FACTORS INVOLVED IN THE INFECTION OF MICE AFTER VACCINATION WITH TYPE II PNEUMOCOCCI.

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In a previous study[1] it was found that vaccination of a group of mice did not protect all the individual animals against subsequent infection with Type II pneumococci. The protection obtained was irregular; some individuals resisted relatively large numbers of pneumococci, while other individuals succumbed to much smaller doses. The present paper deals with a further study of the infection of individual mice after vaccination against Type II pneumococci. The experiments were planned to determine the relative importance of two factors in the postvaccination infections: the previous immunity response of the individual animals, and the dosage of the invading bacteria. The factor of differences in the immunity response of the individual is made especially prominent, for the animals (mice) not only possess no demonstrable natural immunity to the specific bacteria (pneumococci) but the vaccination was done with a type of Pneumococcus known not to produce a highly effective antibacterial serum.

EXPERIMENTAL.

Methods.

Immunization.—70 line-bred females, between 3 and 4 months old, which had been separated from males since weaning were selected for immunization.

The organisms from a 10 hour broth culture of Type II pneumococci (Strain D_{39}, Hospital of The Rockefeller Institute) were resuspended in salt solution and heated at 60°C. for 30 minutes. Each mouse received, subcutaneously, 0.5 cc. of a

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[1] Gaspari, E. L., Fleming, W. L., and Neill, J. M., *J. Exp. Med.*, 1927, xlvi, 101.

diluted suspension of the vaccine (equivalent to 0.1 cc. of broth culture) every 2 days for six doses. 10 days after the last injection a number of mice were tested for acquired immunity. The remainder were given a second course of immunizations with freshly prepared vaccine at 2 day intervals for five doses. After a rest period of 10 days, a number of animals were tested for immunity and the remainder were given a third course of immunizations with fresh vaccine exactly as in the second course; these animals were also tested for acquired immunity 10 days after the last injection.

A number of mice died during the period of immunization, and others were eliminated from the tests because of evident poor condition.

Tests of Immunity of the Animals (Protection Tests).—The tests of immunity of the vaccinated animals were made by intraperitoneal injections of 0.5 cc. of broth containing the desired amount of a 10 hour broth culture of the same virulent strain as that from which the vaccine had been prepared.

Experiments 1, 2, and 3: Influence of Size of Dose upon Infection: Active Immunity Tests of Vaccinated Mice by Injection of Different Numbers of Bacteria.

The first series of experiments (Experiments 1, 2, and 3) consisted of tests of the influence of size of dose upon the occurrence of infections in groups of mice which had previously received vaccination against Type II pneumococci. The doses used in these active immunity tests were 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} cc. of broth culture of pneumococci; this range of doses includes the numbers of pneumococci against which passive protection is usually effective. The mice used in Experiment 1 had received one course of vaccination before the immunity tests; the animals used in Experiments 2 and 3 had received, respectively, two and three courses. The purposes of the investigation are met by presenting the three experiments collectively, since each animal had received the same immunization treatment as the other individuals used in the same experiment. The results of of Experiments 1, 2, and 3 are presented in Table I; a summary of these results is collected in Table II.

Injections and Virulence Controls.—The vaccinated mice were injected as described under "Methods." The normal mice which were injected for virulence controls invariably succumbed to all amounts of culture tested—*i.e.*, from 10^{-7} cc. to 10^{-3} cc. The results of the virulence controls are presented collectively in the last column of Table I.

Control of Possible Effect of Weight of Individual Animals.—All the mice used in

TABLE I.

Tests of the Active Immunity of Vaccinated Mice.

Amount culture injected	Experiment 1. Immunity tests on first group of mice (after one course of vaccinations)	Experiment 2. Immunity tests on second group of mice (after two courses of vaccinations)	Experiment 3. Immunity tests on third group of mice (after three courses of vaccinations)	Virulence controls* (non-immunized mice) on Experiments 1, 2, 3
cc.				
10^{-3}	D 24–48 hrs. S 5 days	D 24–48 hrs. D 24–48 hrs. D 24–48 hrs.	D 24–48 hrs. D 24–48 hrs. D 24–48 hrs. D 24–48 hrs. D 48–72 hrs. S 5 days S 5 days	D 24–72 hrs. D 24–72 hrs.
10^{-4}	D 24–48 hrs. S 5 days	D 48–72 hrs. S 5 days S 5 days	D 48–72 hrs. D 48–72 hrs. D 48–72 hrs. D 48–72 hrs. D 48–72 hrs. D 48–72 hrs. S 5 days	D 24–72 hrs. D 24–72 hrs.
10^{-5}	D 24–48 hrs. S 5 days	D 24–48 hrs. D 24–48 hrs. S 5 days	D 24–48 hrs. D 48–72 hrs. D 48–72 hrs. D 48–72 hrs. D 48–72 hrs. S 5 days S 5 days	D 24–72 hrs. D 24–72 hrs.
10^{-6}	D 24–48 hrs. S 5 days	S 5 days S 5 days S 5 days	D 24–48 hrs. D 24–48 hrs. D 24–48 hrs. D 48–72 hrs. D 48–72 hrs. D 48–72 hrs. S 5 days	D 24–72 hrs. D 24–72 hrs.
10^{-7}	No tests	No tests	No tests	D 24–72 hrs. D 24–72 hrs.

D = died of pneumococcus septicemia.

S = survived.

* Presented in composite form; duplicate mice were injected with each amount of culture in each of the three experiments.

the experiments were in excellent physical condition but varied in weight from 24 to 31 gm. To rule out any possible influence of weight upon the resistance to infection, the mice of different weights in Experiments 2 and 3 were matched so that the tests of the different amounts of culture were made with a series of animals of comparable weight. The results showed that there was no correlation between weight of the animal and its susceptibility.

Survival in Table I.—Since all the virulence controls died in less than 72 hours after injection the protection of the vaccinated mice is based upon survival for 5 days after the test injection. Three of the mice reported as "Survived" in Table I died between 10 and 14 days after injection.

The most obvious fact in Table I is the irregularity in the survival and death of the vaccinated mice. In each of the three experiments individual mice were infected by doses smaller than the doses which

TABLE II.

Summary of Tests for Active Immunity of All the Vaccinated Mice: Proportion of Animals Protected against Different Doses of Pneumococci.

Amount of culture injected.	Number of vaccinated mice tested	Number of mice protected	Proportion of mice protected
cc.			*per cent*
10^{-3}	12	3	25
10^{-4}	12	4	33
10^{-5}	12	4	33
10^{-6}	12	5	42
10^{-3} to 10^{-6}	48	16	33

other mice survived and *vice versa*. This lack of relationship between the number of pneumococci injected and the infection of the vaccinated animals is emphasized in the summary of the experiments given in Table II. There it is seen that about the same proportion of the vaccinated mice survived the injection of each of the different doses; in fact, any deviation from the average proportion of one-third survivors represents the survival or death of only one mouse.

Since these facts indicate that within this range of dosage (10^{-6} to 10^{-3} cc.) the size of the dose does not influence the infection of the vaccinated animals, the infection or resistance of individuals would logically be attributed to differences in individual immunity. In these experiments the immunity of the animals was limited to that

acquired by vaccination. Therefore, within the range of dosage used in these experiments, the previous immunity response of the individual appeared to be the factor determining the postvaccination infections.

Obtaining a "Selected Group" of Actively Immune Mice.

The preceding results indicated that only a certain proportion of the mice respond effectively to pneumococcus (Type II) vaccine. This fact made it impossible to carry out additional experiments until we obtained a selected group of vaccinated mice. It seemed that a "selected group" of individuals known to possess an effective active immunity could be obtained by collecting all the mice which had survived the previous injections of live bacteria in Experiments 1, 2, and 3. These mice were given the following additional vaccination treatment.

Survivors of Experiment 1A.—10 days after the injection made in Experiment 1, all the surviving mice were put upon a vaccination schedule similar to that described under "Methods;" in addition to the immunization preceding the tests of Experiment 1, they received three vaccination courses of five injections each. *Survivors of Experiment 2A.*—These mice received, in addition to the immunization preceding the tests of Experiment 2, two courses of the described vaccination. *Survivors of Experiment 3A.*—These received one additional course of vaccinations. A few mice died during the immunization period.

These mice represent a "selected group" of animals known by previous tests to possess some degree of active immunity against Type II pneumococci and were used as described in the following experiments.

Occurrence of True Mass Infection in Actively Immune Mice.

It is an established fact that no amount of antipneumococcus serum can passively protect mice against overwhelmingly large numbers of virulent pneumococci. This phenomenon can properly be termed "true" mass infection, in that the passive immunity furnished by the antibacterial serum is known to be sufficient to protect against infection by smaller numbers of the bacteria. Experiments were made to determine whether active immunity likewise fails when the vaccinated animal is invaded by doses beyond the zone within which passive protection is effective. In view of the previously demonstrated differences in the response of individual mice to vaccination, the tests for "mass infection" were limited to animals known to have acquired sufficient active immunity to resist invasion by smaller numbers of pneumococci.

Four mice from the previously described "selected group" of actively immune mice were injected with 0.02 cc. of broth culture of Type II pneumococci. Although these mice possessed a fairly high degree of antipneumococcus active immunity, they all died of pneumococcus septicemia. (That all of them possessed

at that time sufficient immunity to protect them against at least 10^{-4} cc. of broth culture is shown by the fact that all other individuals of the "selected" group survived that dosage when tested in a simultaneous experiment.)

The results of this experiment showed that, even in a selected group of actively immune mice, infection invariably follows invasion by overwhelmingly large numbers of pneumococci regardless of the previous immunity response of the individual. This experiment presents a definitely controlled example of the true mass infection of actively immunized animals, in that the infected individuals were known to possess sufficient immunity to protect them against smaller numbers of pneumococci.

Comparison of the Occurrence of Infections in an "Unselected" and in a "Selected" Group of Vaccinated Mice When All the Individuals Are Injected with the Same Number of Pneumococci (Type II).

The importance of the factor of individual immunity response in postvaccination infection was further illustrated by the following comparison of the percentage of infections which occur when a constant number of bacteria is injected into two different groups of vaccinated mice.

In one group, the factor of differences in individual immunity response was eliminated by including only animals known by previous test to have sufficient immunity to resist the invasion of at least a small number of pneumococci; in the other group, this factor was uncontrolled (as in Experiments 1, 2, and 3), and although all the animals had received the same vaccinations, no previous tests had been made to determine the effectiveness of their immunity response. The same number of pneumococci was injected into each individual animal; the dose employed (10^{-4} cc. of broth culture) was below the zone of true mass infection.

The tests were carried out with the following three groups of mice:

1. The "selected" group of vaccinated animals which had been found in previous tests to have sufficient active immunity to resist infection against at least a small number of Type II pneumococci. These mice had received the vaccination treatment indicated in the preceding description of the procedure employed in obtaining the "selected population." (In addition to the heat-killed vaccine,

they had received an injection of living bacteria in connection with Experiment 1, 2, or 3.)

Five mice from this group were inoculated with the test dose.

2. The "unselected" group of vaccinated animals which, although vaccinated, had never been tested to see whether or not they had developed any immunity. (This group is analogous in this respect to the animals used in Experiments 1, 2, and 3.) The routine of immunization was the same as that described previously under "Methods," with the exception that the mice in this group received one additional course of vaccinations.

Nine mice from this group were inoculated with the test dose.

3. A group of normal animals was included to furnish evidence of the invariability of infection of non-vaccinated mice when invaded by the test dose of

TABLE III.

Occurrence of Infection in a "Selected" and "Unselected" Group of Vaccinated Mice When Injected with the Same Dose of Type II Pneumococci.

Groups of mice tested		Number of animals injected	Number survived	Percentage of animals protected
Vaccinated mice	"Selected" group (individuals previously tested for acquired immunity)	5	5	100
	"Unselected" group (individuals not previously tested for acquired immunity)	9	2	22
Normal (not vaccinated) mice	"Selected" group (animals possessing no natural immunity)	6	0	0

bacteria. Six mice received the test dose of 10^{-4} cc.; in addition, the usual duplicate virulence controls were made with 10^{-5}, 10^{-6}, 10^{-7} cc. of culture.

The results of the experiment are summarized in Table III.

The results of this experiment (Table III) furnish further evidence of the importance of the previous immunity response of the individual in the determination of the occurrence of the postvaccination infections. The two groups of vaccinated mice, although they had received approximately the same previous immunization treatment, represent respectively, an "unselected" and a "selected population." In the "selected" group where the factor of marked differences in individual immunity response had been eliminated, the protection was regular and no infections occurred. On the other

hand, in the "unselected" group (where the actual immunity response of the vaccinated animals had not been tested) some mice were infected and some were not, in spite of the fact that each individual was known to have been invaded by the same, reasonably small number of bacteria. Whether or not infection occurred in the "unselected" group probably depended upon the response of the respective individuals to the previous vaccination.

The chief interest of these results is due to the fact that two factors were controlled which are impossible to control in bacterial infections outside the laboratory: (1) dosage or numbers of the invading bacteria; (2) differences in individual immunity response to the vaccination. When the first of these two factors is constant, the occurrence of the infection depends upon the second factor, and the percentage of infection within the group depends upon the percentage of the individuals that had previously responded effectively to the vaccination. When the second factor is kept constant (as in the "selected population" of known immunes) the occurrence of infection depends upon the numbers of bacteria, and all the individuals are protected if the dosage is kept below the zone of true mass infection.

Type Specificity of Active Immunity of Mice Vaccinated with Type II Pneumococci.

The type specificity of the active immunity of mice vaccinated with Type II pneumococci was tested as follows: Four mice were taken from the described "selected population" of Type II-immune individuals and injected respectively with one of the following amounts of virulent Type I organisms, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} cc. of broth culture. (That the animals actually possessed active immunity toward the homologous pneumococci (Type II) was controlled by simultaneous tests of the homologous protection of other individuals from this "selected" group of mice.) Duplicate normal animals were injected with the same amounts to serve as virulence controls of the culture.

The mice which possessed a high degree of active immunity to Type II pneumococci died at approximately the same time as did the normal (unvaccinated) animals, and thus showed no evidence of immunity against the heterologous (Type I) pneumococci. This experiment is of interest as evidence that antipneumococcus (Type II) active immunity of mice is just as type-specific as the immunity in passive protection tests.

DISCUSSION.

The preceding experiments have dealt with the relative value of dosage and individual immunity response as factors in the infection of mice which had previously been vaccinated with Type II pneumococci. After the completion of vaccination the resistance of the individuals was tested by the intraperitoneal injection of different doses of live bacteria. The results of the tests of active immunity can be analyzed to best advantage according to two zones of dosage.

In the first zone (between 10^{-6} and 10^{-3} cc. of broth culture) the infection or protection of the vaccinated animals was irregular, and certain individuals succumbed to doses much smaller than those which other individuals resisted. The differences in the number of live bacteria injected within this zone seemed to be entirely without influence in the determination of infection or protection since the percentage occurrence of infection was approximately the same in the groups injected with each of the different doses although the maximum dose (10^{-3} cc.) was 1000 times the minimum dose (10^{-6} cc.) employed in the experiments. This apparent absence of any relation between size of dose and infection indicates that the irregularities in infection and protection are due to differences in the immunity of the individuals. Since the mice possessed no demonstrable natural immunity to the specific bacteria, the differences in the degree acquired must be referred to differences in individual response to the same vaccination. Hence, in this zone of dosage (10^{-6} to 10^{-3} cc. of culture), the occurrence of postvaccination infection was determined by the lack of previous immunity response of the individual rather than by the numbers of the invading bacteria.

In the second zone of dosage, the relations were reversed and the injection of overwhelming numbers of the bacteria invariably resulted in infection in spite of the fact that the animals were known to possess a fairly high degree of active immunity. The infection of the mice tested in these controlled experiments can properly be termed true "mass infection;" but it is well to recognize that the same term is often applied to postvaccination infections where the actual immunity of the individual is as unknown as is the dosage.

The above demonstration of the relative value of these two factors

in the infection of mice after vaccination with Type II pneumococci is of some interest in the question of postvaccination infections in general. In our experiments, the factor of differences in individual response to the vaccination was made especially prominent by choosing animals possessing no demonstrable natural immunity and vaccinating them with a type of Pneumococcus known not to give a high degree of passive immunity. However, the same factor is probably involved, if much less prominently, in the effectiveness of the vaccination of other animals against other bacteria. The failure of one attack of typhoid fever to render certain individuals immune must be evidence of differences in the immunity response of men to typhoid bacilli. Variations in the passive protective value of convalescent serum from pneumonia and other diseases probably represent examples of the same phenomenon.

SUMMARY.

A group of mice was vaccinated against Type II pneumoccci and subsequently tested for immunity against different numbers of the live bacteria. The immunity tests were conducted within two zones of dosage. In the first zone where the doses were kept within reasonable limits (10^{-6} to 10^{-3} cc. of culture), the number of invading bacteria was without influence and the occurrence of infections was determined by the previous immunity response of the individual. In the second zone of dosage (where passive protection also fails), these relations were reversed, and invasion by overwhelming numbers of the bacteria invariably produced infection regardless of the previous immunity response of the individual.

These results present an extreme example of the importance of the immunity response of the individual as a factor always concerned in the effectiveness of vaccination.