EXPERIMENTAL MENINGOCOCCUS INFECTION OF THE CHICK EMBRYO*

By G. JOHN BUDDINGH, M.D., AND ALICE D. POLK

(*From the Department of Pathology, Vanderbilt University Medical School, Nashville*)

PLATES 37 AND 38

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Experimental infection of the developing chick embryo with the meningococcus has been described in a previous report (1). The chorio-allantois of 12 day old embryos was found to be a suitable environment for the propagation in serial passage of a strain of meningococci obtained directly from the spinal fluid of a patient. No change in the type specificity, fermentation reactions or the original virulence of the strain for the embryo took place during 100 daily passages from membrane to membrane.

Not only was the chick embryo a suitable medium for the continued propagation of the meningococcus but it was also found to be susceptible to infection by this organism. It could be demonstrated by bacteriological culture and microscopic section that in the 12 day old embryo the meningococcus invades the blood stream at the site of inoculation on the membrane. Widespread hemorrhagic lesions, associated with capillary and venous thrombosis developed in the embryo. The meningococci could usually be found in and about these areas. The infected embryos died uniformly within a period of 24 to 48 hours after membranal inoculation.

It has been suggested by Goodpasture (2) that the chick embryo might prove to be a suitable experimental host for the study of bacterial infections. With Anderson he reported (3) observations related to the problem of infection by the study of the lesions produced in the chorio-allantois and in some instances in the embryo itself by several pathogenic bacteria. The significant studies of Gallavan (4) on the meningoencephalitis produced in chick embryos by *Haemophilus influenzae* and those of Gallavan and Goodpasture (5) on the production in the embryo of the lesion of whooping cough by *H. pertussis* further confirmed the apparent potentialities of this method.

The scope of the experimental approach to studies of this nature with the chick embryo was greatly enlarged by a concurrent investigation in this laboratory of the introduction of complement and hemolytic ambo-

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ceptor into chick embryos (6). From these studies, as also from an increasing familiarity with the chick embryo technique, methods were developed by which other routes than the chorio-allantois could be utilized as portals of entry for various infectious agents. Thus intravenous inoculations could be performed; and the mouth and nasopharynx of the embryo were rendered available as portals of entry by infecting the amniotic fluid. Direct inoculation into the body wall or into the cranial cavity could also be carried out. Furthermore techniques were devised whereby the various fluids surrounding the embryo, its organs and tissues, as also the heart blood, could be collected and tested bacteriologically for determining the distribution of the infecting agent following the various routes of inoculation.

Another factor which heretofore had not been taken into consideration in these studies was the age of the chick embryo. Gallavan and Goodpasture had observed that the lesions of whooping cough could only be produced with *H. pertussis* in embryos of at least 14 to 15 days incubation at which time the tracheal and bronchial epithelium first developed cilia. As the study of the meningococcus infection progressed it was noted that 12 day old embryos were much more susceptible to the infection than were 15 day old embryos; and day old hatched chicks resisted direct intracerebral inoculations with comparatively large doses of the organism.

The study of meningococcus infection was therefore continued on a broader basis. Various methods of inoculation and their effect on embryos of different ages were studied and a more thorough microscopic and bacteriological analysis of the infection was made. This report will be concerned with the results obtained from such an enlarged experimental approach to this infection in the chick embryo.

Methods and Material

General.—The general method for preparing and inoculating the chick embryo has been described by Goodpasture and Buddingh (7). In this work the cover-slip technique was used throughout. The techniques for the various methods of inoculation are described by Polk, Buddingh and Goodpasture (6).

Embryos at various stages of the infection were sacrificed for microscopic study by fixing them *in toto* in Zenker's fixative (10 per cent acetic acid). Blocks for embedding were cut in cross-section through the embryo so as to include all the significant organs. Sections were stained by Wright's method to demonstrate bacteria as described by Goodpasture and Anderson (3).

The method for collecting the fluids, heart blood and organs of the infected chick has also been described (6). These were tested for the presence of the meningococci by inoculating samples on 10 per cent ascitic agar plates.

Source and Strain of Organisms.—Several stock laboratory strains and the four Gordon type strains of meningococci which had been grown on artificial culture media

for long periods of time were found to be completely avirulent for the chick embryo. They could be maintained by serial transfer in the embryo with difficulty through only a few passages.

The strain of meningococcus used in this study was another than the one used in the preliminary investigations. It was isolated from the spinal fluid of a patient with the typical clinical features of cerebrospinal meningitis. Its morphological and cultural characteristics were typical as were its fermentation reactions. It was agglutinated by a 1 in 200 dilution of polyvalent meningococcus antiserum. Against the 4 Gordon types of monovalent antiserum no agglutination occurred.

The infection of the chick embryo was initiated by the first culture obtained from the spinal fluid. A stock strain of the organism was maintained by daily transfer *in vivo* to the amniotic fluid of 12 to 14 day old embryos. Its virulence for the embryo was such that 0.05 cc. of amniotic fluid infected 24 hours previously inoculated into the amnion of fresh 12 day old embryos would kill within 24 to 48 hours. Frequent agglutination and fermentation tests and also culture on ascitic agar and microscopic examination of stained smears served to control the purity of the strain.

During the summer months of July and August when fertile hen's eggs are difficult to obtain the strain was cultured on 10 per cent ascitic fluid agar. These relatively short periods of culture on this type of artificial media did not seem to cause any appreciable loss of virulence for the chick embryo.

It has been maintained in this manner without loss of its original virulence or other essential characteristics for a period of 2½ years.

For the purpose of obtaining comparable inocula for many of the experiments a loopful of the infected chorio-allantoic or amniotic fluid from the embryos inoculated 24 hours previously with the stock strain was inoculated on ascitic agar slants and incubated at 37°C. for 18 hours. The 18 hour growth was then suspended in 5 cc. of Ringer's solution. From this stock suspension various dilutions for inoculation were prepared.

Effect of Inoculating the Chorio-Allantois

12 and 15 day old embryos were compared in their reaction to inoculation with 0.05 cc. of the 18 hour culture suspension on the chorio-allantois. 72 twelve day old embryos were inoculated in this manner. Of these, 50 survived 24 hours, 18 survived 48 hours, 12 survived 72 hours and 10 survived 96 hours and longer. Of thirty 15 day old embryos inoculated in the same way, 28 survived 24 hours, 27 survived 48 hours and 25 survived 72 hours and longer.

Membranes were fixed for microscopic study at 3 hour intervals up to 24 hours after inoculation and at 48, 72 and 96 hours. At 24, 48, 72 and 96 hours the embryos were also fixed for study and tested for the presence of meningococci by culture of the heart blood.

In the 12 day old embryo the membranal lesion is characterized by the early development of hemorrhage from the capillary bed lying directly beneath and within the ectoderm. The meningococci can be seen to proliferate rapidly on the surface and from the 6 hour period onward a moderate inflammatory exudate consisting chiefly of mononuclears and a few polymorphonuclears is present in the mesoderm and within and on the surface

of the ectoderm. The hemorrhages become more extensive as the infection progresses. From the 12 hour period onward thrombosis of the surface capillaries occurs in many foci with resulting necrosis of the overlying ectoderm. In these areas leukocytes and red blood cells are seen in abundance and form a layer of necrotic cellular debris in which the organisms proliferate in great numbers. From such foci they extend into the mesoderm and at the 18 to 24 hour period they are present in large numbers in the areas of hemorrhage in the mesoderm and especially on the endothelial surfaces of the membranal veins. At this stage the lumina of the vessels contain meningococci.

12 day old embryos examined at the 24 hour or 48 hour stage following membranal inoculation in which the meningococci have invaded the mesodermal vessels in the membrane show widespread hemorrhagic lesions. The meningococci can be cultured from the blood stream in such cases. Large areas of hemorrhage are present within the meninges, associated with the presence of the diplococci on the endothelial surface of the veins and capillaries and growing free among the extravasated red blood cells, and in the tissue spaces of the pia arachnoid. Very few if any inflammatory cells are present in these areas in embryos of this age. Small hemorrhages of this type are present in the cerebral tissues and choroid plexus. They are also present in great numbers in the subcutaneous tissues, the lungs, myocardium and within striated muscles. The glomeruli of the kidneys often stand out as large blue staining masses of organisms which have completely occluded the capillaries. In a few instances they have been seen in and on the surface of the Kupffer cells of the liver.

The membranes of 12 day old embryos surviving 72 hours and longer show only a thin layer of necrotic cellular debris on the ectodermal surface. No organisms are cultivable from the surface at this stage. These embryos apparently survive either because of variations in the inoculum, so that fewer meningococci are introduced and are therefore successfully localized by the scant inflammatory exudate followed by autolysis, or because of variations in susceptibility in individual embryos.

In the 15 day old embryos the meningococcus proliferates much more slowly on the surface of the membrane. There is an early response, within 5 to 12 hours with large numbers of polymorphonuclears. Hemorrhages in the mesoderm are quite infrequent. After 12 to 18 hours many large mononuclears, which apparently arise from the perivascular connective tissue in the mesoderm, wander out onto the surface of the membrane and phagocytose the majority of the meningococci. At 48 hours the exudate on the surface consists chiefly of necrotic cellular debris in which occasional

degenerated forms of the diplococci can be seen. By 72 to 96 hours only a thin layer of hyaline necrotic cellular material is present on the surface and the meningococci have disappeared.

In the 15 day old embryo no internal lesions develop after membranal inoculation. The cocci do not seem to gain entry to the blood stream and cannot be demonstrated there by microscopic examination or by culture. With the maturation of the embryo there is a much more active leukocytic response which perhaps localizes the infection to the membranal surface. The membrane also seems to be a definitely less favorable environment for the growth of the meningococcus than that of the 12 day old embryo.

Effect of Intravenous Inoculation

Intravenous inoculations were performed in 13 and 15 day old embryos. Following inoculation into the membranal vein of twenty-four 13 day old embryos with 0.025 cc. of a 1 in 100 dilution of the 18 hour culture suspension only 5 embryos survived 24 hours and 1 survived 96 hours. The only survival apparently was due to a technical error. Of twelve 15 day old embryos receiving 0.025 cc. of a 1 in 10 dilution of the 18 hour culture suspension only 1 survived 24 hours and none longer than 48 hours.

Culture of the heart blood of 9 of the embryos of this series, of which 4 were tested at 18 hours and 5 at 24 hours, failed to demonstrate the presence of the organism. Microscopic examination of the embryo revealed no lesions.

When injected directly into the blood stream the meningococcus apparently does not survive for any length of time. Presumably it rapidly disintegrates and the toxic products thus released are sufficient to cause the death of the embryo.

Effect of Intracranial Inoculation

Intracranial inoculations were performed in 14, 15 and 16 day old embryos. The response to this procedure is much the same in the embryos of various ages. Following inoculation with 0.025 cc. of the 18 hour culture suspension directly into the brain in 45 embryos only 10 survived 24 hours, none survived as long as 48 hours. In each of 4 embryos of this series examined the meningococci could be cultured from the brain, but in only one could they be recovered from the heart blood.

Microscopic study of the 24 hour infected embryo shows a widespread purulent meningitis in every instance. It is apparent that the meningococcus finds a most favorable environment for its growth within the meninges and cerebrospinal fluid since great masses of organisms can be found in these areas. There is a heavy infiltration into the meninges with great numbers of polymorphonuclears and mononuclears. Great numbers of the cocci can be seen within the actively phagocytic mononuclear and in smaller numbers within the polymorphonuclear cells. There are large areas

of hemorrhage in the meninges and within the cerebral tissues. Massive necrosis of the cerebral tissues also takes place. Within the ventricles the cocci are present abundantly with large numbers of inflammatory cells and a varying amount of hemorrhage.

It is only by direct intracerebral inoculation that a typical purulent meningitis can be produced consistently in every instance.

Effect of Inoculation into the Body Wall of the Embryo

The body wall of embryos was also chosen as a route of inoculation. In these experiments the exact point of injection was not controlled and it involved penetration of the amniotic sac which would often result in infection of this fluid. Embryos of 12, 13 and 15 days incubation were subjected to this procedure.

12 day old embryos were found to be very susceptible to infection following inoculation with 0.05 cc. of the 18 hour culture suspension into the body wall. Of the 10 embryos of this age only 3 survived 24 hours, 2 survived 48 hours and 1 survived 96 hours and longer. Of this series 3 embryos, one each at 24 hours, 48 hours and 96 hours were examined. At 24 and 48 hours meningococci could be recovered by culture from the blood stream and the amniotic fluid. The 96 hour survival yielded negative cultures.

Microscopically the 24 and 48 hour infected embryos showed a type of lesion which was characterized by hemorrhages of greater or less extent in the brain, meninges and lungs and subcutaneous tissues. In the 24 hour lesion only a few meningococci could be detected in and about the capillaries from which the hemorrhages occurred. The 96 hour survival showed no demonstrable lesions.

The 13 day old embryos were slightly more resistant to this route of inoculation than were the 12 day old embryos. 75 embryos of this age received 0.05 cc. of the 18 hour culture suspension into the body wall. Of these 17 survived 24 hours, 4 survived 48 hours and 1 survived 72 hours. 6 embryos of this series were examined by culture and microscopically.

Of the 4 embryos sacrificed 24 hours after inoculation all showed positive cultures from the heart blood, the amniotic fluid and the brain. In each of them microscopic examination revealed a purulent meningitis, with numerous diplococci growing free in the intercellular spaces of the meninges. Cocci could also be detected in and around the blood vessels. Coincident with the meningeal lesion there were numerous petechial hemorrhages in the cerebral tissues associated with thrombosis of small arterioles in which diplococci could be seen in many instances. In one of these embryos a purulent exudate with many organisms was present in the paranasal sinuses and in 2 instances cocci were present in the lungs without the presence of

an inflammatory exudate. The sinuses and lungs were most likely infected by organisms growing in the amniotic fluid.

The 13 day old embryo sacrificed 48 hours after inoculation showed positive cultures from the heart blood, amniotic fluid and the brain. Microscopically a widespread purulent meningitis with numerous petechial hemorrhages into the brain was present. Meningococci could be seen in moderate numbers both intra- and extracellularly. There was also a purulent sinusitis present in this embryo. The 72 hour survival showed a positive culture from the amniotic fluid. Cultures from the heart blood and brain were negative. Microscopically a purulent sinusitis and a purulent exudate in the bronchi and alveoli of the lungs were present. A few diplococci were present but these were mostly degenerate forms.

15 day old embryos receiving 0.05 cc. of the 18 hour culture suspension into the body wall showed a tendency to survive somewhat longer than 13 day old embryos. Of 54 embryos in this series, 27 survived 24 hours, 9 survived 48 hours and 4 survived 96 hours and longer.

Of the 24 hour survivals 4 were examined by culture and microscopically. From one negative cultures were obtained; one showed positive cultures from the heart blood, brain and amnion; another only from the heart blood and brain; from the fourth positive cultures were obtained from the amnion and brain and a negative culture from the heart blood. Each showed infection of the cranial sinuses, 3 showed infection of the lungs and none showed signs of meningeal infection.

5 of the embryos surviving 48 hours were examined. Of these 3 showed negative cultures throughout; one had a positive blood culture and one showed positive cultures from the amniotic fluid, heart blood and brain. All showed infection of the sinuses; 3 showed infection of the lungs. The one with positive cultures from the blood and brain showed a widespread purulent meningitis with numerous meningococci present.

From the single 72 hour survival examined a positive culture was obtained from the amniotic fluid only. Microscopically only a purulent sinusitis was present. The 96 hour survival gave negative cultures but showed a purulent sinusitis on microscopic examination. Only a few degenerate forms of the cocci were present in this lesion.

Effect from Inoculation of the Amniotic Fluid

By inoculating the amniotic fluid of the chick embryo advantage is taken of circumstances in which the mouth and nasopharynx are available as a portal of entry for the infection. Embryos of 12 and 15 days incubation were used for this study.

12 day old embryos were found to be highly susceptible to infection by this route. Of 103 embryos inoculated with 0.05 cc. of the 18 hour culture suspension, 23 survived 24 hours, 10 survived 48 hours and 7 survived 72 hours and longer.

During the first 24 hours following inoculation groups of 3 embryos each were sacrificed at 3 hour intervals and examined by culture and microscopically. Meningococci could be cultured from the amniotic fluid at the 3 hour period but not from the heart blood and the brain. From the 6 hour period onward cultures from the heart blood and brain were positive in every instance. Microscopically the chief lesion which developed was one of capillary hemorrhage. These appeared as early as 6 hours after inoculation in the subcutaneous tissues, meninges and the lungs, and with the progression of the infection, became more extensive and numerous. In embryos sacrificed at the 12 to 18 hour period, at which time the majority of them died from the infection, these lesions were most pronounced and very extensive in the meninges, cerebral tissues, the ventricles and the lungs. Diplococci were only occasionally seen in the lesions at this period.

In the embryos surviving 24 hours the meningococci could be seen in large numbers within the blood vessels and on the endothelial surfaces of the capillaries and veins as well as growing free in the tissues in and about the areas of hemorrhages.

From 12 day old embryos surviving 48 hours and longer no positive cultures could be obtained and no lesions could be demonstrated microscopically. These survivals evidently represented embryos in which no infection was established due to faulty technique, or they may have represented individual differences in susceptibility on the part of the embryos themselves.

Seventy-three 15 day old embryos were inoculated into the amniotic sac with 0.05 cc. of the 18 hour culture suspension. Of these 58 survived 24 hours, 40 survived 48 hours and 26 survived 72 hours and longer.

From this series groups of 3 embryos each were sacrificed at 3 hour intervals during the first 24 hours following inoculation. Up to the 18 hour period the meningococci had not proliferated in the amniotic fluid of these embryos to such an extent that their presence was demonstrated by culture. Heart blood and brain cultures were also negative. From 3 embryos examined at the 24 hour period cultures of the amniotic fluid and heart blood were positive in each case. Cultures from the brain were negative. Microscopic examination showed each of these to have an infection of the cranial sinuses and 2 of them showed diplococci in the lungs. There was no meningeal involvement at this period.

From 4 embryos sacrificed 48 hours after inoculation cultures from the amnion and heart blood of 3 were positive. 2 of these showed a positive culture from the brain. The 4th embryo showed negative cultures throughout. Of the 3 embryos showing positive cultures from the amnion and heart blood each had a purulent sinusitis with numerous diplococci present intra- and extracellularly. In 2 of these an infection of the lungs was present. A purulent meningitis was present in 2 showing positive cultures from the brain. The embryo with negative cultures showed no microscopic lesions.

3 embryos were examined from those surviving 72 hours. 2 of them showed negative cultures from the heart blood, amniotic fluid and brain. From the remaining embryo positive cultures were obtained from all three sites. The 2 embryos from which negative cultures were obtained showed a purulent sinusitis, a purulent inflammatory reaction in the bronchi and alveoli of the lungs and a meningitis. Diplococci could not be found microscopically. In the one from which positive cultures were obtained a purulent sinusitis, infection of the lungs and a meningitis were found with the diplococci present in moderate numbers both intra- and extracellularly.

From 3 embryos examined 96 hours after inoculation negative cultures were obtained. In each of them a purulent sinusitis was found and in 2 a mild, possibly a residual, inflammatory process was present in the meninges. No organisms were present in the areas involved.

In a few of the 15 day old embryos inoculated by the amniotic route which showed a pulmonary infection, organisms were also present in the peritoneal cavity accompanied by small collections of inflammatory cells. This infection most likely entered the peritoneum by way of the abdominal air sacs of the embryo.

DISCUSSION

To review the numerous and extensive experimental studies which have been performed with the meningococcus in the usual laboratory animals during the past 50 years does not fall within the scope of this report. These researches have sufficiently well established the fact that the meningococcus possesses a relatively low virulence for the usual laboratory animals. Large doses of the microorganism have usually been required to produce any appreciable effects, and in the opinion of most investigators these have been due to a toxemia produced by the liberation of endotoxins from the lysed cocci rather than to actual infection by them.

Serial transfer of the meningococcus from animal to animal has on the whole met with little success and in most instances attempted culture of

the microorganism from animals dead of the experimental infection has met with conflicting results in the hands of various investigators. That a typical purulent meningitis can be induced by the direct intracranial inoculation of virulent organisms into various animals has been demonstrated by practically everyone interested in this problem. Such experiments have in recent years been successfully performed by Zdrodowski and Voronine (8) and Branham and Lillie (9, 10) in rabbits, and in guinea pigs by Branham, Lillie and Pabst (11). However in these experiments there was no selective localization in the meninges as a result of introduction of the meningococcus at a more distant focus.

The enhancement of the virulence of meningococcus for mice by coating the organism with gastric mucin as introduced by Nungester, Wolfe and Jourdonais (12) and by Miller (13) has proved of definite value in the assay of protective antisera but has not greatly advanced the study of the infection itself.

The chick embryo is an experimental host in which the meningococcus finds a favorable environment for growth. Younger embryos of 10 to 12 day incubation period are most suitable for the purpose of serial transfer of the organism and the maintenance of its virulence and cultural characteristics. As the embryo progresses in its development it becomes a less suitable medium for the meningococcus.

Following inoculation of the chorio-allantois of 12 day old embryos invasion of the blood stream by the meningococcus takes place at this site. The resulting septicemia is accompanied by the development of widespread hemorrhagic lesions in the embryo itself. The lesions are characterized primarily by capillary and venous thromboses and hemorrhage. Following inoculation into the body wall or into the amniotic fluid of 12 day old embryos the same type of invasion of the blood stream occurs with the development of generalized hemorrhages. There is also very little if any inflammatory response to the presence of the meningococci in embryos of this age.

Older embryos, 14 to 15 days of age, respond to infection of the membrane with an inflammatory reaction which tends to localize the microorganism at the site of inoculation. When these embryos are inoculated directly into the cranial cavity a purulent meningitis develops. Inoculation into the body wall or into the amniotic fluid is followed by specific localization in only a few tissues which apparently provide favorable environmental and nutritional factors for the growth of the meningococcus. Of these the cranial sinuses, the lungs and meninges are the most outstanding. Follow-

ing such localization, multiplication of the meningococcus takes place rapidly. The increase in the number of microorganisms, of which many undergo autolysis, and the presence of the inflammatory exudate which tends to undergo degeneration evidently change these environments from favorable to unfavorable ones for the growth of the meningococcus. The microscopic study of these lesions seems to indicate that phagocytosis *per se* does not destroy the meningococcus for in the earlier stages of the lesions intracellular cocci appear to be in a good state of preservation and apparently multiplying. When autolysis of the extracellular cocci takes place degeneration of the inflammatory cells and also of the intracellular microorganisms occurs. The infectious process seems to be a self limiting one and the lethal effect on the host seems largely to be determined by the amount of noxious substances released by the lysed microorganisms.

The factors which seem to determine the increasing insusceptibility of the embryos as they progress in their development are those of maturation which express themselves in species specificity. The younger embryos, being less species specific, provide in the first place a most favorable nutrient medium for the growth of the meningococcus. There are no effective barriers to entry of the microorganism into the blood stream and they do not encounter unfavorable influences to their growth or survival in this medium. As the embryo matures conditions develop which are gradually less favorable for the proliferation of the meningococcus.

During the latter part of its embryonic development and at the time of hatching, the factors of species specificity have developed to such a degree that in the case of the meningococcus, which has acquired a highly specialized parasitism in relation to perhaps only a single host, man, environmental conditions are of such a nature as to be unsuited for its growth requirements.

Thus the chick embryo during a definite stage of its development is a suitable medium for the growth of the meningococcus and is susceptible to infection by it. The observations here recorded demonstrate that the types of lesions encountered in man, namely a meningitis, sinusitis and pulmonary infection, are reproduced in the chick embryo. Moreover the embryo at various stages of its development exhibits varying grades of susceptibility to this infection. In the 12 day old embryos a meningococcus septicemia develops with the production of generalized lesions closely simulating those of fulminating meningococcemia in man. In older, 14 to 15 day old embryos, a more selective localization takes place in the cranial sinuses, lungs and meninges with the development of an acute inflammatory reaction.

The chick embryo is the first experimental animal to our knowledge in which such selective localizations of the meningococcus occur, involving the utilization of portals of entry other than direct inoculation into the cranial cavity. These circumstances have made possible an analysis of the factors concerned in the pathogenesis of this infection in the embryo which will be presented in a subsequent paper.

These observations have also served as a basis for a study of the usefulness of the embryo as a test animal for the assay of therapeutic antisera and for an analysis of certain factors concerned in the protection conferred by these substances against infection by the meningococcus.

SUMMARY

1. A strain of meningococci obtained directly from the spinal fluid of a patient has been propagated in serial passage in 10 to 12 day old chick embryos without change in its essential characteristics.

2. The chick embryo is susceptible to infection with the meningococcus, and, depending on its stage of development, reacts to the infection with more or less specific lesions.

3. In chick embryos of 15 days incubation, following the utilization of definite portals of entry, such as the nasopharynx, or by inoculation of the amniotic fluid or by inoculation of the body wall, the meningococcus is localized in specific areas, namely in the cranial sinuses, the lungs or meninges, or in all of these areas.

4. The lesions of the meningococcus infection in man, a septicemia, sinusitis, pneumonia and meningitis can be reproduced in the chick embryo by choosing embryos at the proper state of development and utilizing the various portals of entry experimentally available.

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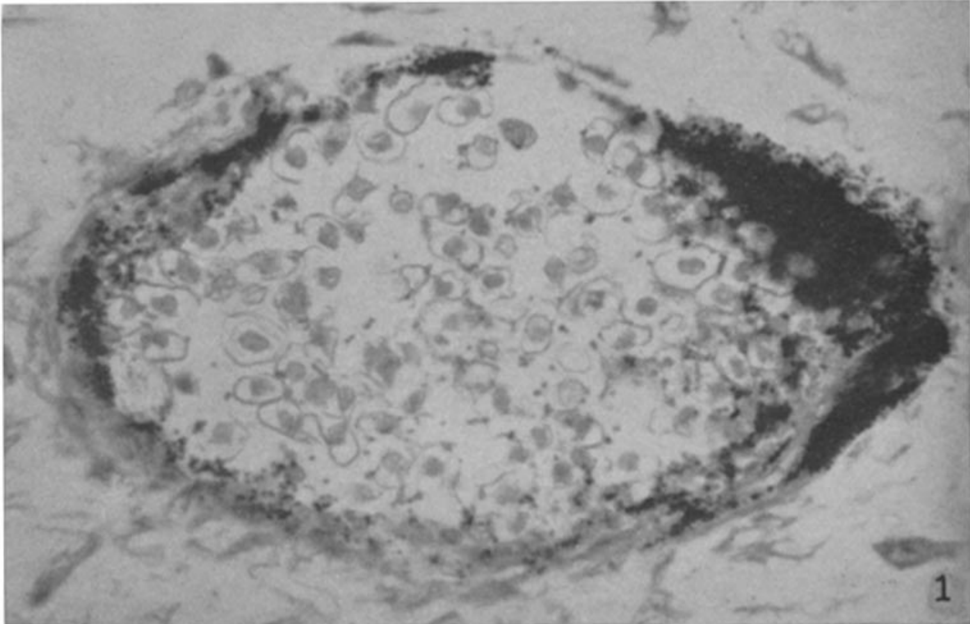
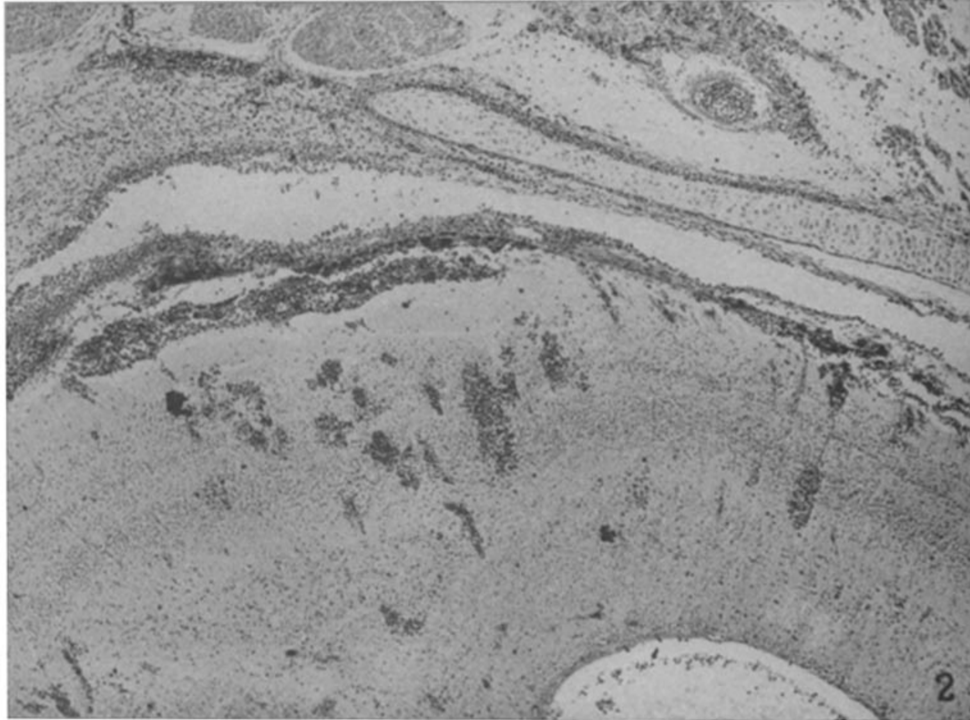
EXPLANATION OF PLATES

PLATE 37

FIG. 1. Membranal infection; 12 day old embryo; 24 hours. Showing meningococci in the lumen and on the endothelial surface of a membranal vein. Wright's stain. $\times$ 1000.

FIG. 2. Meningeal infection following injection of meningococci into the body wall of 15 day old embryos (48 hours). Note masses of organisms in meninges with widespread inflammatory exudate, hemorrhages in the cerebrum and inflammatory reaction in the ventricle. Wright's stain. $\times$ 55.



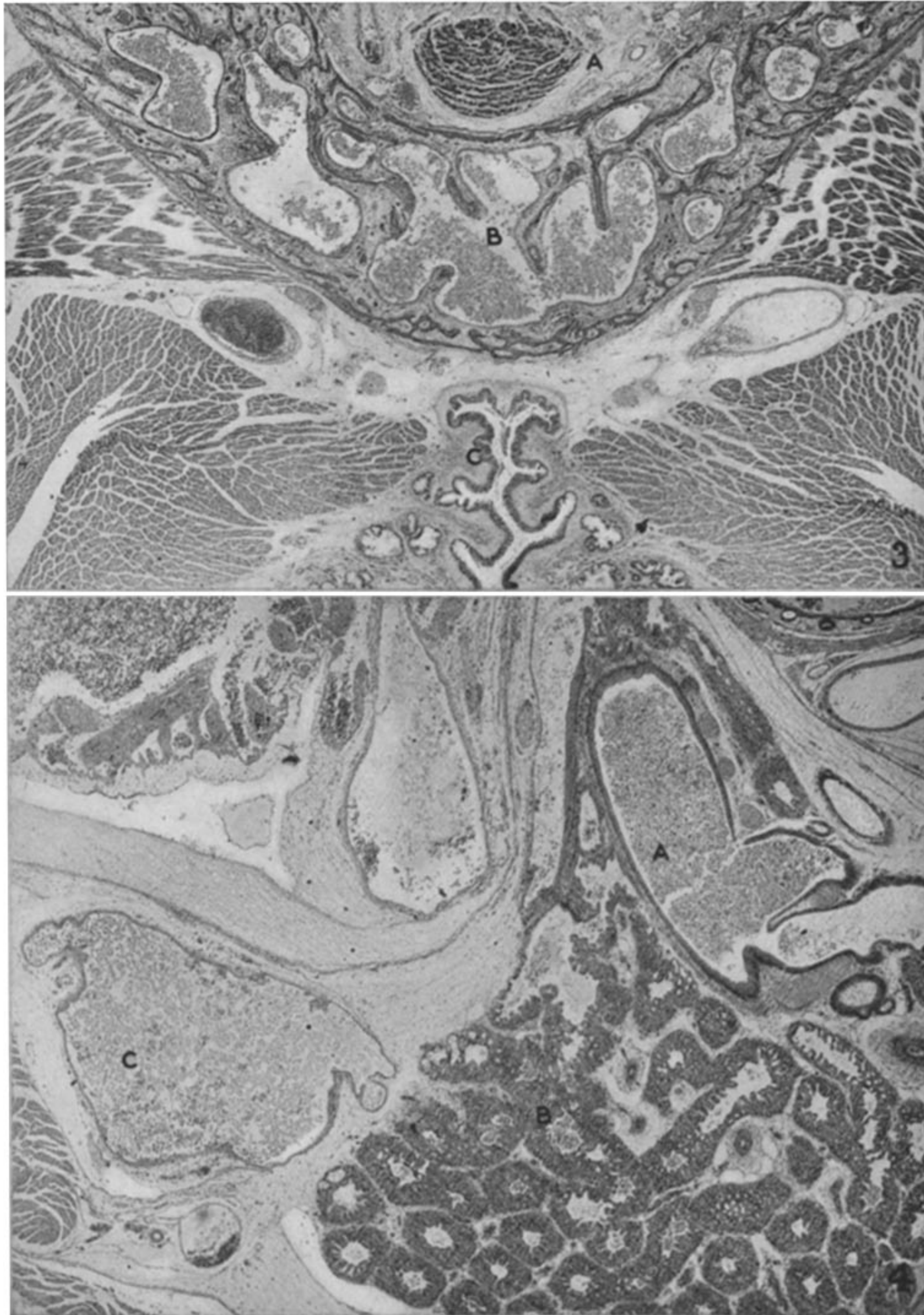


(Buddingh and Polk: Meningococcus infection of chick embryo)

PLATE 38

FIG. 3. Infection in 15 day old embryos following amniotic injection. 48 hours. A, meninges; B, cranial sinus with purulent exudate; C, nasopharynx with exudate. Wright's stain. × 40.

FIG. 4. Infection in 15 day old embryo following amniotic injection. A, bronchus with purulent exudate; B, pulmonary alveoli filled with exudate; C, dilated air sac filled with exudate and inflammatory reaction extending to surrounding blood vessels. Wright's stain. × 30.



(Buddingh and Polk: Meningococcus infection of chick embryo)