THE TRANSMISSION OF PERIODIC OPHTHALMIA OF
HORSES BY MEANS OF A FILTERABLE AGENT

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PLATES 27 TO 31

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In a previous communication (1) one of us together with Burky re-
ported the results of a bacteriological study of periodic ophthalmia in
horses. Both aerobic and anaerobic cultures of eyes freshly enucleated
from horses with both active and quiescent periodic ophthalmia, were
uniformly negative. The disease, however, has been shown by others
to be definitely transmissible by inoculation into a normal eye of an
exudate from an eye that was the seat of active disease. The purpose
of this paper is to report the pathology of the disease as observed in
the epidemic which we have investigated and to publish observations
indicating that in this particular epidemic the disease was caused by
a filterable agent.

In the fall of 1926 there occurred a sudden outbreak of periodic
ophthalmia among thorough-bred horses on a farm located in high-
rolling country in northern Maryland. The clinical appearance of
the disease in this epidemic was identical with that observed elsewhere,
and did not differ from that seen in sporadic cases which have come
to our attention. The disease is essentially a recurrent serous uveitis.
The onset is characterized by lachrymation, oedema of the periorbital
fold, slight haziness of the cornea, the occurrence of a gelatinous,
mucopurulent exudate in the anterior chamber, and the usual signs
of iritis with synechiae and exudations into the vitreous. Exudates
in the retina occurred, but were difficult to study on account of the
disease of the anterior portions of the uvea.

The initial attack usually lasts from 4 to 10 days and then subsides,
resulting at first in little permanent damage. The period of remission
is irregular, varying usually from 3 to 6 weeks, after which a fresh attack occurs. The attacks recur one after the other with increasing or decreasing severity. In a few horses the eyes recover from the attacks and show only small vitreous opacities, occasional posterior synechiae and varying amounts of retinal atrophy. In a few of the horses in which such recovery occurred there was almost complete retinal atrophy with resultant blindness, while in others the only evidence of the disease was limited to several vitreous opacities. The commonest outcome, however, is for the attacks to occur with increasing severity, with the formation of complete posterior annular synechiae, secondary glaucoma, cataract and ultimately moderate phthisis bulbi.

Pathology

The pathology of the disease has been described by several authors, and most recently by Heinrich Jakob (2). These descriptions, however, do not correspond exactly with the picture we have constantly observed in the horses studied.

The characteristic lesion we have observed appears to be a focal mononuclear infiltration of the uveal tract, the pigment epithelium, the retina and the optic nerve.

This focal infiltration spreads, cyclitic membranes may form, and scarring with complete detachment of the retina may take place. The mononuclear infiltration occurs throughout the optic nerve and in focal points throughout the retina. In advanced, severe cases a heavy cyclitic membrane covers the ciliary process. The cyclitic membrane is densely infiltrated with small round cells. Follicular accumulations of round cells, which superficially resemble lymph follicles, are found throughout the stroma of the uveal tract and in the cyclitic membrane. The essential features of the pathological picture are illustrated in the micro-photographs.

Figs. 1, 2, 3, 6, 7, 10, 11 show, under different magnifications, typical lesions from a number of different horses whose vision was destroyed by the natural disease.

One of the most notable features in the pathological picture is the formation of follicular collections of round cells, which superficially resemble lymph-follicles. These may occur in the stroma of the iris, or outside the normal tissues of the eye, lying in the inflammatory cyclitic membrane. Fig. 12 shows the typical appearance of these follicular collections of cells. While these collections superficially resemble lymph-follicles, they appear, on closer inspection, to differ from them. They have no germinal centers; capillaries are present, and both large and small mononuclear
cells are found, together with plasma and epithelioid cells. Further, we could find no sinuses, such as are found in true lymph-follicles. Stained with the Foot stain, the reticulum characteristic of true lymph-follicles could not be demonstrated.

Whether the follicular collections of cells are pathognomonic of this disease cannot be said as yet. They have been constantly observed in all the horses with periodic ophthalmia that we have studied, and we have not found them in the eyes of horses with uveitis caused by bacteria. Further study of uveitis in horses produced by other agents will be necessary to determine whether or not the collections of cells are to be considered as a specific feature of periodic ophthalmia.

EXPERIMENTAL

Our first studies on the etiology of this disease were concerned with an attempt to cultivate a bacterium from the diseased eyes. All aerobic and anaerobic cultures from eyes showing the disease in both active and quiescent form were entirely negative. We were forced therefore to disagree with Rosenow's (3) conclusion that a Gram negative flavobacterium is the etiological agent of the disease. After numerous attempts to demonstrate other possible causes such as spirochetes, plasmodia, and tuberculin hypersensitivity, none of which were successful, we considered the possibility of a filterable agent as the specific etiologic factor.

A horse in the acute stage of the disease was sacrificed and the eyes removed. One eye was saved for histological study, and the other, affected with the disease in the acute stage, was opened under aseptic precautions, the aqueous and vitreous humors as well as the retina and uveal tract removed and ground up in a sterile mortar with sterile sand and several cubic centimeters of physiological salt solution. The supernatant fluid was then passed through a Berkefeld N filter. This filtrate was tested for sterility by aerobic and anaerobic cultures on rabbit blood agar, glucose beef infusion broth and cooked meat medium under vaseline seal. Under butyn anaesthesia 0.5 cc. of this filtrate was then injected into the vitreous humor of a normal horse. Within 24 hours the horse developed the clinical picture typical of periodic ophthalmia, with oedema of the periorbital fold, lachrymation, photophobia, ciliary congestion, exudation in the anterior chamber, vitreous opacities and the evidence of an acute iritis. The attack lasted about 10 days, then subsided and recurred several times.

This experiment indicated that it was possible to recover from the eyes of horses with periodic ophthalmia a filter-passing agent which
was capable of producing the clinical picture of the disease in a normal horse, but gave no indication as to whether or not the agent was a living virus. In order to throw light on this point it was decided to attempt to transmit the disease to other species with the hope of carrying it through several transfer-generations of animals, because of the expense entailed in the use of horses. Rabbits were selected for this purpose, and a second horse with periodic ophthalmia in the acute stage was sacrificed and the eyes enucleated. One eye was saved for histological study and a filtrate was prepared from the second eye in the manner described above. Inasmuch as the same technique for preparing and testing the filtrate for sterility was used throughout all the transmission experiments we shall, for the sake of brevity, refer to these procedures as the "usual technique." The clear filtrate, proven sterile by culture, was injected directly into the vitreous of each eye of a series of six rabbits. All injections were made while the rabbits were under the influence of an anaesthetic.

There quickly developed in these rabbits a clinical picture which, while not similar in every respect to that observed in horses, was nevertheless quite constant.

There was no visible involvement of the anterior uvea, but within 24 hours there developed an acute retinitis with fluffy exudates throughout the retina. In some of the animals the lesions tended to recur after a remission of 2 to 3 weeks. The histology of these lesions is described below. Control inoculations into normal rabbits' eyes with filtrate of emulsions from normal horses' eyes failed to produce such lesions, and aerobic and anaerobic cultures of the diseased eyes were sterile.

These facts warranted the surmise that the disease had been transmitted successfully to rabbits by the intra-vitreous injection of a filtrate from the eyes of a horse with periodic ophthalmia.

Acting on this assumption, an attempt was next made to carry the disease from the rabbit back to the horse.

A normal horse (No. 2) was inoculated directly into the vitreous with 1 cc. of a filtrate prepared according to the usual technique from one diseased eye of each of three of the experimentally infected rabbits. Within 24 hours after inoculation the horse showed the typical picture of periodic ophthalmia observed in horses with the natural disease. The acute symptoms persisted for 10 days and then subsided. 3 weeks later the disease recurred and there was subsequently a second recurrence.
This experiment suggested that the filterable agent was capable of propagation in the rabbit’s eye, but as the transmission had been made through a single transfer-generation of rabbits from the naturally diseased horse to the experimentally infected eye of the horse, it was necessary to elaborate the original experiment in order to establish this theory.

An attempt was made therefore to transmit the disease from horses to rabbits and to carry it through several transfer-generations of rabbits in succession before making the crucial experiment of transmitting it to the horse.

Accordingly another horse with periodic ophthalmia was sacrificed and a filtrate prepared with the usual technique from the acutely diseased eye. This filtrate, proven sterile by aerobic and anaerobic cultures, was injected into the vitreous of each eye of a series of four rabbits. All four rabbits developed a retinitis similar to that observed in the preceding experiments. After an interval of 2 weeks, two of these rabbits were sacrificed, the eyes removed, cultured by both aerobic and anaerobic methods, and a filtrate prepared from them. A second series of four rabbits was inoculated by intravitreous injection with this filtrate. The remaining two rabbits were kept for further observation and for histological study.

In this manner the disease was carried through six successive transfer-generations of rabbits with the constant occurrence of the typical retinitis. Aerobic and anaerobic cultures were made of all eyes removed for preparation of filtrates. These cultures were uniformly negative. Occasionally a rabbit was encountered which showed no lesions after inoculation. Such rabbits were not used for transfer. These rabbits which appeared refractory to inoculation were the exception, for the occurrence of the retinitis was remarkably constant throughout.

A filtrate from the eyes of three rabbits of the sixth transfer-generation was then inoculated into the vitreous of the left eye of each of two normal horses (Nos. 3 and 4). Both of these horses developed a clinical picture similar to that observed in the natural disease.

The initial symptoms were oedema of the periorbital fold, lachrymation, photophobia, ciliary congestion and the occurrence of a gelatinous, mucopurulent exudate in the anterior chamber. The signs of inflammation were noted 24 hours after inoculation, and they reached their height within 48 hours. The initial attack lasted from 1 to 2 weeks and then diminished, leaving only slight evidence
PERIODIC OPHTHALMIA OF HORSES

of inflammation. In each horse the disease recurred after periods of from 2 to 4 weeks. One horse (No. 3) was apparently very susceptible. The injected eye showed an unusually severe reaction. The attacks recurred with increasing severity, and the final picture was similar to that observed in cases of severe natural disease—complete posterior synechiae, cataractous lens, and moderate phthisis. The other horse (No. 4) had four attacks, each less severe than the preceding one. The attacks finally ceased spontaneously, and the picture was then similar to that observed in the eyes of horses in which the natural disease had healed normally, as the only evidence of the disease was some clouding of the vitreous and a few posterior synechiae. None of the experimental horses had any symptoms of involvement of the control or uninjected eye.

Controls

The following control experiments were performed.

1. A filtrate was prepared in the usual manner from the eye of a normal horse and injected into the vitreous of each eye of four rabbits. The eyes of these animals did not exhibit the retinitis observed in the eyes of rabbits injected with filtrates from the eyes of horses with periodic ophthalmia. Histological study of these eyes showed no pathological lesions.

2. The eye of a normal horse was injected with 2 cc. of sterile filtrate obtained from normal rabbits' eyes. This horse showed a moderate reaction following the injection which lasted about 48 hours. Within 4 days the eye appeared entirely normal, and remained so for a period of 3 months observation. The horse was then sacrificed. Histological examination of the inoculated eye showed no pathological lesions resembling, even remotely, those of periodic ophthalmia.

3. In order to exclude a possibility that the experimental lesions were due to traumatism alone, salt solution was injected into the vitreous of the eyes of normal rabbits and of a normal horse. Clinical and histological examination of these eyes revealed no pathological lesions.

Pathology in Experimental Rabbits

The pathological lesions found in the experimental rabbits were less extensive than those found in the horses. They consisted chiefly in focal areas of acute retinal inflammation with disorganization of the normal retinal picture by round cell infiltration, which was fundamentally the same as the pathological lesion found in horses. No follic-
ular collections of cells were observed. These lesions were seen both in the acute stage and the stage of repair. Figs. 8 and 9 show such lesions. Occasionally lesions in the choroid were found, although as a rule the uveal tract was remarkably free from abnormalities.

The optic nerve was also involved in a few rabbits showing mononuclear infiltration of the stroma and of the tissues over the surface of the nerve. Fig. 13 illustrates such lesions.

One rabbit showed a definite infiltration in the sheath of the optic nerve, such as is found in meningitis, the cells near the nervehead being chiefly mononuclear in type, while polynuclear cells were found posteriorly in the sheath of the nerve (Fig. 4).

We have as yet made no intra-cerebral injections of this filterable agent, and the brains of the experimental rabbits and horses have not yet been studied, so that we have no information as to the condition of the central nervous system.

Pathology of Disease in the Inoculated Horse

The lesions in the disease produced experimentally were uniformly of the same character as those found in the natural disease.

Figs. 5 and 14 to 17 illustrate the various pathological lesions found in Horse 3 in which the attack recurred with increasing severity until the disease reached the end stage: mononuclear infiltration of the iris with collections of round cells (Fig. 14); acute lesions of the choroid and retina with proliferative changes, and disorganization and infiltration of the retina by mononuclear cells (Fig. 15); follicular-like collections, chiefly of mononuclear cells, lying both in the stroma of the anterior uvea and in the cyclitic membrane (Figs. 16 and 17) and optic neuritis with mononuclear infiltration of the nerve stroma (Fig. 5).

The eyes of the other two horses (Nos. 2 and 4) in which the clinical picture of the disease was produced experimentally, finally healed, and at the time the horses were sacrificed the eyes showed but little clinical abnormality. Pathological study of the eyes of these horses, as might be expected, did not show the pronounced changes found in the eyes which were the seat of advanced disease.

One horse (No. 2) showed several focal areas of round cell infiltration throughout the stroma of the anterior uvea, while the other horse (No. 4) showed some diffuse cellular infiltration around the pectinate ligament and the root of the ciliary processes. We have, unfortunately, no histological preparations of eyes from horses that have recovered from the natural disease, but clinical examination of such eyes gives no reason to suppose that more extensive pathological lesions could be found than these illustrations show. The lesions found in these two
horses illustrated in Figs. 18 and 19, were what might be expected in horses in which the disease had healed after several acute attacks.

**COMMENT**

These experiments were designed to solve the problem of whether periodic ophthalmia in horses can be successfully transmitted by means of a filter-passing agent. We believe it has been. The reasons upon which we base our belief are three-fold. First, the experimental disease is indistinguishable clinically from the disease as it is observed in nature, not only in the actual appearance of the lesions but in the relapsing course which they pursue. Secondly, the pathological lesions in the experimental disease and in the natural disease are identical. Thirdly, we could find no evidence that bacteria produced these lesions. Aerobic and anaerobic cultures made from eyes in the acute stage of the disease gave uniformly negative results, and sections of such eyes, stained by special methods did not show either bacteria or spirochetes. Furthermore, the infiltration with mononuclear cells, and the almost complete absence of polymorphonuclear cells in the exudates would be unusual in a disease caused by bacteria such as are usually found in pyogenic inflammations. We believe therefore these experiments to indicate that this epidemic of periodic ophthalmia in horses is caused by a filterable agent, and that the disease can be transmitted to other animals by the intra-vitreous inoculation of this agent. The successful transmission of the disease to the horse after successive transfers through six series of rabbits indicates that the agent is capable of propagation in this species and belongs to the group of filterable viruses. In the main this agent appears to attack the optic nerve, retina, pigment epithelium, and the uveal tract. Whether the involvement of the uveal tract is an extension of the inflammation from the retina and pigment epithelium of the ciliary processes cannot be stated. In rabbits the disease produced by this filterable agent is limited almost entirely to the nervous structures of the eye, while in horses the onset and involvement of the anterior uvea are so sudden and abrupt that it is impossible to tell where the initial lesion starts.

It is also by no means certain that the action of this filterable agent is limited solely to the eyes. Two horses and a number of rabbits
were autopsied and sections of the viscera studied histologically, but no lesions were found. The brains and spinal cords, however, were not examined in any of the animals. Sections of the optic nerves, both of the horses and rabbits, suggest the possibility that disease of the central nervous system may also occur. None of the horses under observation died from natural causes, but there was a low mortality among the experimental rabbits. It has been noted that horses affected with the disease appear irritable and tend to throw their heads up unduly. The rabbits were observed at regular intervals, but we did not see in them any symptoms of central nervous system involvement. We can therefore hazard no opinion as to this possibility, other than that the sections of the eyes suggest that there may have been such involvement. This is a point for future investigation.

We have not been able to demonstrate inclusion bodies. In a number of sections we have found granules in cells which suggested inclusion bodies, but in no instance could these be definitely identified as such. An explanation of our failure to find such bodies may be that they are found chiefly in the early stages of diseases caused by filterable agents; our sections of eyes with the natural disease were all obtained from blind horses in which the disease had reached the end stage. Likewise, our experimental horses were all in the late or healed stages of the disease at the time of autopsy, for in the transmission experiments it was necessary to observe the horses for a long period of time in order to establish the clinical picture of recurrent attacks.

Not all horses appear uniformly susceptible to the disease. The morbidity rate in a herd is relatively low. There was a marked variation in the severity of the clinical picture both in the natural disease and in that experimentally induced. While the eyes of a few horses affected with the natural disease eventually healed with little or no final damage to the eyes, the disease usually progressed until there was more or less complete scarring of the eyes with concomitant loss of vision. The comparative mildness of the experimental disease in a few instances may possibly be attributable to a partial natural immunity. The low morbidity of the disease in a herd is in harmony with this conception. Moreover, not all rabbits appear to be susceptible to the experimental disease. A few were encountered which showed no reaction to the intra-vitreous injection of the filterable agent.
PERIODIC OPHTHALMIA OF HORSES

We have no knowledge of the mode of infection or the natural method of transmission of the disease. Intravenous inoculation of filtrates into four rabbits gave negative results. We cannot state whether or not this filterable agent is similar to any of the other filterable viruses so far studied, or if it constitutes a specific entity in itself. We have as yet made no studies on the possible protective or therapeutic properties of convalescent serum. These are all points for further investigation.

CONCLUSIONS

A filterable agent has been obtained from the humors and tissues of the eyes of horses suffering from active periodic ophthalmia. The intra-vitreous injection of this filtrate produced in normal horses the same clinical and pathological picture observed in the natural disease. This filtrate injected into rabbits produced a different clinical picture, but the essential pathological lesions closely resembled those found in horses. After passage of the filterable agent through six generations of rabbits, it again produced the clinical and pathological picture of the natural disease when injected into the eyes of normal horses.

It appears, in this epidemic at least, that this filterable agent was the specific etiological factor of the periodic ophthalmia.

In conclusion we desire to express our thanks to Major G. L. Stryker for the gift of horses with the disease, and for his cooperation and assistance throughout the course of this investigation, without which it could not have been conducted. We are indebted to Dr. F. B. Kindell and Dr. S. S. Blackman for autopsy examination of horses, to Dr. Jonas Friedenwald and Dr. F. H. Verhoeff for their valuable assistance in the examination of the histological specimens of the eyes, and to Dr. T. B. Turner for aid in the manipulations incident to transfer.

BIBLIOGRAPHY


**EXPLANATION OF PLATES**

**PLATE 27**

**FIG. 1.** (Natural disease.) Mononuclear infiltration of stroma of iris and follicular accumulation of round cells (high power).

**FIG. 2.** (Natural disease.) Monocellular infiltration over ciliary processes.

**FIG. 3.** (Natural disease.) Optic neuritis.

**FIG. 4.** (Experimental disease in rabbit.) Mononuclear infiltration in sheath of optic nerve.

**FIG. 5.** (Experimental disease in Horse 3.) Optic neuritis with mononuclear infiltration of nerve stroma.

**PLATE 28**

**FIG. 6.** (Natural disease.) Focal lesion in retina; mononuclear infiltration.

**FIG. 7.** (Natural disease.) Disorganization and atrophy of retina in advanced disease.

**FIG. 8.** (Experimental disease in rabbit.) Acute lesion of retina.

**FIG. 9.** (Experimental disease in rabbit.) Healing lesion of retina with fibrosis.

**PLATE 29**

**FIG. 10.** (Natural disease.) Acute lesion over pigment epithelium and follicular accumulation of mononuclear cells.

**FIG. 11.** (Natural disease.) Mononuclear infiltration of choroid.

**FIG. 12.** (Natural disease.) Follicular accumulation of mononuclear cells (low power).

**FIG. 13.** (Experimental disease in rabbit.) Optic neuritis.

**PLATE 30**

**FIG. 14.** (Experimental disease in Horse 3.) Mononuclear infiltration of iris and follicular collection of cells.

**FIG. 15.** (Experimental disease in Horse 3.) Acute lesion in choroid with dense mononuclear infiltration.

**FIG. 16.** (Experimental disease in Horse 3.) Infiltration and follicular accumulation of mononuclear cells in anterior uvea (low power).

**PLATE 31**

**FIG. 17.** (Experimental disease in Horse 3.) Follicular-like collection of mononuclear cells lying in the inflammatory cyclitic membrane (low power).

**FIG. 18.** (Experimental disease in Horse 2.) Focal area of mononuclear infiltration in anterior uvea (high power).

**FIG. 19.** (Experimental disease in Horse 4.) Diffuse mononuclear infiltration about pectinate ligament.
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