A STUDY OF PASSIVE IMMUNITY TO MENINGOCOCCUS INFECTION IN THE CHICK EMBRYO*

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In the accompanying reports (1, 2) the experimental infection of chick embryos by the meningococcus is described. A strain of the microorganism, obtained directly from a clinical case of meningitis was found to be pathogenic for chick embryos at various stages of their development. Its virulence was such that a relatively small dose killed the embryos in 24 to 72 hours following inoculation. The meningococcus proliferated rapidly and could be maintained indefinitely in its virulence and essential characteristics in serial passage from embryo to embryo.

Not only could the meningococcus be cultivated in this manner but infection of the embryo resulted, inducing specific, well recognized pathological lesions characterized by a localization of the microorganism and an inflammatory reaction in the nasopharynx, cranial sinuses, lungs and meninges.

Of the various routes of inoculation used in producing the infection in the embryo that of introducing the meningococcus into the amniotic fluid was of particular significance. The mouth and nasopharynx were thus utilized as portals of entry.

In embryos of 14 and 15 days incubation the meningococcus found a favorable environment for growth in the nasopharynx, cranial sinuses and the lungs, in which areas a typical inflammatory response developed. These localities were involved as a result of a direct contact with the infected amniotic fluid. From these primary foci it was shown that infection of the meninges occurred in a large proportion of the embryos indirectly as a result of the transportation of the meningococci by way of the blood stream.

This type of experimental infection offered opportunities for the study of passive protection induced by specific antisera, both antibacterial and antitoxic. Previous experimental work (3) had shown that immune bodies such as hemolytic amboceptor could be introduced into embryos by the

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intravenous route, and be recovered from the blood stream for several days in significant quantities.

Experiments were therefore performed to determine whether the course of meningococcal infection could be altered or prevented by introducing specific antibodies of various types into the blood stream of the chick embryo before and after inoculation. An homologous antiserum from domestic hens and cocks, a commercially prepared polyvalent antiserum and a commercial meningococcus antitoxin were used and their effects compared. A bacteriologic and microscopic study was also undertaken of embryos receiving antibodies before infection and of those treated with antibodies after the infection had been established.

**Material and Methods**

**Strain of Meningococcus.**—The strain of meningococcus used throughout this experiment was the same one we used in the study of the mechanism of infection in the chick embryo (1, 2). Introduced into the embryo directly following isolation from a clinical case of meningitis, its virulence for the chick embryo has been maintained by daily passage from amnion to amnion in 12 to 14 day old chick embryos from 2 years except for a single intervening period of 2 months when it was grown on 10 per cent ascitic fluid agar. Its agglutination and fermentation reactions have not changed during this period. It was agglutinated by polyvalent antimeningococcus serum in dilutions up to 1 in 200. Against each of the four Gordon types of monovalent antiserum no agglutination occurred.

**Types of Antisera Used.**

1. **Homologous Antiserum.**—Full grown domestic cocks and hens were given a series of 7 weekly intravenous injections of increasing doses of suspensions of living meningococci prepared from the experimental strain. The suspensions were prepared by washing off with 5 cc. of saline the 18 hour growth on ascitic agar slants seeded with a loopful of allantoic fluid from 24 hour infected embryos. For the first injection 0.25 cc. of the suspension was used. This was increased to 0.5 cc. for the second injection; to 0.75 cc. for the third and fourth injections, and 0.1 cc. was used for the fifth, sixth and seventh injection. The birds were bled a week to 10 days following the last injection. The agglutinating titre of such serum varied from 1 in 100 to 1 in 300. This serum will be referred to as homologous antiserum.

2. **Polyvalent Concentrated Meningococcus Antiserum.**—This was a commercial product. The concentrated serum usually represents about ¼ of its original volume. This serum had an agglutinating titre of 1 in 400 against the experimental strain.

3. **Meningococcus Antitoxin.**—Throughout most of the experiments embryos of 14 days incubation were used. The antiserum was injected intravenously 8 to 12 hours before inoculation of the amniotic fluid with the test dose of organisms. The details of

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1 Supplied by the Gilliland Laboratories.
2 This was supplied by Dr. N. S. Ferry of Parke, Davis and Company.
the technique of intravenous injection of the chick embryo are described elsewhere (3). During the 8 to 12 hours intervening between the administration of the serum and inoculation with meningococci a small number of the embryos died as a result of uncontrollable hemorrhage. Such embryos were discarded.

The allantoic fluid of 12 to 14 day old embryos, which had been inoculated into the amnion with the experimental strain 18 to 24 hours previously, provided a convenient suspension of the meningococci for initiating the infection in the embryos to be tested. The meningococci are accidentally introduced into this fluid at the time of inoculation and proliferate in it at a fairly uniform rate. It was found that 0.025 cc. of this fluid injected into the amnion of untreated 14 day old embryos would kill 95 to 100 per cent of them in 48 to 72 hours. Smears of the infected amniotic fluid contained innumerable meningococci. All embryos in this experiment were inoculated with this amount of infected allantoic fluid. This dose will therefore be referred to as the test dose.

A series of experiments was performed for the purpose of analyzing the course of the infection in embryos receiving homologous antiserum before inoculation and in embryos receiving the same treatment after inoculation. The distribution of the meningococci was determined by culture of the heart blood, amniotic fluid and brain on 10 per cent ascitic agar. These embryos were then fixed in toto in Zenker’s fluid (10 per cent acetic acid). Cross-section blocks of the entire embryo were embedded in paraffin and Wright’s stain was used to demonstrate the microorganisms in the tissues (4).

The Effect of Intravenously Administered Homologous Antiserum on Amniotic Meningococcus Infection

The unconcentrated undiluted homologous antiserum was tested in a group of 118 fourteen day old embryos. The serum was given in 0.1 cc. amounts 8 to 10 hours before inoculating the amniotic fluid with the test dose. For control purposes a group of 66 embryos of the same age was given 0.1 cc. of normal chicken serum intravenously and inoculated in the same manner. A group of 62 embryos was also inoculated with meningococci alone.

24 hours after inoculation 97 of the embryos which had received antiserum were still alive; 18 of those which had received normal serum were alive and 6 of those which had received no serum were alive. 95 embryos of the group which had received antiserum survived 96 hours. Of those receiving normal serum 7 survived 48 hours, 5 survived 72 hours and 4 survived 96 hours. Of those receiving no serum only 2 survived beyond the 48 hour period to 96 hours.

The result of this experiment is represented in Chart 1.

The homologous serum was then titrated by injecting intravenously dilutions of 1 in 4, 1 in 20, 1 in 80 and 1 in 160 in saline in 0.1 cc. amounts into 14 day old embryos 8 to 10 hours before inoculating the test dose into the amnion. The results of these experiments are represented in Chart 2.

It is seen from this chart that the protection conferred by the homologous antiserum is diminished by dilution and that the embryo is a fairly sensitive indicator of the amount of antibodies present in a given amount of
CHART 1. Protection by unconcentrated monovalent homologous antiserum against amniotic meningococcus infection.

CHART 2. Protection by unconcentrated monovalent homologous antiserum against amniotic meningococcus infection.
serum. In the lower dilutions of 1 in 4 and 1 in 20 the protective action of the serum is still marked but rapidly falls off in dilutions of 1 in 80 and 1 in 160. As will be seen in the following sections these titrations were valuable in comparing the protective effect of the other types of sera used.

**The Effect of Intravenously Administered Polyvalent Commercial Antimeningococcus Serum on Amniotic Infection**

To serve for a comparison with the unconcentrated homologous serum the concentrated polyvalent serum was first diluted 1 in 4 in saline. This was done because the concentrated product usually represents approximately 1/4 by volume of the original serum. The subsequent dilutions of the serum were made on this basis and the undiluted antiserum referred to in Chart 3 represents the 1 in 4 dilution. The serum was administered in 0.1 cc. amounts intravenously 8 to 10 hours before inoculating the amniotic fluid with the test dose. Undiluted, 1 in 4, 1 in 20, 1 in 40, 1 in 80 and 1 in 160 dilutions were tested. These corresponded roughly to the dilutions of the homologous serum used. As a control for protective effect commercially prepared concentrated antipneumococcus serum was used undiluted and in a 1 in 4 dilution to be comparable with the concentrated antimeningococcus serum.

The results of this experiment are represented in Chart 3.

It is seen that the polyvalent commercial antiserum has approximately the same protective value as the homologous serum. The relative differences in the amount of protection conferred by the various dilutions are about the same for both. That the control serum has a slight protective action is also evident but this is rapidly lost on dilution.

**The Effect of Intravenously Administered Meningococcus Antitoxin on Amniotic Infection**

The antitoxin was given in 0.1 cc. amounts intravenously in undiluted form and in 1 in 2, 1 in 4 and 1 in 10 dilution. For control purposes 0.1 cc. of diphtheria antitoxin was used. The embryos were subsequently inoculated in the same manner as in the above experiments.

The results are represented in Chart 4.

The meningococcus antitoxin confers definite protection against infection when undiluted, but not to the extent of the homologous or polyvalent antiserum. The protective action is rapidly lost on dilution, a 1 in 10 dilution conferring less protection than does the 1 in 160 dilution of the antisera. The diphtheria antitoxin conferred practically no protection. This is in contrast to the slight protection conferred by the concentrated antipneumococcus serum.

It is evident from these experiments that passive immunity to subsequent meningococcus infection can be induced in the chick embryo by the intra-
A. GIVEN 0.1 CC. UNDILUTED ANTITOXIN INTRAVENOUSLY
B. GIVEN 0.1 CC. 1-4 DIL. ANTITOXIN INTRAVENOUSLY
C. GIVEN 0.1 CC. 1-16 DIL. ANTITOXIN INTRAVENOUSLY
D. GIVEN 0.1 CC. 1-64 DIL. ANTITOXIN INTRAVENOUSLY
E. GIVEN 0.1 CC. 1-256 DIL. ANTITOXIN INTRAVENOUSLY
F. GIVEN 0.1 CC. 1-1024 DIL. ANTITOXIN INTRAVENOUSLY

CHART 3. Protection by concentrated polyvalent commercial antiserum against amniotic meningococcus infection. For comparison with unconcentrated monovalent serum all titrations were made from 1 in 4 dilution.

A. GIVEN 0.1 CC. UNDILUTED ANTITOXIN INTRAVENOUSLY
B. GIVEN 0.1 CC. 1-2 DIL. ANTITOXIN INTRAVENOUSLY
C. GIVEN 0.1 CC. 1-4 DIL. ANTITOXIN INTRAVENOUSLY
D. GIVEN 0.1 CC. 1-16 DIL. ANTITOXIN INTRAVENOUSLY
E. GIVEN 0.1 CC. 1-64 DIL. ANTITOXIN INTRAVENOUSLY

CHART 4. Protection by commercial antitoxin against amniotic meningococcus infection.
venous administration of meningococcus antiserum either in the homologous or polyvalent form and by meningococcus antitoxin. Titrations of the antisera or antitoxin can be made and differences in the protective action of these substances can be ascertained by this method.

The Reaction of Passively Immunized Embryos to Meningococcal Infection

The course of the amniotic meningococcus infection in 14 day old embryos passively immunized by intravenous injection of homologous antiserum was followed by sacrificing embryos at various intervals following infection and culturing the amniotic fluid, heart blood and brain, and by making a microscopic study of the lesions.

It was found in every instance that meningococci could be cultured from the amniotic fluid at all times up to 96 hours after infection by the amniotic route. In a number of cases they could be cultured from the heart blood and on rare occasions from the brain.

By microscopic examination the diplococci were found to be present in the cranial sinuses and lungs in the early stages of the amniotic infection of passively immunized embryos. Up to 24 hours there was less inflammatory response in these sites to the presence of the microorganism than in the controls; and on the whole the cocci were present in smaller numbers. At 48 and 72 hours following infection the sinuses and lungs of the immunized embryos usually contained a marked inflammatory reaction, the diplococci were present in large numbers but there was only a slight amount of phagocytosis. The cells of the exudate were well preserved and showed no tendency toward degeneration or necrosis as compared with those of the exudate in embryos receiving normal serum. At 96 hours few if any cocci were present in these areas and the exudate was rapidly disintegrating.

Other embryos were infected by the amniotic route and after the infection had been established antiserum was administered intravenously. The majority of embryos treated in this manner survived 96 hours and longer. Cultures of the amniotic fluid 24 to 72 hours after the intravenous administration of the antiserum yielded meningococci but those of heart blood and brain were sterile. A sinusitis and infection of the lungs had been established in these embryos. Diplococci were present in these sites in moderate numbers but not in the abundance found in embryos treated with normal chicken serum. The cells of the exudate were well preserved and there was less phagocytosis of cocci as compared with the normal serum-treated controls.

In embryos given antiserum intravenously and then injected several hours later intracerebrally, the meningococci were found to grow much
more slowly than in normal serum-treated controls. Whereas all the embryos treated with normal serum died within 24 hours after intracerebral injection a large proportion of those receiving antiserum survived as long as 72 hours. The meningeal exudate in the protected embryos was much scantier, the cells were better preserved, and phagocytosis of the cocci by large mononuclears of the “fixed” variety was more marked than in unprotected embryos. Infection of the sinuses and lungs usually occurred by way of the amniotic fluid into which the organisms were introduced unavoidably at the time of intracerebral injection. The reaction at these sites was the same as in embryos inoculated directly into the amnion. Meningococci were cultured from the brain and amniotic fluid of embryos treated in this manner but the heart blood was sterile.

Embryos given an intracranial injection of 0.1 cc. of homologous antiserum and several hours later an injection of meningococci in the same site developed practically no meningeal reaction although the meningococci proliferated in the meninges and ventricles in moderate numbers. The large mononuclear fixed phagocytes of the meninges actively phagocytosed large numbers of the cocci. The number of cocci in the cranial cavity was never very large and 48 to 72 hours after injection they disappeared. The sinuses and lungs of these embryos developed an infection in the same manner as did embryos which were not protected, due presumably to an insufficient concentration of antibodies in the blood stream following intracranial injection of antiserum. Meningococci were cultured from the amniotic fluid, heart blood and brain of embryos treated in this manner.

**DISCUSSION**

In these experiments 14 day old chick embryos, which are susceptible to meningococcus infection, were passively immunized by the intravenous administration of antiserum and of antitoxin. Following the introduction of a test dose of meningococci into the amniotic sac 95 to 100 per cent of untreated embryos or those given normal serum died from the infection in 48 to 72 hours. The intravenous administration of undiluted unconcentrated homologous antiserum prepared in chickens, or of polyvalent commercial antiserum diluted 1 in 4 protected at least 80 per cent of the embryos against death from the infection.

Titrations of the sera and antitoxin in increasing dilutions in saline indicated that the polyvalent commercial antiserum had approximately the same protective value as did the homologous antiserum, while the commercial meningococcus antitoxin in its undiluted form did not have as great a protective action as did the antisera. This difference in protective
qualities between antiserum and antitoxin has been noted by Cohen (5) in mice. Branham (6), however, found that antitoxins compared favorably with antisera in their protective action for mice.

The nature of the mechanism responsible for protection against meningococcal infection by the antiserum in chick embryos is still obscure. The observations made indicate that the antiserum has a relative inhibitory effect on the growth of the meningococci. Actual infection is not prevented since sinusitis and infection of the lungs and occasionally a blood stream invasion with the development of a meningitis occurs in treated embryos. The fixed mononuclears of the meninges are actively phagocytic following intracerebral injection. The antiserum possibly exerts a neutralizing effect on the toxins or endotoxins released by the meningococci, since the cells of the inflammatory exudate remain well preserved for a much longer period of time in the protected embryos as compared with those receiving normal serum.

Although the rate of growth of the meningococci within the cranial sinuses, the lungs and meninges of protected embryos is much slower than in the non-protected ones their disappearance seems to be due largely to their own natural autolytic processes. The presence of the antiserum seems in a large measure to have a neutralizing effect upon injurious products, so that there is less stimulation to inflammatory reaction and less necrosis. These observations are only of a preliminary nature and it is felt that further experimentation of this type is needed to elucidate many factors concerned in this problem. It seems evident, however, that complement is not necessary to the protective activity of immune sera.

There have not been enough different types of antiserum used to enable us to state whether significant differences in protective value will be brought out in others. The results are, nevertheless, highly suggestive and there are many apparent advantages over the protective tests now in use. That the mouse protection test with mucin-coated meningococci is not an ideal one is pointed out by Branham (7). In the chick embryo by the method outlined here, the virulence of the meningococcus can be maintained without the addition of a foreign substance such as gastric mucin. The embryo also responds to an actual infection with the meningococcus in a well defined manner, closely simulating the natural human disease. The antiserum can be injected intravenously and if necessary titrated by dilutions. Its protective action can be measured by its capacity to prevent death in a highly susceptible laboratory animal.

The technique is relatively simple and large numbers of embryos are easily and economically obtained. The indications from these experi-
ments are that the chick embryo should prove to be a fairly sensitive indicator of the potency of meningococcal antisera and antitoxins.

SUMMARY

1. 14 day old chick embryos are protected against subsequent meningococcus infection through the amniotic route by the intravenous administration of an homologous antiserum produced in hens, by a commercial concentrated polyvalent meningococcus antiserum and by a commercial meningococcus antitoxin.

2. Titrations of the different antisera indicate that the homologous and commercial polyvalent sera have approximately the same protective value and are much more effective than the commercial antitoxin. The titrations also show that the chick embryo is a sensitive indicator of the amount of antibodies present in a given amount of serum.

3. The mechanism of the protective action of the antisera is not apparent from these experiments except that in the treated embryos there is a relative inhibition of the growth and presumably a neutralization of the injurious products of the meningococci.

BIBLIOGRAPHY