in the various experiments, from 5 to 300 milligrams of a dried culture of bacteria as the immunizing dose in one injection.

Since this earlier work was finished, we have extended our experiments using smaller quantities of bacteria. Otherwise the experimental procedure and materials were the same as those previously employed, and the reader is referred to our earlier paper on this subject for such information, as the present short report will not repeat the description given there.

Ten sheep were employed in the present experiments. They were divided into five groups of two, which received 6, 3, 1.5, 0.75, and 0.37 mg of dried culture, respectively. Each dose was suspended in 1 ml of sterile mineral oil containing 0.3 per cent phenol. The injections were given on December 8, 1948. We followed the development of infiltrates at the site of injection, the rise in antibody as measured by the complement-fixation test, and the development of cutaneous allergy to avian tuberculin.

Nine of the ten sheep developed fever (40 C or higher) at one time or another from the first to the eighth day. After this their temperatures remained below 39.7 C, and usually below 39.5, for another six days. None of the sheep showed signs of illness at any time during this period. All the animals developed infiltrates of considerable size at the site of injection. These came slowly and were first definitely noticeable on the ninth day, and then only in 6 or 7 of the 10 sheep. The infiltrates continued to increase in size for two months, after which time they remained stationary until the experiment was terminated 11 months after the vaccine had been administered. The infiltrates were of the same general appearance as in our earlier experiments.

At autopsy only one infiltrate was found to adhere to the underlying muscle. In the remaining nine cases they were movable in the subcutaneous tissue. They thus belonged to the first type as described in our earlier paper. The one exception in which the infiltrate was found to adhere slightly to the muscle (second type) was in one of the two sheep that had received the largest dose (6 mg). Table 1 gives the average weight of the infiltrates in each group of sheep. The infiltrates were somewhat smaller than those found in the earlier experiments, but their size this time did not appear to be related to the weight of injected bacteria.

TABLE 1

The weight of infiltrates produced by the various quantities of dried bacteria injected

	WEIGHT OF DRIED BACTERIA INJECTED				
	0.37 mg	0.75 mg	1.5 mg	3.0 mg	6.0 mg
Average weight of infiltrates in each group of sheep.....	53.0 g	28.5 g	70.0 g	55.0 g	40.5 g

TABLE 2

The results of skin sensitivity tests using one intracutaneous injection of avian tuberculin (The injections were given on December 8)

SHEEP NO.	MG INJECTED	DATE OF TEST										
		Dec. 10	Dec. 17	Jan. 10	Feb. 10	March 17	Apr. 24	June 7	July 2	Aug. 15	Sept. 9	Oct. 10
89	6.0	-	-	+	+	+	+	+	+	+	+	+
93	6.0	-	-	-	(+)	+	-	(+)	+	(+)	(+)	-
91	3.0	-	(-)	+	+	+	+	+	+	+	+	+
92	3.0	-	-	+	+	+	+	+	+	+	+	(+)
94	1.5	-	-	+	+	+	+	+	+	+	(+)	+
95	1.5	-	-	-	+	+	+	+	+	+	+	(+)
86	0.75	-	-	(+)	+	+	+	-	(+)	(+)	(+)	+
88	0.75	-	-	(+)	+	+	+	+	+	-	(-)	-
87	0.37	-	-	+	+	(+)	(+)	(+)	+	+	+	+
90	0.37	-	+	+	+	+	+	+	+	+	+	(+)

TABLE 3

The outcome of the complement-fixation tests with sera taken at varying intervals (The figures indicating the strength of the reaction are comparable, as the reading of the reaction was standardized as previously described)

SHEEP NO.	MG INJECTED	DATE ON WHICH SERUM WAS TAKEN										
		Dec. 9	Dec. 18	Jan. 11	Feb. 10	March 17	Apr. 22	June 7	June 30	Aug. 16	Sept. 22	Nov. 2
89	6.0	-	4.5	14.5	18.5	19.0	16.5	14.0	15.5	10.5	7.0	8.0
93	6.0	-	8.5	16.5	16.5	18.5	18.5	20.0	19.0	15.5	13.0	8.5
91	3.0	-	14.5	20.5	21.0	20.0	20.5	19.0	18.0	12.0	8.0	7.0
92	3.0	-	14.5	21.0	22.5	24.5	25.0	23.0	21.5	19.5	21.0	20.5
94	1.5	-	10.5	17.0	18.5	16.5	16.5	16.0	16.0	13.0	7.0	7.0
95	1.5	-	9.0	16.5	19.0	22.0	19.0	17.0	18.0	15.0	14.0	15.0
86	0.75	-	4.5	14.5	18.0	17.5	20.5	21.0	22.0	22.0	19.5	19.0
88	0.75	2.0	4.5	14.5	20.5	22.5	22.5	22.0	19.5	18.0	11.0	9.0
87	0.37	1.5	8.0	14.0	16.5	15.5	16.5	16.5	12.5	12.0	9.0	10.0
90	0.37	-	14.5	16.5	16.5	18.5	18.5	9.5	10.0	10.0	3.5	4.0
Average strength.		0.35	9.3	16.6	18.8	19.5	19.4	17.8	17.2	14.8	11.3	10.8

The internal iliac lymph glands on the injected side were found to be enlarged in 7 cases. The average weight of these enlarged glands was 6.1 grams, with a

maximum of 18 grams. In three cases their weight was normal, i.e., less than 3 grams. The average weight of the glands on the side opposite the injection was 1.7 grams, the highest value being 3.0 grams. Acid-fast bacilli were found in 8 of the 10 infiltrates, but their number was small. The infiltrates proved sterile on ordinary bacteriological media.

The skin sensitivity of the sheep to avian tuberculin was tested 2 and 9 days after the injection of the "vaccine" and after that approximately once a month, 11 times in all. Table 2 gives the outcome of these tests. It will be seen that the sheep all developed a positive reaction, and that most of them maintained it until the end of the experimental period.

Every time that the sensitivity to tuberculin was tested samples of serum for the complement-fixation test were also taken. Regarding the technique of this test and the method for reading it, we refer to our earlier paper. Table 3 shows the outcome of these antibody titrations in the 10 sheep. It will be seen that the titer rose slowly at first and continued to rise for about 2 months. Toward the end of the experimental period it began to fall again, but this fall was no more pronounced in the animals that had received the smaller quantities of bacteria in the beginning. The lesions did not appear to affect the general health of these sheep at all.

DISCUSSION AND SUMMARY

The present short report is an addendum to an earlier, more extensive paper in which it was reported that heat-killed *Mycobacterium paratuberculosis* in mineral oil injected into sheep, in doses varying from 5 to 300 mg, would elicit a powerful and protracted immunological response, including an infiltration of considerable size at the site of injection.

In the present experiments smaller doses were used, i.e., from 6 to 0.37 mg of dried bacteria per sheep. The results obtained were essentially similar to those produced by the larger doses, but the infiltrates at the site of injection were smaller and did not penetrate into the muscle, except very slightly in one case.

A small number of apparently intact acid-fast bacteria were found in the lesions of 8 of the 10 sheep employed.

The skin sensitivity to avian tuberculin developed at least as regularly, and it lasted approximately equally long, as after the larger doses. The sheep developed complement-fixing antibodies in extremely high titers, which were comparable to those observed after the larger doses. It seems, however, that the titer started to fall somewhat earlier in this series than in the earlier experiments with the larger doses. The possible usefulness of this kind of vaccine for producing resistance to infections with *Mycobacterium paratuberculosis* is being investigated.

REFERENCE

- SIGURDSSON, BJÖRN, AND TRYGGVADÓTTIR, A. G. 1949 Immunization with heat-killed *Mycobacterium paratuberculosis* in mineral oil. *J. Bact.*, **58**, 271-278.