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LOCALIZATION OF PNEUMOCOCCI IN THE LUNGS OF PARTIALLY IMMUNIZED MICE FOLLOWING INHALATION OF PNEUMOCOCCI

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In preceding papers[1] it has been shown that when mice are intoxicated with alcohol and then sprayed with a culture of virulent Type I or Type II pneumococci many of the animals die with a general blood infection, but there rarely occurs any localization of the infection in the lungs. On the other hand, when mice, which have previously been exposed to inhalations of pneumococci of either type, are alcoholized and sprayed, pulmonary localization of the infection with manifest lesions in the lungs often occurs. There are at least two possible explanations of this phenomenon. It is possible that as a result of the first inhalation the tissues of the lungs become sensitized, or altered, so that on the second exposure the infection becomes localized in this organ. The second possibility is that by the first inhalation there is induced a state of general immunity, even of mild grade, and, as is well known, infections in immune animals are likely to be localized at the site of injection. Previous experiments have shown that after repeated inhalations of living virulent Type I pneumococci mice become increasingly resistant to infection with these organisms.

Experiments were undertaken, therefore, to determine whether pulmonary lesions may occur following the inhalation of virulent pneumococci by alcoholized mice that have previously been specifically immunized by routes other than the pulmonary one. In these mice a state of pulmonary sensitization cannot be assumed to be present. Mice were given intraperitoneal injections of (1) normal horse serum (2) Type I or Type II antipneumococcus serum or (3) Type I or Type

[1] Stillman, E. G., *J. Exp. Med.*, 1924, **40,** 353, 567.

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II pneumococcus vaccine, and after 10 days they were alcoholized and exposed to a spray of homologous or heterologous pneumococci.

EXPERIMENTAL

Methods.—Normal horse serum or antipneumococcus horse serum diluted in normal salt solution was injected intraperitoneally into mice in amounts varying from 0.1 cc. to 0.0000000001 cc., the total quantity of fluid injected in each case being 0.5 cc. 3 hours later the animals were alcoholized and exposed to a spray of pneumococcus culture. Other mice were injected intraperitoneally with a suspension of heat-killed pneumococci in doses varying from 0.1 cc. to 0.0000000001 cc. The vaccine was so prepared that 1 cc. of the suspension was equal to 10 cc. of broth culture. 10 days later these mice were intoxicated with alcohol and exposed to a spray of a culture of virulent Type I pneumococci.

All the mice were alcoholized by the intraperitoneal injection of 1.5 cc. of a 10 per cent solution of alcohol in salt solution. 1 hour after the administration of the alcohol the animals were placed in the chamber and sprayed with 50 cc. of broth culture of virulent Pneumococcus Type I. They were exposed in the spray box for 1 hour. In every instance in which fatal infection occurred, cultures were made from the heart's blood, and at least one lobe of the lung was fixed in Zenker's fluid for histological study.

Pulmonary Lesions in Infected Mice Which Had Received Normal Horse Serum

In order to determine if the injection of a foreign protein as such would so change the reactivity of mice that pneumococci would localize in the lungs, 201 mice were first injected intraperitoneally with normal horse serum, then alcoholized and exposed to infection by the spray method. Although twenty-five, or 12 per cent of these mice, died of pneumococcus septicemia, no localized lesions in the lungs were present.

Pulmonary Lesions in Passively Immunized Mice

215 mice were passively immunized by a single intraperitoneal injection of Type I antipneumococcus serum, various amounts being employed. They were then alcoholized and sprayed with an homologous culture of Type I pneumococcus. Of the mice so treated, forty-eight, or 22 per cent, died of pneumococcus septicemia, and twelve, or 25 per cent of those dying, showed definite, localized, pulmonary lesions.

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In order to determine if pulmonary localization could be produced by the use of immune serum of a heterologous type, mice were similarly injected with Type II antipneumococcus serum, alcoholized and sprayed with a culture of Type I pneumococci. Of the 224 mice so treated, seventeen, or 7 per cent, died of pneumococcus septicemia, but in no instance was there any evidence of pulmonary localization. It appears, therefore, that the injection of heterologous immune serum does not afford sufficient immunity to cause a localization of the pneumococcus infection in the lungs.

Pulmonary Lesions in Actively Immunized Mice

Of 211 mice, previously injected intraperitoneally with varying amounts of a suspension of heat-killed Type I pneumococci and subsequently exposed to a spray of a living culture of pneumococci of the homologous type, thirty-two, or 15 per cent, died of pneumococcus septicemia, and pulmonary localization occurred in four, or 12 per cent of those dying.

Pulmonary Lesions in Mice Actively Immunized by the Injection of Suspensions of Heat-Killed Pneumococci of a Heterologous Type

In order to determine if localization would occur in mice actively immunized by the injection of a suspension of heat-killed pneumococci of a heterologous type, 215 mice were injected with a suspension of heat-killed Type II pneumococci, and 10 days later all the animals were alcoholized and sprayed with a culture of virulent Type I pneumococcus. 52, or 24 per cent of these mice, died of pneumococcus septicemia but in none was there any evidence of an attempt to localize the infection.

DISCUSSION

Table I shows in a condensed form the results obtained in these experiments. It is seen that considerable variation occurred in the frequency of death. At first sight it is difficult to see why only 12 per cent of the animals receiving normal horse serum died, while of those that received immune serum 24 per cent died, and only 7 per cent of those that received heterologous serum. It must be remembered, however, that in infecting animals by the inhalation method it

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is impossible to control the dosage accurately. There must be great variation in the number of organisms that lodge in different parts of the respiratory tract and also in the number of bacteria that actually invade the tissues. The mice in each experiment were divided into groups, each group of fifteen to twenty mice being treated exactly alike, but the mice of the different groups received different amounts of normal, or immune serum, or heat-killed bacteria. When all these circumstances are taken into consideration it is not surprising that there should have resulted considerable irregularity in the results, so far as protection is concerned, though it is not believed that these results invalidate the conclusions regarding the localization of the infection in the lungs.

TABLE I

The occurrence of pulmonary lesions in mice alcoholized and sprayed with culture of Type I pneumococci after receiving injections of normal serum or homologous or heterologous immune horse serum, or after active immunization by injections of homologous or heterologous heat-killed pneumococcus cultures.

Treated with	No. of mice	No. died	Per cent	No. having pulmonary lesions	Per cent of these dying
Normal horse serum	201	25	12	0	0
Homologous immune horse serum	215	48	24	12	25
Heterologous immune horse serum	224	17	7	0	0
Homologous vaccine	211	32	15	4	12
Heterologous vaccine	215	52	24	0	0

The fact that 25 per cent of the animals which died after receiving homologous immune serum and 12 per cent of those receiving homologous vaccine exhibited localized pulmonary lesions, while none of those previously treated with normal horse serum, heterologous serum or killed bacteria showed pulmonary lesions can hardly be explained by chance alone. The experiments seem to show that slight grades of specific immunity are important in causing the organisms to localize in the lungs and to produce lesions there.

CONCLUSIONS

1. When mice are passively immunized by the intraperitoneal injection of antipneumococcus horse serum or actively by the injection

of heat-killed pneumococcus cultures, and are then alcoholized and sprayed with a culture of pneumococci of the same type as that of the bacteria employed in immunization, a considerable number die with localized lesions in the lungs.

2. If instead of injecting immune serum of the type corresponding to that of the bacteria employed in producing the infection, normal horse serum or immune serum of a heterologous type be injected, or if the animals be previously immunized by the injection of killed pneumococci of a heterologous type, none of the animals which die show any evidence of localization of the infection in the lung.

3. The occurrence of pulmonary lesions in alcoholized mice after spraying with a culture of pneumococci is the consequence of a general immunity of a very mild grade.