

## THE EFFECT OF THE ROUTE OF IMMUNIZATION ON THE IMMUNITY RESPONSE TO PNEUMOCOCCUS TYPE I

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It has been shown that, following repeated inhalations of living Type I pneumococci, agglutinins and protective antibodies are demonstrable in the serum of rabbits (1). By the spray method employed, however, the number of bacteria lodging within the respiratory tract or invading the tissues cannot be controlled. The following study was undertaken in order to determine the immunity response of rabbits injected with fixed amounts of heat-killed pneumococci following (1) intravenous, (2) intraperitoneal, (3) intramuscular, and (4) subcutaneous injection of varying amounts of suspension of heat-killed pneumococcus Type I. The duration of active immunity and the length of time that agglutinins and protective antibodies persist in the serum will be dealt with in a subsequent paper.

### *Method*

The inoculum was composed of washed heat-killed Type I pneumococci suspended in salt solution. 1 cc. was equivalent in bacterial content to 10 cc. of an 18 hour broth culture. The rabbits were injected at 4 day intervals. The first 2 doses given were 0.5 cc., the next 2 doses 1 cc. each, and thereafter 1.5 cc. Different groups of rabbits received a total of 1, 3, 6, 9, 12 and 15 cc. of the inoculum. 10 days after receiving the last dose the animals were bled from the ear vein.

The presence of agglutinins was determined by a modified thread reaction. To 1 cc. of immune rabbit serum diluted in salt solution was added 0.2 cc. of an actively growing broth culture of pneumococcus Type I. The tubes were incubated for 2 hours in the water bath at 37°C., placed in the ice box overnight, and the reactions read the next morning. Agglutinins were recorded as present in the serum only when the reactions were positive in a dilution of at least 1:10.

The presence of protective antibodies in the blood of the immunized rabbits was demonstrated by determining the capacity of a given amount of serum, to protect white mice against intraperitoneal injection of homologous pneumococcus, the virulence of which was such that 0.000,001 cc. killed control mice within 48 hours. The immune serum and culture were administered simultaneously.

TABLE I  
Agglutinins and protective antibodies in rabbits inoculated by

No. of inoculations	Total amount of inoculum	Intravenous			Intraperitoneal			Intramuscular			Subcutaneous		
		Agglutins	Amount of culture against which 0.5 cc. of sera protected		Agglutins	Amount of culture against which 0.5 cc. of sera protected		Agglutins	Amount of culture against which 0.5 cc. of sera protected		Agglutins	Amount of culture against which 0.5 cc. of sera protected	
2	1 cc.	A 1	—	.01	B 1	—	.001	C 1	—	.01	D 1	—	.1
		A 2	—	.01	B 2	—	.001	C 2	—	.001	D 2	—	—
		A 3	—	.01	B 3	1.50	.01	C 3	—	.01	D 3	—	—
4	3 cc.	A 4	1.100	.1	B 4	1.50	.1	C 4	1.10	.1	D 4	—	.000,1
		A 5	1.50	.1	B 5	—	.001	C 5	—	—	D 5	—	.01
		A 6	1.40	.1	B 6	—	.1	C 6	—	—	D 6	—	.01
6	6 cc.	A 7	1.100	.1	B 7	—	.001	C 7	—	.001	D 7	—	.001
		A 8	—	.1	B 8	—	.01	C 8	1.10	.1	D 8	—	.001
		A 9	1.50	.1	B 9	1.20	.1	C 9	—	—	D 9	—	.000,01
8	9 cc.	A 10	1.40	.1	B 10	1.10	.01	C 10	—	.001	D 10	—	.000,01
		A 11	1.50	.1	B 11	—	.01	C 11	1.10	.000,01	D 11	—	.000,01
		A 12	1.100	.1	B 12	1.100	.1	C 12	1.10	.000,1	D 12	—	.000,01
10	12 cc.	A 13	1.500	.1	B 13	1.50	.1	C 13	1.20	.000,1	D 13	—	.000,01
		A 14	1.100	.1	B 14	1.50	.1	C 14	—	.001	D 14	—	.1
		A 15	1.500	.1	B 15	1.50	.1	C 15	—	.01	D 15	—	.1
12	15 cc.	A 16	1.100	.1	B 16	1.40	.1	C 16	—	.01	D 16	—	.000,1
		A 17	1.500	.1	B 17	1.500	.1	C 17	1.10	—	D 17	—	—
		A 18	1.500	.1	B 18	—	.01	C 18	—	—	D 18	—	.001
		A 19	1.400	.1	B 19	1.40	.01	C 19	1.10	.001	D 19	—	—
		A 20	1.200	.1	B 20	1.10	.01	C 20	—	.000,1	D 20	—	—
							C 21	—	—	—	D 21	—	—

Before inoculation a test bleeding was taken from all the rabbits used. None of the normal rabbit sera contained agglutinins nor did the normal sera protect mice against intraperitoneal injections of 0.000,001 cc. of pneumococcus.

### *Agglutinins*

From Table I it is seen that 16 of the 20 rabbits which received, during the course of treatment, from 1 to 15 cc. of killed culture intravenously, developed agglutinins, as tested by the method employed, ranging in titre from 1-40 to 1-500. 3 of the 4 animals in whose serum agglutinins were not demonstrable had received only 2 doses totaling 1 cc. of original culture. Within the limits defined, the agglutinin titre of the immune sera rose as a rule in direct proportion to the amount and number of inoculi which the rabbits had received.

Of the 20 rabbits injected intraperitoneally, 12 developed demonstrable agglutinins. Although 1 of the rabbits which had received a total of 1 cc. developed agglutinins, the percentage was not notably increased among the rabbits that had received 3 and 6 cc. and 1 rabbit failed to show agglutinins even after the administration of a total of 15 cc. The titre of the agglutinins was also lower, being as a rule no higher than 1-50. Only 2 rabbits developed really active agglutinating sera, of 1-100 and 1-500 respectively.

In the case of the rabbits injected intramuscularly the percentage of those showing agglutinins in the blood is less. Of 21 rabbits treated intramuscularly only 7 showed serum agglutinins. In these instances, the agglutinin titre bore little relationship to the amount of vaccine injected. Although the sera of 2 rabbits which had received in all 3 and 6 cc. respectively showed agglutinins, only 1 of the 4 rabbits which had received a total of 15 cc. produced agglutinins. In all instances the agglutinin titre remained low. In only one instance were agglutinins demonstrable in a dilution as high as 1-20.

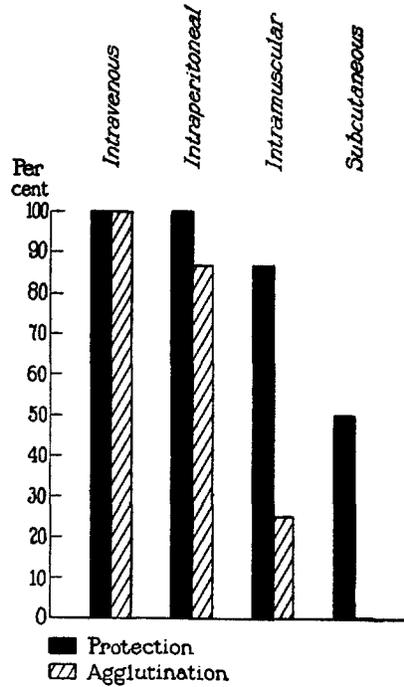
In the case of the 21 rabbits vaccinated subcutaneously none formed demonstrable agglutinins.

### *Protective Antibodies*

From Table I it is seen that all of the 20 rabbits inoculated intravenously developed protective antibodies. The serum of the 3 rabbits which received a total of 1 cc. protected mice against 0.01 cc.

of virulent Type I pneumococci while the sera of the remaining rabbits protected mice against 0.1 cc. of this culture.

The serum of the intraperitoneally injected rabbits also afforded protection in all instances. The amount of protection, however, in most instances was not so great as that exhibited by the serum of the



TEXT-FIG. 1. Comparison of immunity response of rabbits inoculated by various routes with 12 or 15 cc. of heat-killed pneumococcus vaccine

intravenously treated rabbits. In only 9 instances did the rabbit serum protect mice against 0.1 cc. of virulent culture.

The serum of 80 per cent of the intramuscularly injected rabbits afforded some degree of passive protection, but the protective power of the serum was in general less than that of the rabbits inoculated by the intravenous or intraperitoneal route. There was even more irregularity in the amount of protection afforded by the serum from the rabbits which had received the larger amounts of killed culture. The

serum of 2 rabbits which had received respectively a total of 3 and 6 cc. protected against 0.1 cc. of culture. Of the 4 rabbits which had received 15 cc., 1 failed to show any protective antibodies, and 3 protected mice only against 0.001 cc. of pneumococcus culture.

The differences just noted were even more conspicuous in the case of rabbits injected subcutaneously. Although the serum of 71 per cent of these rabbits showed some degree of protection, in most instances this was merely sufficient to protect mice against 0.001 cc. of culture.

The differences in the antibody response of rabbits inoculated by various routes is graphically shown in Text-fig. 1. 12 cc. of the saline suspension of heat-killed pneumococci, Type I, administered to rabbits intravenously proved to be a sufficient amount to insure the development of a high proportion of agglutinins and protective antibodies. In Text-fig. 1 the percentage of rabbits that had received 12 or 15 cc. of inoculum by various routes is graphically shown. The rabbits injected intravenously or intraperitoneally all developed protective antibodies in their serum. Even the rabbits immunized by intramuscular and subcutaneous injection developed protective bodies in most instances. The differences in formation of agglutinins in these same rabbits were striking. Whereas 100 per cent of the intravenously immunized rabbits showed agglutinins, only 87 per cent of the intraperitoneally and 25 per cent of the intramuscularly immunized rabbits produced agglutinins. Those immunized subcutaneously showed none.

#### *Relation between Agglutinins and Protective Antibodies*

From Table I it is also seen that 35 or 42 per cent of the 82 rabbits injected in various ways developed both agglutinins and protective antibodies. The serum of 37 animals showed only protective antibodies; while in 10 instances there was no demonstrable antibody response. In no instance were agglutinins present without protective antibodies. If the serum contained agglutinins, it would also, as a rule, protect mice against 0.1 cc. of culture. This occurred in 26 instances. But in 9 instances a serum containing agglutinins only protected mice against from 0.01 cc. to 0.000,01 cc. of pneumococcus culture. On the other hand, 5 sera which contained no demonstrable agglutinins protected mice against 0.1 cc.; and 12 sera protected against 0.01 cc. of virulent culture.

## DISCUSSION

Since the first demonstration that the serum of animals injected with pneumococci possessed immune properties a great deal of work has been done on the production of pneumococcus immunity. This work has recently been reviewed by Barach (2). Comparatively little attention, however, has been paid to different routes of inoculation. Cecil and Stephen (3) found that intravenous inoculation of small quantities of pneumococcus Type I completely protected monkeys against infection by the homologous organism whereas larger intramuscular doses failed. They also found that intratracheal inoculation would produce immunity but that spraying the throat did not produce complete immunity. Cooper (4) found that submucous inoculation of rabbits in the cheek afforded protection but that a similar inoculum subcutaneously or intradermally in the abdominal wall did not protect rabbits.

Lister (5) showed that the serum of rabbits inoculated intravenously contained agglutinins and opsonins but that the sera from other rabbits inoculated with similar amounts subcutaneously contained no agglutinins and that the opsonic index was only slightly raised.

The present experiments show that while agglutinins and protective antibodies are often both present in an immune serum for pneumococcus Type I their amounts do not necessarily vary together nor are both always found. Intravenous inoculation of rabbits will most regularly give rise to sera containing the two in quantity. Intraperitoneal and intramuscular inoculation elicit them to a less degree. But, following subcutaneous inoculation, according to the method here described, rabbits develop protective antibodies only.

## SUMMARY

1. The sera of 80 per cent of the rabbits intravenously inoculated with fixed amounts of heat-killed pneumococci contained agglutinins and all showed protective antibodies.
2. The sera of 60 per cent of the intraperitoneally inoculated rabbits contained agglutinins and all showed protective antibodies.
3. The sera of 33 per cent of the intramuscularly inoculated rabbits contained agglutinins and 86 per cent also showed protective antibodies.

4. None of the sera of the subcutaneously inoculated rabbits contained agglutinins although protective antibodies were present in 71 per cent.

5. Although there is a close relationship between the presence of agglutinins and protective antibodies in a given immune serum, these do not run parallel.

## REFERENCES

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