A MENINGO-ENCEPHALITIS IN CHICKS PRODUCED BY THE INTRACEREBRAL INJECTION OF FOWL POX VIRUS

By G. JOHN BUDDINGH, M.D.

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

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(Received for publication, February 28, 1938)

The lesions of fowl pox are readily produced experimentally by the inunction of infectious material into the scarified or slightly injured epithelium of susceptible fowls (1). The characteristic eruption which closely resembles that of the natural disease will then appear at the site of inoculation in the course of 3 or 4 days. The virus is found to be present in greatest abundance in the local lesions. It has a specific predilection for multiplying within the susceptible epithelial cells which at the sites of infection become hyperplastic and hypertrophied, followed in the later stages of the disease by necrosis and ulceration.

The presence of the virus within the susceptible epithelium is morphologically recognizable in the pathognomonic intracytoplasmic inclusions. These structures have been shown by Woodruff and Goodpasture (2) to be composed of a mass of smaller elementary or Borrel bodies which are surrounded by a lipoid capsule. The elementary bodies are now generally considered to represent the actual virus.

A generalized distribution of the virus throughout the organism of the infected host seems to take place during the course and long after recovery from the spontaneous as well as the natural disease. Inoculation of the blood or suspensions of the various organs and tissues of infected fowls into the skin of other susceptible birds has demonstrated this fact (3–5). In spite of the widespread and prolonged dissemination, actual proliferation of the virus, as indicated by the presence of the specific inclusion bodies, seems to take place only within the epithelium of the skin and the mucous membranes of the upper respiratory tract and conjunctivae.

The specific affinity of the virus for epithelial cells can be demonstrated by intravenous injection of infectious material. A typical eruption will then develop at the sites of predilection for the natural disease, namely the comb and wattles, angles of the beak, conjunctivae and the upper respiratory tract. If, following this method of inoculation, the skin in any particular area is injured by scarification or plucking the feathers the eruption will appear at this site (6). This specific affinity of the virus of fowl pox for epithelial cells, or in other words its epitheliotropism, is perhaps its most distinct characteristic. This affinity is
apparently not readily altered in the course of the natural or experimental infection. In the natural infection the portal of entry for the virus is presumably the mouth or upper respiratory tract, situations in which the virus is immediately introduced into its natural environment, the epithelial cells. By the usual experimental inoculations the same conditions are more or less closely repeated.

Introduction into a foreign environment, particularly by intracerebral inoculation into susceptible animals, may markedly alter the behavior of certain viruses. Thus Theiler (7) was the first to show that by repeated intracerebral passage in mice the virus of yellow fever was greatly enhanced in its neurotropic potentialities. A "fixed" virus was thus obtained which on intracerebral inoculation caused the death of the majority of mice within 4 or 5 days after inoculation. It has further been shown by the work of numerous other investigators that the fully fixed neurotropic virus differs from the original pantropic virus in that its affinities for nervous tissues are greatly enhanced while that for other tissues, particularly its hepatotropism, is greatly reduced.

The behavior of vaccinia virus can also be greatly changed by intracerebral passage in rabbits as shown by Levaditi and Nicolau (8). After testicular passage in the rabbit the virus was by repeated intracerebral passage completely adapted to this environment. This neurovaccine was shown by Douglas, Smith and Price (9) to be greatly increased in its virulence as compared with dermovaccine for the skin and viscera of rabbits. In a comparative analysis of the lesions produced by neurovaccine and dermovaccine in the chorio-allantoic membrane of chick embryos (Buddingh, 10), the former was distinguished by an increased capacity for infecting cells of mesodermal origin as well as by a marked increase in virulence for ectodermal epithelium.

Levaditi and Nicolau (8) undertook the intracerebral inoculation of fowl pox virus into several adult hens. The lesion which developed was described as being closely analogous to that produced in rabbits by the intracerebral inoculation of vaccinia virus. Perivascular proliferation, infiltration and parenchymatous alterations were found. They did not report the presence of intracytoplasmic inclusions in the cells in and around these lesions. Lipschutz (4) also reported similar attempts with pigeons without definite results.

The following report will be concerned with our experience and observations on the disease and pathological lesions produced in young chicks by the intracerebral inoculation of fowl pox virus.

EXPERIMENTAL

The strain of fowl pox virus used in these experiments was one which had been maintained in this laboratory for many years by occasional transfer in the skin of chicks. Between transfers it was stored in a dried state over calcium chloride.

A short time before undertaking this experiment this strain of virus was propagated in the chorio-allantoic membrane of chick embryos in pure culture through
10 generations. Microscopic study of the membranal lesions showed the infection to be limited chiefly to the ectodermal epithelium and to a slight extent to the entodermal epithelium. No tendency for infection of the mesodermal elements of the membrane was observed.

Lesions from the 10th generation of the virus propagated in the chorio-allantoic membrane were carefully triturated in a sterile mortar with 10 parts of normal saline. This suspension was centrifuged at low speed for several minutes to throw down the coarser particles. The supernatant material was tested in infusion broth and in anaerobic deep meat infusion for bacterial contamination, and was found to be sterile.

2 or 3 day old chicks were used for intracerebral inoculation. The bacterially sterile virus suspension was injected intracranially in 0.05 cc. amounts by means of a size 24 needle fitted to a tuberculin syringe. Vaseline kept just above the melting point was first applied to the feather down over the head to prevent infection of the skin at the site of injection. Immediate death from injury due to the injection often resulted. Of 10 or 12 chicks inoculated 6 or 8 would usually survive without immediate severe reactions.

Symptoms from the infection usually made their appearance on the 4th or 5th day after inoculation. By the 7th or 8th day the majority of the chicks were dead, or in the last stages of the disease.

The virus was carried through serial intracerebral passage in brain tissue by sacrificing chicks in the last stages of the disease. The brains were removed aseptically and triturated in a sterile mortar with about 5 volumes of sterile normal saline. After we were assured of the bacterial sterility of this suspension by appropriate culture in aerobic and anaerobic media another lot of baby chicks was inoculated in the manner described. The virus was thus propagated in pure culture through 14 intracerebral passages at which point the series was terminated.

Material for microscopic study was collected from chicks in the last stages of the disease, i.e., at the 7th and 8th day following inoculation, as well as on the 3rd, 4th, 5th and 6th day of the infection. Normal chicks of the same age were used for comparison and control. The entire heads of the chicks were removed and fixed in toto in Zenker's fluid containing 10 per cent acetic acid. Good fixation and complete decalcification of the skull bones was usually effected within 48 hours. Blocks for paraffin embedding were cut through the entire head in cross section. Sagittal sections were also made from several samples. By this procedure the meninges remained intact and practically all the regions of the brain as well as the surrounding structures of the cranium could be examined microscopically. Sections were usually stained with hematoxylin and eosin, dehydrated in alcohol, cleared in xylol and mounted in balsam. A few samples were stained by Ranson's pyridine silver method and others by osmic acid for fat.

In Table I the plan of the entire experiment is graphically reproduced, showing the number of chicks inoculated, the course of the
TABLE I
The Experimental Work with Intracerebral Inoculation of Chicks with Fowl Pox Virus

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- ○ - INOCULATED CHICK WITHOUT SYMPTOMS
- Θ - CHICK WITH SYMPTOMS OF DROWSINESS
- ▪ - CHICK WITH SYMPTOMS OF SOMNOLENCE AND PARALYSIS
- ♦ - CHICK SACRIFICED FOR MICROSCOPIC STUDY
- ♦ - BRAIN USED FOR PASSAGE THROUGH NEXT GENERATION
- ● - CHICK DEAD FROM INFECTION
- O - CHICK SURVIVED
- ❄ - BRAIN SAVED FOR FURTHER EXPERIMENTS
disease and the material collected for microscopic study. It will be seen that 96 chicks survived the immediate effects of the injection. Of these, 22 were sacrificed at various stages of the disease for microscopic study and 14 were used as material to carry the virus through successive passages. Of the remainder all but one died from the resulting infection.

Clinical Course of the Disease

During the first 3 days following intracerebral injection of the virus the chicks show no symptoms. The first symptom, drowsiness, appears on the 4th or 5th day. This drowsiness increases by the 6th day to somnolence in which all interest in food and water disappears. At this time spastic paralysis of the leg muscles develops which usually reaches such an extent as to make standing impossible. On the 7th or 8th day the majority of the chicks die. Shortly before death generalized convulsions and marked opisthotonus are usually observed (Fig. 1).

Pathological Changes

Gross Findings.—Very few gross changes can be observed in the brains removed during the last stages of the infection. The dura is markedly thickened, contains small areas of hemorrhage, focal areas of necrosis and is generally quite adherent to the skull bones. The brain appears somewhat edematous and occasional scattered pin-point hemorrhages are present.

Microscopic Findings.—As will be seen from Table I, most of the material for microscopic study was collected during the last stages of the infection, although one complete series collected at daily intervals beginning the 3rd day following inoculation was obtained from which the development of the lesions could be ascertained.

The intracranial structures involved are chiefly the choroid plexus, the meninges and the perivascular connective tissues of the brain. Outside the cranial cavity, lesions are also found within the marrow cavity of the skull bones, the paranasal sinuses, mastoid cells and some of the structures of the orbital cavity.

A few general observations must be made before discussing in detail the lesions in the various structures. In the usual infection of the
skin and mucous membranes the virus induces a marked hyperplasia and swelling of the epithelial cells. The specific intracytoplasmic inclusions are distinct and easily recognized. There is no great disturbance in the spatial relationship of the infected cells to each other. Not until the lesion is well advanced, about the 9th or 10th day following inoculation, is there much necrosis of the infected cells.

In the intracranial infection there is also a marked proliferation of the cells of the susceptible tissues but this is accompanied by a rapidly developing necrosis and a profound disturbance of the shape of the infected cells and their relationship to each other. The inclusion bodies do not stand out as distinctly as in the usual lesion of the epithelium but much more intimately involve the entire cytoplasm of the cell. They seem to be much more loosely constructed and are composed apparently of numerous small refractive globules. Much of the virus seems to be forming more rapidly in proportion to the lipoid capsular material. Fat stains such as Sudan III or osmic acid in appropriately prepared material from the lesions seem to substantiate this fact.

A uniform change in the shape of infected cells takes place. They all become globular with the nucleus pushed to one side. With this change in shape separation from the surrounding cells or basement membrane occurs and the infected cells rapidly disintegrate. All types of infected cells assume this globular appearance and become separated from each other.

These processes seem to take place with relative rapidity and are present in the earliest lesions studied. The difference in the early and more advanced stages of the disease is one of extent of this rapid process rather than a slow progressive change in the infected tissues over a large area.

The choroid plexus is perhaps most profoundly affected of all the intracranial structures (Fig. 10). The normally single layer of epithelial cells has undergone rapid proliferation. Practically every cell contains inclusion bodies. Separation from the basement membrane, rapid degeneration into globular cells lying free within the ventricle and rapid disintegration take place (Figs. 11 and 12).

Marked thickening of the leptomeninges and the dura due to hyperplasia of the connective tissue cells takes place (Fig. 2). There is
marked focal necrosis in many areas surrounded by a zone of cells of which the majority are globular in shape and contain inclusion bodies (Fig. 4). The focal necrosis is evidently due to the rapid destruction of the cells by the virus.

Within the brain proper the capillaries stand out very prominently (Fig. 5). Hyperplasia of the connective tissue cells, of which the majority are swollen and globular in shape and contain inclusion bodies, is observed (Fig. 6). Small hemorrhages around these capillaries are frequent. This is possibly due to destruction of the vessel walls by the virus. Degeneration and necrosis of focal areas of brain substance in the neighborhood of these vessels is common and is secondary to the vascular lesions.

Focal collections of large round cells containing the specific cytoplasmic inclusions are found also within the marrow cavity of the bones of the skull (Figs. 7 and 8). The centers of these areas usually show necrosis. The exact nature of the cells involved is somewhat obscure but since they are most frequently found where active bone formation is taking place they are quite likely osteoblasts.

There is also a marked involvement of the epithelial lining of the paranasal sinuses and mastoid cells during the latter stages of the disease. Marked proliferation, with practically every cell containing inclusion bodies, and a rapidly developing necrosis, characterize the infection of these structures.

Within the orbital cavity the connective tissues of the sheaths of the extraocular muscles show the same general changes as found in the meninges. The infection can be traced back in successive sections along the sheath of the optic nerve along which route it has apparently spread from the initial foci in the brain (Fig. 3).

Careful study of the neurons and supporting glial cells of the brain proper presents no evidence of actual infection of these cells by the virus, therefore there is no indication of an acquired neurotropism. The ependymal cells lining the ventricles also show no changes. Such areas of necrosis or degeneration which are present in the brain are secondary to the infection of the meninges and the perivascular lesions or are directly due to injury produced by the inoculating needle.

There is a minimum of inflammatory reaction observed in all the lesions described. Where a considerable amount of necrosis has
occurred a moderate number of actively phagocytic mononuclears are present. Polymorphonuclear leukocytes are present in very small numbers.

Microscopic study of the visceral organs of several of the infected chicks reveals no lesions. The process is evidently quite local in its development.

**DISCUSSION**

The pathological lesions produced in chicks by the intracerebral injection of the virus of fowl pox are of interest from two points of view. In the first place the injection produces an experimental disease with the virus which differs markedly from the spontaneous or the usual experimental infection of the skin and mucous membranes. The lesions of this disease are essentially an infection of the meninges, the choroid plexus, the paranasal sinuses, the mastoid cells and of the marrow of the cranial bones. It is almost invariably fatal and presents a uniform development of the symptoms of somnolence, spastic paralyses, convulsions and death.

The symptoms of the disease can be definitely related to the development of the infection of the meninges, perivascular tissues of the brain and the choroid plexus. The widespread meningeal involvement accounts for the meningismus observed. The somnolence or stupor are perhaps expressions of the spread of the infection and alterations in intracranial pressure. The secondary involvement of the brain proper may account for the progressive development of the spastic paralyses.

In the second place this method of propagation has produced marked changes in the behavior of the virus. What the actual nature of the conditions in this environment is which is responsible for this change is not readily amenable to analysis. However its effect on the virus can be observed in the types of cells involved and the nature of the changes produced in these cells.

A marked increase in the virulence of the virus for epithelial cells is one expression of this change in behavior of the virus. This is most clearly seen in the involvement of the choroid plexus. Not only a rapid proliferation, but a rapid destruction of these susceptible cells
is effected by the virus. This increase in virulence is perhaps due to an acquired capacity for rapid formation of the virus within these cells. On the other hand the virus has acquired the capacity for infecting cells of mesodermal origin. The connective tissue cells of the meninges and the perivascular tissues as well as mesenchymal elements of the bone marrow are widely affected by the virus. In the natural or experimental disease in which the epithelium of the skin and mucous membranes of the upper respiratory tract are the site of the disease this feature does not appear to be prominent. Reischauer (11) in a detailed study of the natural disease has observed an occasional inclusion body in the connective tissue and cartilage cells of the orbit.

The capacity for infecting nervous tissue *per se* in the sense of a true neurotropism does not develop. Our observations give no indications which might explain why this tissue is refractory to infection by the virus.

The fact that young chicks instead of mature hens were used in this study may account for the type of lesions obtained. Those produced by Levaditi and Nicolau (8) in mature hens apparently were of a much milder nature. This problem however deserves further experimental investigation.

In the third place the virus has acquired in this environment the property of similarly altering all types of infected cells regardless of their origin so that they become spherical in shape and detached from each other. The infected cells also apparently undergo necrosis much more rapidly than the epithelial cells infected with the original dermal virus.

The variation in behavior of the virus brought about by the intracerebral environment takes place with apparent rapidity and does not seem to be greatly enhanced by repeated passage. The symptoms and lesions produced in the chicks inoculated intracerebrally at the first passage with the original strain of virus did not differ markedly from those observed at the 14th passage. In this respect fowl pox virus differs from vaccinia and yellow fever virus in that in the latter numerous passages are required to produce a maximum or fixed variation. In the case of fowl pox this maximum variation or fixation of
new characteristics is apparently acquired on the first intracerebral passage and does not seem to increase greatly by repeated passage.

The results of these studies have served to interest us in a more detailed analysis of the variation produced in fowl pox virus by intracerebral passage. The findings of these further studies are the subject of an accompanying report.

SUMMARY

1. Intracerebral inoculation of fowl pox virus in young chicks produces a disease characterized by the development of drowsiness and somnolence 4 to 5 days after inoculation. This is followed by spastic paralysis and convulsions on the 6th and 7th day. The majority of inoculated chicks die on the 7th or 8th day.

2. The pathological lesions are found chiefly in the meninges, perivascular structures, the choroid plexus, paranasal sinuses, mastoid cells, the bone marrow of the cranial bones, and the orbital tissues. No affinity for nervous tissue per se develops.

3. In this environment the virus has a high virulence for the choroidal plexus epithelium and acquires the capacity for infecting cells of mesodermal origin. All infected cells of whatever origin undergo a similar structural change. Fowl pox inclusions can be demonstrated within them and they become spherical in shape and detached from each other.

4. The virus has been carried through 14 successive intracerebral passages. The symptoms and lesions in the chicks inoculated with the 14th passage showed no marked difference from those of the first passage. No enhancement of the changes brought about in the virus by the intracerebral environment seems to take place upon repeated passage.

BIBLIOGRAPHY


EXPLANATION OF PLATES

All sections were stained with hematoxylin and eosin.

PLATE 40

FIG. 1. Photograph of chick 7 days after intracerebral inoculation with fowl pox virus. Marked opisthotonos and spastic paralysis of legs and wings.

FIG. 2. Meningeal lesion, 7 days. Marked cellular hyperplasia and focal necrosis. ×75.

FIG. 3. Extension of infection along the sheath of the optic nerve. 7 day lesion. ×75.

FIG. 4. High power of meningeal lesion (Fig. 2). Separation of connective tissue cells and increase in their size due to inclusion bodies. ×1000.

FIG. 5. Cerebral lesion, 7 days. Marked perivascular hyperplasia and infiltration. ×75.

FIG. 6. High power of perivascular lesion in Fig. 5. Swelling and spherical shape of cells due to infection with the virus. ×1000.
(Buddingh: Fowl pox virus producing meningo-encephalitis)
PLATE 41

FIG. 7. Marked focal lesion in bone marrow. 7 day lesion. ×75.
FIG. 8. High power of Fig. 7. Involvement of osteoblasts with fowl pox inclusions. ×250.
FIG. 9. High power of Fig. 8. Osteoblasts with inclusion bodies. ×1000.
FIG. 11. High power of Fig. 10. Hyperplasia, and desquamation of choroidal epithelium with involvement of the cells with inclusion bodies. ×250.
FIG. 12. High power of Fig. 11. Choroidal epithelium swollen with fowl pox inclusions. ×1000.
(Buddingh: Fowl pox virus producing meningo-encephalitis)