EPIDEMIC TREMOR, AN ENCEPHALOMYELITIS AFFECTING YOUNG CHICKENS*

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During the last 4 years there has appeared in New England flocks a disease of young chickens hitherto unrecognized. This disease was described in a preliminary report (Jones, 1932) as "An encephalomyelitis in the chicken." Its increasing frequency among commercial flocks in the last 2 years has made necessary the use of a more descriptive name. In view of the striking symptom which differentiates it from other nervous disorders, and because of its appearance in large numbers of chickens within a flock, the name "epidemic tremor of chickens" has been chosen. The present paper is a report of studies carried on in field epidemics and in the laboratory since its first appearance.

The disease has been transferred experimentally to normal chickens by intracerebral inoculation of suspensions of brain and spinal cord. During the course of twenty passages, the virulence has been materially increased. Whereas in early experiments only a few of the inoculated birds contracted the disease, in recent transfers it has not been unusual for an entire series to become infected. The average incubation period has likewise been shortened in the course of these passages, and the severity of the brain lesions has increased. The condition has many of the characteristics of a virus disease and has been tentatively classed as such.

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Clinical Course

The first symptom observed in affected flocks is usually the constant trembling of certain individuals. Attention is called to these birds by the rapid vibration of the head, but on handling them, it is apparent that muscles in addition to those of the neck are affected to a variable degree. The tremor becomes aggravated when the birds are handled or when the flock is in any way excited, but tends to subside when the birds are undisturbed, and disappears in sleep. On further examination of a flock, other chickens less severely affected are often found. Such birds may exhibit a fine tremor which is scarcely noticeable until the bird is picked up. Ataxia is also a symptom which usually is seen in conjunction with tremor, but has occasionally been the only symptom to appear. When ataxia is the first manifestation of the disease, tremor may develop later. More frequently, ataxia appears simultaneously with, or at varying intervals subsequent to, the development of tremor. Those chickens which are affected by tremor alone are apparently not seriously incapacitated, since they walk about, and are able to eat and drink in spite of the constant head tremor. Even in the most advanced cases, in which a coarse tremor is constantly present, the birds are still able to peck at food, and do not appear greatly inconvenienced by their condition. All of the birds affected with tremor alone seem capable of survival for an indefinite period under laboratory conditions. Those chickens which have ataxia associated with the tremor, or ataxia alone are seriously handicapped in their efforts to move about, and as the ataxia is progressive, the birds ultimately are unable to reach sufficient food to maintain themselves even in confinement.

Pathology

The pathology of the disease was studied in detail in the birds received during the outbreak in 1931. We are indebted to Dr. M. M. Canavan of the Department of Pathology for painstaking examination of the brains and spinal cords of the birds of this series, and for the original description of the lesions of the nervous system. Similar detailed studies were made in birds from epidemics in 1932 and 1933, and in a series of inoculated birds. Routine examination of birds from field epidemics and of inoculated birds to correlate pathology with symptoms is now confined to sections of the brain alone.

Distribution of Lesions.—The characteristic lesions of the disease are microscopic and are found scattered throughout the brain and spinal cord. No lesions which could be detected in the gross have been found in the central nervous system or viscera. There have been no tumors noted, and no changes in eye coloring or vision.

In addition to the involvement of the nervous system, microscopic

lesions are found in the viscera of birds suffering from natural infections, but rarely in chickens which have been inoculated.

The distinctive lesions of the brain and spinal cord of birds from field epidemics consist of microscopic collections of neuroglia cells (Figs. 1 and 2). These are composed of macroglia and oligodendroglia with an occasional microglia cell. They are found clustered around capillaries. Cells are seen in mitosis in these foci, which indicates the proliferative nature of the lesion. Foci of glia cells are often found near the ventricles of the brain in association with degenerating nerve cells. Similar lesions are found thoughout the brain and spinal cord. In the earlier series examined, they appeared to be more numerous in the cerebrum than in the cerebellum, but in more recent cases the lesions of the cerebellum seem to be of greater severity. The number of foci is also greater, and the foci are larger than in the earlier series. This is particularly true in the inoculated birds.

Perivascular infiltration around the larger vessels of the brain is also found in many instances, but varies greatly in severity. Degeneration of Purkinje's cells is often severe.

The lesions of the brain and spinal cord of inoculated birds are similar to those described above. In many cases, however, they are far more severe than anything encountered in spontaneous cases of the disease. The lesions are largely perivascular. The focal collections of neuroglia cells, which are the typical lesions, are most often found surrounding the small vessels in the deeper portions of the brain. The cells composing these collections are macroglia, oligodendroglia, and microglia, as in the lesions of spontaneous cases, and in addition there is often an astrocyte involved (Figs. 3, 4, and 5).

In inoculated birds infiltration around the larger vessels of the brain is sometimes extremely severe. In certain cases this is largely lymphocytic in character. Lymphocytic infiltration is also present at times around the meningeal vessels. It is apparently greater around veins than their accompanying arteries.

Studies of the peripheral nerves have revealed neither gross nor microscopic lesions.

The typical lesions of the visceral organs of spontaneous cases are microscopic foci of infiltration with cells of the lymphoid series. The foci are of two types, one rounded and sometimes sharply circumscribed and encapsulated, the other, irregular, with ill defined boundaries. The circumscribed lesions are most commonly and strikingly found between the alveoli of the pancreas, often in the vicinity of blood vessels (Fig. 6). The blue staining of their cells is pale in contrast to the irregular pinkish islands of Langerhans, and the intense blue of the cells of the alveoli. Similar areas are also found in the spleen and infrequently in the heart. The infiltrations in the spleen vary greatly in appearance, and comparison of sections from the spleens of normal and diseased birds has led to the conclusion that in many cases it is impossible to distinguish between normal and pathological accumulations of cells in this organ.

The rounded, circumscribed lesions are largely composed of cells resembling the lymphoblasts. The nuclei are large, with coarse granules of chromatin. There are mitotic figures in many of the lesions, and numerous pycnotic nuclei. The areas are often surrounded by a narrow band of connective tissue, and certain of these areas are joined by irregularly shaped infiltrations of cells of similar type. The irregular areas of infiltration are found most frequently in the heart. They also occur in the pancreas and spleen and are occasionally present in the liver, kidney, lung, ovary, and other organs. They are composed chiefly of cells resembling large lymphocytes, and no mitotic figures have been seen among them. In the heart, such infiltrations often push apart the muscle bundles (Fig. 7). There seems to be no degeneration of the muscle fibers, but in some cases the heart shows other evidences of chronic inflammation.

Since lymphoid cell infiltrations of similar nature are encountered in many pathological conditions in chickens, these lesions are not considered in any way distinctive of epidemic tremor, although the infiltration in the pancreas is unusual. The fact that they do not occur in the inoculated birds may indicate a more strictly localized reaction to the infective agent, possibly due to its mode of introduction.

Epidemiology

Our first experience with the disorder was in May, 1930, when nine Rhode Island Red chickens about 2 weeks of age were brought to us from a commercial flock. These birds were affected with a fine tremor which did not abate during a period of observation extending over 3 months. The group was abnormally excitable. No ataxia developed among them, nor to our knowledge was there any ataxia in the flock from which they were taken.

The disease was not seen again until April, 1931, when eleven Rhode Island Red chickens were brought to the laboratory from a different source. Six of the birds were 4 weeks old and had been kept on the farm where they were hatched. The other five birds were from the same stock but hatched 1 week later. They had been sold as day old chicks and consequently had been under different environmental conditions since hatching. The older birds were less severely affected than the younger ones. All showed tremor but of varying degrees of severity. Some of the birds in both groups were ataxic on their arrival in the laboratory and some developed ataxia later.

In January, 1932, severe outbreaks occurred on one poultry farm in early hatches of eggs from one source. Forty-two birds from 2 to 6 weeks of age were sent to the laboratory. It was said that certain birds on this farm had exhibited tremor on the 2nd day out of the incubator. All of these birds exhibited tremor when sent to us, and many of them were likewise ataxic.

The occurrence of a similar condition in several other flocks was reported to us during the spring of 1932, but no specimens were sent to the laboratory until May when forty chickens were sent in from a flock unrelated to that in which the January outbreak had occurred. All but one of these chickens were ataxic, and twenty-seven of them were also affected with tremor.

The epidemics of 1930, 1931, and spring of 1932 all occurred in Massachusetts. After the May, 1932, epidemic, no cases were heard of until November, 1932, when a serious epidemic was reported in New Hampshire. This was followed by outbreaks in other New England states until by the first of June, 1933, there had been reported to this laboratory as having occurred during the winter and spring hatching season of 1932–33, eight epidemics from Massachusetts, four from New Hampshire, two from Maine, and one from Connecticut. There have doubtless been other affected flocks which have not come to our attention. No cases of the disease have been reported during the months from June to November, 1933. Affected birds in the majority of these flocks have exhibited tremor, ataxia, or both. Birds from two flocks were affected with ataxia only.

From epidemics in 1932, there was some evidence that the disease was more frequent among chicks of the first hatch of the season than in later hatches. Further experience, however, has proved that the appearance of the disease in relation to hatch is a matter of chance. Certain epidemics have been confined to the first hatch of the season; other outbreaks have affected the first three hatches; while still others have appeared in the middle or toward the end of the breeding season, and have affected from one to three hatches.

There have been some cases of recurrence of the disease in flocks in the 2nd year. No recurrence has been noted for 2 successive years in the flock involved in the 1931 epidemic.

The disease may appear in young chickens during the 1st week or subsequently up to 5 or 6 weeks of age. In one case tremor is said to have appeared at 3 days, and in another, at 5 days. The usual time of onset, however, is at 3 weeks of age. No epidemics have been reported among adult birds. Epidemics have occurred in Rhode Island Red, New Hampshire Red, Barred Plymouth Rock, and White Plymouth Rock flocks.

Etiology

Early in the study of these outbreaks, experiments were undertaken to determine the nature of the disease. Efforts were made to transmit the conditions to normal birds and to cultivate an organism from the tissues of diseased birds. In addition, the possibility of the disease being a nutritional disorder, or a form of food poisoning was considered.

Chemical analysis for calcium and phosphorus was made of the blood of two birds with severe tremor. The analysis showed 11 mg. calcium and 8.7 mg. phosphorus which was considered[1] a normal value for young chickens.

[1] We are indebted to Dr. Joseph C. Aub for the calcium and phosphorus determinations and for their interpretation.

No evidence of rickets has been found.

Influence of Vitamins.—The occurrence of nervous disorders thought to be due to the absence or deficiency of various vitamins in the diet has been reported by several investigators (Hughes, Lienhardt, and Aubel, 1929, Hogan and Shrewsbury, 1930, Wolf and Pappenheimer, 1931). To determine whether vitamin deficiency was a cause of epidemic tremor, certain affected chickens sent to us during the 1931 epidemic were given a diet supplemented with whole milk, lettuce, hard boiled egg, and a commercial vitamin B concentrate. No influence of diet was discerned.

Attempts to Cultivate an Organism.—All attempts at cultivation of an organism of etiological significance in the disease have been unsuccessful. Cultures on agar slants were made of brain suspensions used for inoculation. With the exception of occasional obvious contaminants, the cultures were negative. Cultures of brain and spinal cord from birds received from field epidemics were made on a variety of media including broth, agar, agar plus normal chicken serum, blood agar, and chopped meat media. Cultures were also made of heart's blood, liver, spleen, and pancreas from a series of ten spontaneous cases. Broth, agar, agar plus serum, and chopped meat media were used. No organism of etiological importance was isolated in either of these series.

Contact Experiments.—Attempts to transmit the disease to normal birds through contact with affected birds were made in three epidemics. Normal and diseased chicks were kept in the same cages with every opportunity for contamination of the feed and drinking water and exposure to droppings. In one experiment a suspension of brain from a diseased bird was mixed with the drinking water, and some of this suspension was likewise introduced into the nares by means of a fine pipette. No disease developed in any of the exposed birds, nor were lesions found in the brain on microscopic examination.

Tests of Suspected Feed.—The grain supply naturally was suspected by poultry men to be the cause of the disease in several of the early outbreaks. Experiments were carried out (1) to test the grain for contamination with the infective agent, (2) to discover whether the composition of the mash was at fault, and (3) to see whether a toxic substance was produced in mash when stored in a warm place. A commercial broiler ration from farms where it was being fed to young chicks among which the disease had appeared was used in these experiments. Groups of normal chickens in the laboratory were fed this ration (*a*) without autoclaving; (*b*) after autoclaving at 15 pounds pressure for 20 minutes; (*c*) without autoclaving and after storage in a very warm room. None of the fifty chickens in these experiments developed the disease. Microscopic examination was made of the brain from nine birds. No lesions were found.

Tests were also made for the presence of spore-bearing anaerobes in the feed. None were found.

Inoculation Experiments.—Attempts were made in 1930, when the first affected chicks were studied, to transmit the disease to normal

chickens by intraperitoneal inoculations of suspensions of intestine, liver, spleen, and brain. A portion of the intestine with its contents was also made into a suspension in normal saline and given *per os* to a normal chicken. The inoculated birds were kept in the laboratory for 112 days. They developed no symptoms during that time. At autopsy the gross findings were negative in every case. The brains were later studied microsopically. Unequally stained nerve cells were found, slight perivascular infiltration, and rare, small, focal collections of glia cells. No lesions were found in the spinal cords of these birds. In view of the results of later inoculation experiments in which inoculated birds were found to have brain lesions without having shown symptoms of disease, it is possible that these birds acquired the disease, but in so light a form that they exhibited none of the typical signs.

Since these early experiments, few attempts have been made to transmit the disease by other than intracerebral inoculation of suspensions of brain and spinal cord. The following experiments with inoculation by other routes and with other organs may, however, be summarized:

Suspensions of liver, spleen, pancreas, and gall bladder from birds in the 1932 epidemics were inoculated subcutaneously into a small series of normal chicks. No symptoms were observed in any of the birds so inoculated, nor were any lesions found in the brain. Suspensions of brain and spinal cord of birds in the 1932 epidemic were also given to a group of normal chickens *per os*, intraperitoneally, intracerebrally, and by the two latter routes combined. No symptoms were observed in the inoculated birds and no brain lesions were found.

More recently, intraperitoneal and intracerebral inoculations have been made with a suspension of spleen from a bird inoculated with the eighteenth passage of the virus. As a control for the presence of virus in the bird, a suspension of brain from the same bird was inoculated intracerebrally into four birds. Brain suspension was likewise inoculated intraperitoneally into four birds. None of the seven birds receiving spleen suspension developed symptoms of disease after 5 weeks. Occasional small brain lesions were found in two of those inoculated intracerebrally, and some perivascular infiltration was found in the brain in two of these inoculated intraperitoneally. No lesions were found in the other three birds. Three of the four chicks receiving brain suspension intracerebrally developed symptoms in 27 to 33 days after inoculation. The fourth bird exhibited typical brain lesions. None of the birds inoculated with brain suspension intraperitoneally showed symptoms after 5 weeks. Small foci of infiltration were found in the brains of three of these birds. The fourth was negative.

Intracerebral inoculation of normal birds with suspensions of brain, spinal cord, or both, from diseased birds has proved to be an effective mode of transmission. By this method the disease has now been transferred through twenty successive passages. The following technique was used in the first eight passages and occasionally in some of the later transfers.

Diseased birds were killed by chloroforming, and the brain and spinal cord (if used) removed aseptically. The tissue was ground in a sterile mortar, and normal saline added to form approximately a 1:10 suspension by volume. The coarser particles were allowed to settle, and the supernatant fluid drawn into a tuberculin syringe with a 25 gauge needle. Inoculation was made into the cerebrum, 0.1 cc. to each bird. This procedure was later modified by grinding the tissue with sterile sand, and after centrifugalization, the supernatant fluid was used for inoculation. This method is now used for routine inoculation.

During the outbreak in January, 1932, the intracerebral inoculation of brain and spinal cord of affected chickens into normal chickens was first undertaken. Material from twenty-seven chickens in the January and May epidemics of that year was used in this experiment. Brain, or spinal cord, or both from each bird was made into a suspension, as previously described, and four or more young Rhode Island Red chickens were inoculated with each suspension. A total of 91 chickens were inoculated, of which three developed typical tremor and ataxia. Each of the three chicks to develop symptoms had been inoculated with material from a different bird. Microscopic examination was made of the brains of forty-five of the chickens which had been inoculated but had developed no symptoms of disease. Lesions were found in two, each in a different series. Of the five birds with either symptoms or lesions, two had been inoculated with a suspension of spinal cord, two with a suspension of brain, and one with a suspension of combined brain and spinal cord. The incubation periods in the chicks showing symptoms were 29, 30, and 35 days. The fact that only five birds contracted the disease out of 91 chickens inoculated suggests that the active infective agent was present in the brain in only a small proportion of the chickens at the time they were used in the inoculation experiments.

In subsequent passages, a higher proportion of successful inoculations resulted, and the average incubation period was shortened.

The brain and cord of each of the three chickens which had developed tremor and ataxia after inoculation in the first passage were used for further inoculations. The results of this passage and of the subsequent passages are presented in Table I. Rhode Island Red chickens were used in the majority of these experiments, and White Leghorns in the remainder. Age of birds at inoculation varied from 2 days to 15 days.

These experiments may be summarized as follows:

Epidemic tremor of chickens has been transmitted through twenty passages by intracerebral inoculation of suspensions of brain, spinal cord, or the two combined.

In those series of inoculations in which at least one chicken showed disease, the number of infections has varied from 25 per cent in the early passages to 100 per cent in many of the later ones. The incubation period varied from 6 to 44 days, with the largest number of individuals showing symptoms during the 3rd and 4th weeks.

The infective agent has been shown to be present in saline suspensions of both brain and cord. After centrifugalization, it is present in the supernatant fluid as well as in the sediment of such suspensions. The infective agent in the supernatant fluid is viable for at least 6 hours at room temperature, for 18 hours at 37°C., and for 48 hours at 5°C. Its survival in 50 per cent glycerine will be discussed later.

Combined intracerebral and subcutaneous or intraperitoneal inoculations proved to be no more effective than intracerebral inoculations alone.

The addition of normal chicken serum, 2 per cent starch in salt solution, or minced chicken embryo to the suspensions failed to increase the proportion of infections.

The addition of testicular extract did not shorten the incubation period nor increase the proportion of infections over that of the control group in the one experiment in which it was tried.

Infectivity of Filtrates.—Epidemic tremor was early recognized as having many of the characteristics of a virus disease. A number of experiments have been undertaken to test the filter-passing ability of the infective agent in the brain. Since some of this work is still in progress, a detailed report will be made later. Results of completed experiments may be briefly summarized as follows:

Seitz filters were used in eleven experiments. 95 chickens were

TABLE I

Summary of Nineteen Intracerebral Passages of Epidemic Tremor in Chickens

Passage No.	No. of chicks inoculated	Inoculation	Results			Incubation period
			Cases with typical symptoms	Cases without symptoms		
				Examined microscopically	Lesions present	
		1932				*days*
2	4	Feb. 24 1:10 suspension* brain 391	0	1	0	
	4	Feb. 24 1:10 suspension cord 391	0	1	0	
	10	Mar. 3 1:10 suspension brain 399	2	5	0	28, 29
	11	Mar. 3 1:10 suspension cord 399	0	2	1	
	5	June 18 1:10 suspension brain 641	0	3	1	
	6	June 18 1:10 suspension cord 641	0	3	0	
3	5	Apr. 6 1:5 suspension brain 516	0	1	0	
	5	Apr. 6 1:5 suspension cord 516	1	0		40
	3	Apr. 6 1:5 suspension brain 518	0	1	0	
	5	Apr. 6 1:5 suspension cord 518	1	0		34
4	4	May 13 1:5 suspension brain and cord 600	0	1	0	
		This suspension plus equal volume:				
	4	(*a*) 2% suspension starch in normal saline	0	1	0	
	4	(*b*) Normal chicken serum	0	1	0	
	4	(*c*) 5 day chick embryo (minced)	1	1	0	32
		May 27 1:10 suspension brain 592				
	3	Inoculated intracerebrally	0	1	0	
	3	Inoculated intracerebrally and intraperitoneally	0	1	0	
	3	May 27 1:10 suspension cord 592	0	1	0	

5	4	June 18	1:10 suspension brain 661	2	2	0	24, 30
	5		1:10 suspension cord 661	0	2	0	
6	5	July 20	1:10 suspension brain 694	2	0		31, 41
	5		1:10 suspension cord 694	0	1	0	
	4	July 20	1:10 suspension brain 695	1	0		41
	4		1:10 suspension cord 695	0	1	0	
7	11	Aug. 20	1:10 suspension brain and cord 721	9	0		15, 16, 20, 21, 21, 34, 34, 36, 44
	8	Aug. 20	1:10 suspension brain and cord 722	3	4	4	30, 30, 33
8	10	Sept. 24	1:10 suspension brain and cord 756	4	6	5	20, 23, 33, 44
			Combined intracerebral and intraperitoneal inoculation				
	10	Sept. 24	Inoculated as above with brain and cord 756	6	4	1	23, 27, 28, 29, 37, 41
		Oct. 5	Reinoculated intracerebrally with 1:10 suspension brain and cord 758				
	5	Sept. 24	1:10 suspension brain and cord 746	4	1	0	20, 29, 34, 35
			Combined intracerebral and subcutaneous inoculation				
	3	Sept. 26	1:10 suspension brain and cord 757	1	2	0	17
	5		Supernatant fluid from this suspension after 5 min. centrifugalization	3	2	2	20, 31, 32
	3		Sediment after centrifugalization	2	0		23, 30
	5	Oct. 10	1:10 suspension brain and cord 747	0	0		
9	5	Oct. 21	1:10 suspension brain and cord 786 and 818	4	1	0	25, 27, 28, 38
	5		Supernatant of above suspension after 10 min. centrifugalization	1	4	2	26
	2	Oct. 25	Supernatant of 1:10 suspension brain and cord 797 and 793	2			23, 24
	5	Nov. 10	Supernatant of 1:10 suspension brain and cord 790	3	2	2	23, 25, 27
	4	Nov. 11	Supernatant 790 after 24 hrs. refrigeration	2	2	2	22, 26

* All suspensions were made in normal saline except as noted.

TABLE I—*Concluded*

Passage No.	No. of chicks inoculated	Inoculation	Results			Incubation period
			Cases with typical symptoms	Cases without symptoms		
				Examined microscopically	Lesions present	
		1932				*days*
	2	Nov. 18 1:10 suspension brain and cord 836 and 846 Intracerebral and subcutaneous inoculation	2			10, 19
	4	Dec. 1 1:10 suspension brain 837 after 6 hrs. at room temperature	3	1	1	12, 13, 13
10	2	Dec. 2 Supernatant of 1:10 suspension brain 838	1	1	1	12
	1	Dec. 8 Supernatant of 1:10 suspension brain and cord 903 and 907	1			26
	1	Dec. 9 Supernatant as above after 18 hrs. at 37°C.	1			25
	2	Dec. 12 1:10 suspension brain and cord 905	2			24, 26
	3	Dec. 14 Supernatant of 1:10 suspension brain and cord 930	3			20, 20, 21
11	3	Dec. 15 Supernatant of 1:10 suspension brain and cord 929	2	1	1	19, 35
	3	Dec. 16 Supernatant of 1:10 suspension brain and cord 926	3			19, 19, 35
	3	Dec. 19 Supernatant of 1:10 suspension brain and cord 925	2	1	0	18, 18
		1933				
12	4	Jan. 13 Supernatant of 1:10 suspension brain and cord 1033 and 1038	3	1	0	25, 25, 26
	3	Jan. 17 1:10 suspension brain 957	2	1	0	22, 27
13	4	Feb. 10 1:10 suspension brain 1113	4			20, 20, 20, 20
	3	Feb. 24 1:10 suspension brain 1121	3			19, 21, 27

14	2	Mar. 8	Supernatant fluid of 1:10 suspension brain 1220 and 1228	1	1	1	15
	5	Mar. 8	Supernatant fluid of 1:10 suspension brain 1218, 1224, and 1226	4	1	1	16, 20, 21, 27
	3	Mar. 28	Supernatant fluid of 1:10 suspension brain 1244 and 1245	2	1	1	20, 20
15	9	Apr. 29	Supernatant of 1:10 suspension 1272	9			21, 23, 24, 30, 33, 33, 40, 41, 42
16	8	June 21	Supernatant of 1:10 suspension brain 1398, 1424, and 1426	2	6	3	29, 33
17	3	July 24	1:10 suspension brain 1512	3			21, 21, 28
	5	July 29	Supernatant of 1:10 suspension brain 1475 and 1481 after 48 hrs. in refrigerator	2	3	3	16, 37
18	4	Aug. 31	Supernatant of 1:10 suspension brain 1522, 1524, and 1530	4			15, 19, 21, 25
	5	Sept. 8	Supernatant of 1:10 suspension brain 1523 and 1528 after 48 hrs. in refrigerator	4	1	1	26, 30, 30, 33
19	4	Oct. 7	Supernatant of 1:10 suspension brain 1583	3	1	1	27, 28, 33
20	3	Nov. 9	Equal parts of hormone broth and supernatant of 1:10 suspension in hormone broth of brain 1622 and 1624	3			6, 6, 36
	4		Equal parts testicular extract and supernatant of 1:10 suspension in hormone broth of brain 1622 and 1624	2			7, 22

inoculated with bacteriologically sterile filtrates. Three birds in three different experiments developed the disease, and eleven others in eight different experiments had typical microscopic brain lesions.

Berkefeld filters have also been used in a number of experiments. Typical disease as well as brain lesions have followed the inoculation of Berkefeld N filtrates in two experiments. Inoculation of Berkefeld W filtrates has thus far failed to produce disease.

Survival of the Infective Agent in Glycerine

Infective brain tissue preserved in 50 per cent glycerine for 6, 47, and 69 days has been used to inoculate three series of four chicks each. All of those inoculated with the 47 and 69 day material became ataxic. One of those inoculated with the 6 day material developed disease; two had typical brain lesions; the fourth was normal. The survival of the virus in glycerine for a period of over 2 months has thus been demonstrated.

Attempts to Demonstrate Transmission of the Infective Agent through the Egg

The occurrence of epidemic tremor in chicks less than a week old suggests that the disease may be transmitted through the egg.

A series of inoculations was made in 1933 with brain tissue of embryos from a stock in which epidemic tremor had appeared in the preceding hatch. Unfortunately the disease died out after its first appearance, and the completely negative experiment was hence inconclusive. It is planned to repeat this work.

Breeding experiments were likewise undertaken. A small number of chicks, offspring of affected birds, have been raised in the laboratory. None of these developed disease, nor were they immune at 6 weeks of age to intracerebral inoculation of infective brain.

A serious outbreak of epidemic tremor in chicks of known parentage at an experimental farm[2] in November, 1932, provided an opportunity to carry on breeding experiments on a large scale. The disease appeared only in the first hatch of the season and affected approximately 50 per cent of 400 chicks brooded. Pedigree hatching was then instituted, but no disease developed in chicks from these matings. Breeding pens from these birds and from the survivors of the affected

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[2] We are indebted to the Department of Poultry Husbandry of the New Hampshire Agricultural Experiment Station for the opportunity to observe and the permission to report these experiments.

birds of the first hatch were saved, and all eggs were incubated. No disease appeared in them up to January, 1934. A detailed report of these experiments will be published at a later date.

There is no available evidence that transmission takes place through the egg.

Controls

All of the Rhode Island Red chickens used in laboratory inoculations were obtained as day old chicks from one commercial hatchery. Many hundreds of chicks obtained from this source from time to time in lots of twenty-five or fifty have likewise been used for other experiments in progress in the laboratory throughout the course of the work with epidemic tremor. One chick showed a fine head tremor on arrival at the laboratory, but on examination of the brain no lesions were found. The cause of the tremor in this case remains unexplained. Aside from this one questionable bird, there have been no spontaneous cases of epidemic tremor in our chickens.

Many groups of chickens have been kept as controls for series inoculated with epidemic temor, and the brains of many uninoculated birds have been examined for lesions in the course of these experiments, but no effort has been made to keep a control group for each series of experiments.

DISCUSSION

The occurrence of tremors and ataxia in chicks has been reported in association with a number of diseases of various etiology.

Pappenheimer and Goettsch (1931) in describing the clinical behavior of chicks suffering from "nutritional encephalomalacia" state that many of the birds before they were completely prostrated, became incoordinate and ataxic, and that clonic spasms of the legs and sometimes coarse tremors were also observed. The disease appeared in young chicks on a particular diet. The lesions were found chiefly in the cerebellum, but occurred not infrequently in the cerebrum, midbrain, and medulla as well. The essential lesion is described as ischemic necrosis followed, if the animal survives, by reparative organization of the dead tissue. Dunlap (1932) has also reported an ataxia of chicks associated with nephritis. This disease is apparently similar in its symptom complex to that described by Pappenheimer and Goettsch; namely, muscular incoordination, twitching or tremor of the head and legs, and retraction of the head. No microscopic studies of the central nervous system were reported, but changes in kidneys and proventriculus were noted, both grossly and microscopically. The uric acid content of the blood was increased, indicating impairment of nitrogenous metabolism. It was believed that this disease was of nutritional origin since it was correlated in field cases with high protein intake and forced feeding. It was stated that changes in feeding prevented new cases.

The nutritional etiology of these two conditions is sufficient to differentiate them from epidemic tremor, in which there is no evidence of nutritional origin or influence. Furthermore, the microscopic brain lesions of encephalomalacia and epidemic tremor are distinctive. The absence of nephritic symptoms further differentiates epidemic tremor from the ataxia reported by Dunlap.

Barile (1931) has reported what is apparently a case of lymphomatosis gallinarum. A distinction must be made between this disease and the encephalomyelitis of epidemic tremor. The latter is a disease chiefly of the first month of life. It is unaccompanied by tumors or iritis, and there is no enlargement of dorsal root ganglia, nor infiltration of the peripheral nerves. There is no involvement of the alimentary tract.

In the occasional flock in which ataxia appears unaccompanied by the head tremor, there is difficulty in distinguishing between epidemic tremor and prostrating rickets. On splitting the leg bones of ataxic chickens affected with epidemic tremor, however, a heavy line of calcification may be seen along the line of growing cartilage. Epidemic tremor in our experience has been unaccompanied by rickets, and no rachitic changes have been observed on X-ray examination, nor on section of the tibia in a number of birds from field epidemics and from experimentally inoculated chickens.

Successful transmission of the condition by brain to brain inoculation leads us to the conclusion that we are dealing with a disease of infectious origin. Attempts to cultivate an organism capable of transmitting the disease have been completely negative. Production of the disease following inoculation of bacteriologically sterile filtrates after passage through Seitz and Berkefeld N filters places the causative organism among the filter-passing group. No cell inclusions have been demonstrated.

SUMMARY AND CONCLUSIONS

A new disease having a characteristic and well defined symptom complex is described as occurring in young chickens in four New England states. Tremor, principally of the head and neck, and progressive ataxia are the characteristic symptoms, either or both of which may

be present in a single bird. Age at onset in field epidemics ranges from 3 days to 6 weeks, with a majority of cases reported at 3 weeks. Morbidity in commercial flocks ranges from 5 to 50 per cent; mortality in affected hatches may be 50 per cent. The disease may or may not recur in successive hatches, and in the same flock in successive years. Although birds may survive an attack of the disease, nervous symptoms persist in a majority of cases.

There is no evidence that nutritional factors are involved. Normal chickens have not contracted the disease by contact with affected birds. The disease has been reproduced in normal chickens by intracerebral inoculation of brain and spinal cord from affected birds. Twenty brain-to-brain passages have been made up to the present time. The incubation period in laboratory passages ranges from 6 to 44 days with symptoms appearing usually between 21 and 28 days. The proportion of inoculated birds developing symptoms has increased with successive passages.

The infective agent in the brain has survived in 50 per cent glycerine for 69 days. No organism has been cultivated. The disease has been reproduced after inoculation with bacteriologically sterile filtrates obtained with Seitz and Berkefeld N filters.

Attempts to demonstrate the presence of the infective agent in the chicken embryo have been inconclusive. Chicks hatched from eggs laid by birds which had survived the disease were not infected, nor were they immune to inoculation at 6 weeks of age.

The characteristic lesion of the disease consists of microscopic focal collections of glia cells, perivascular infiltration, degeneration of Purkinje's cells, and degeneration of nerve cells. Foci of infiltration are present throughout the brain and spinal cord. In the viscera of birds from field epidemics, microscopic focal infiltrations of cells of the lymphoid series are often found. Their presence is most notable in the pancreas and heart. No cell inclusions have been demonstrated.

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EXPLANATION OF PLATES

PLATE 51

FIG. 1. Ventricular area in brain of spontaneous case showing focal collections of glia cells. Cresylecht violet stain. × 65.

FIG. 2. Spinal cord of spontaneous case. Note collections of cells in gray matter. Cresylecht violet stain. × 60.

FIG. 3. Optic lobe of inoculated bird. Note perivascular infiltration. Eosin-methylene blue stain. × 65.

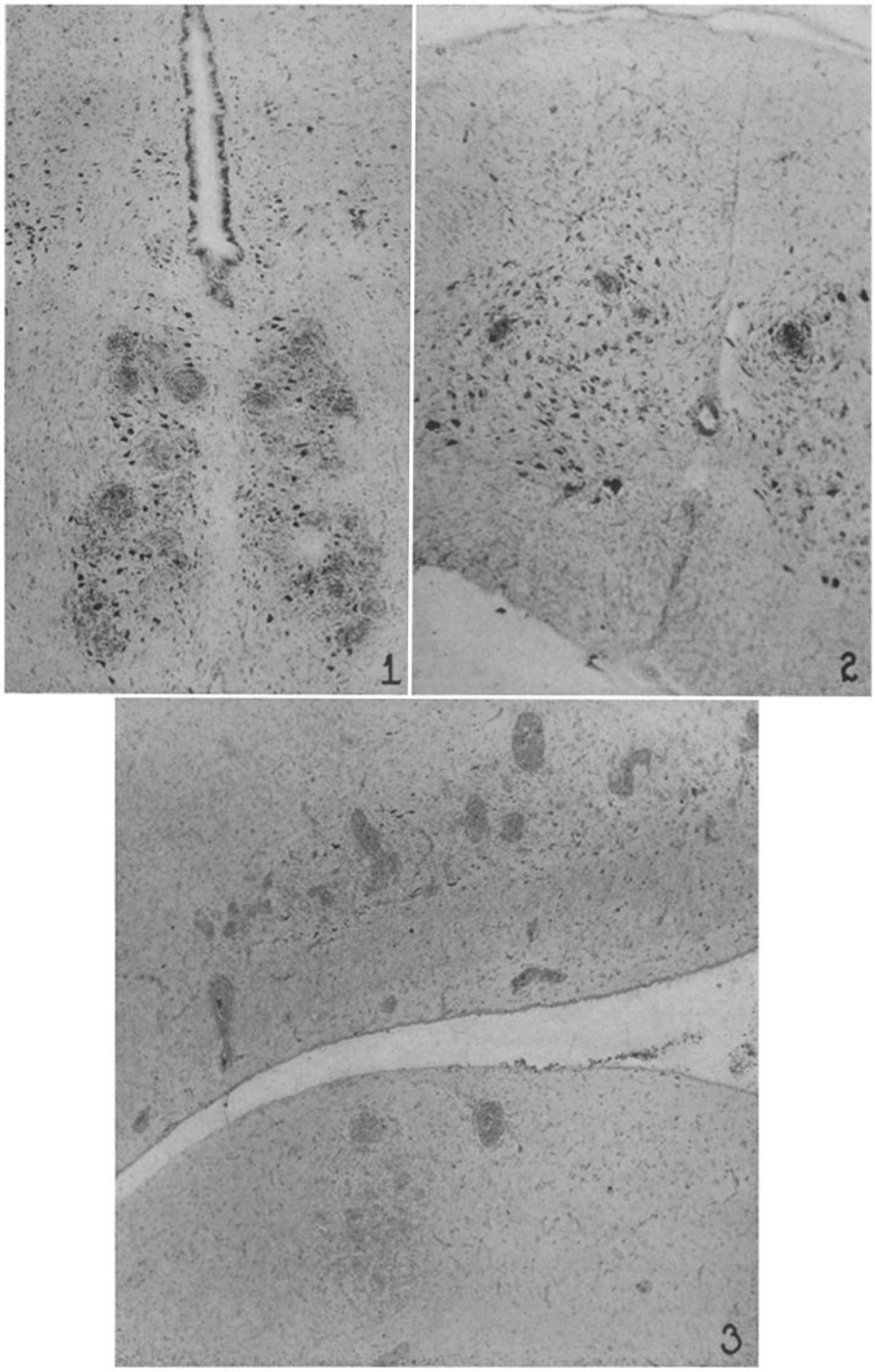
PLATE 52

FIG. 4. Cerebellum of inoculated bird. Severe focal infiltration. Eosin-methylene blue stain. × 65.

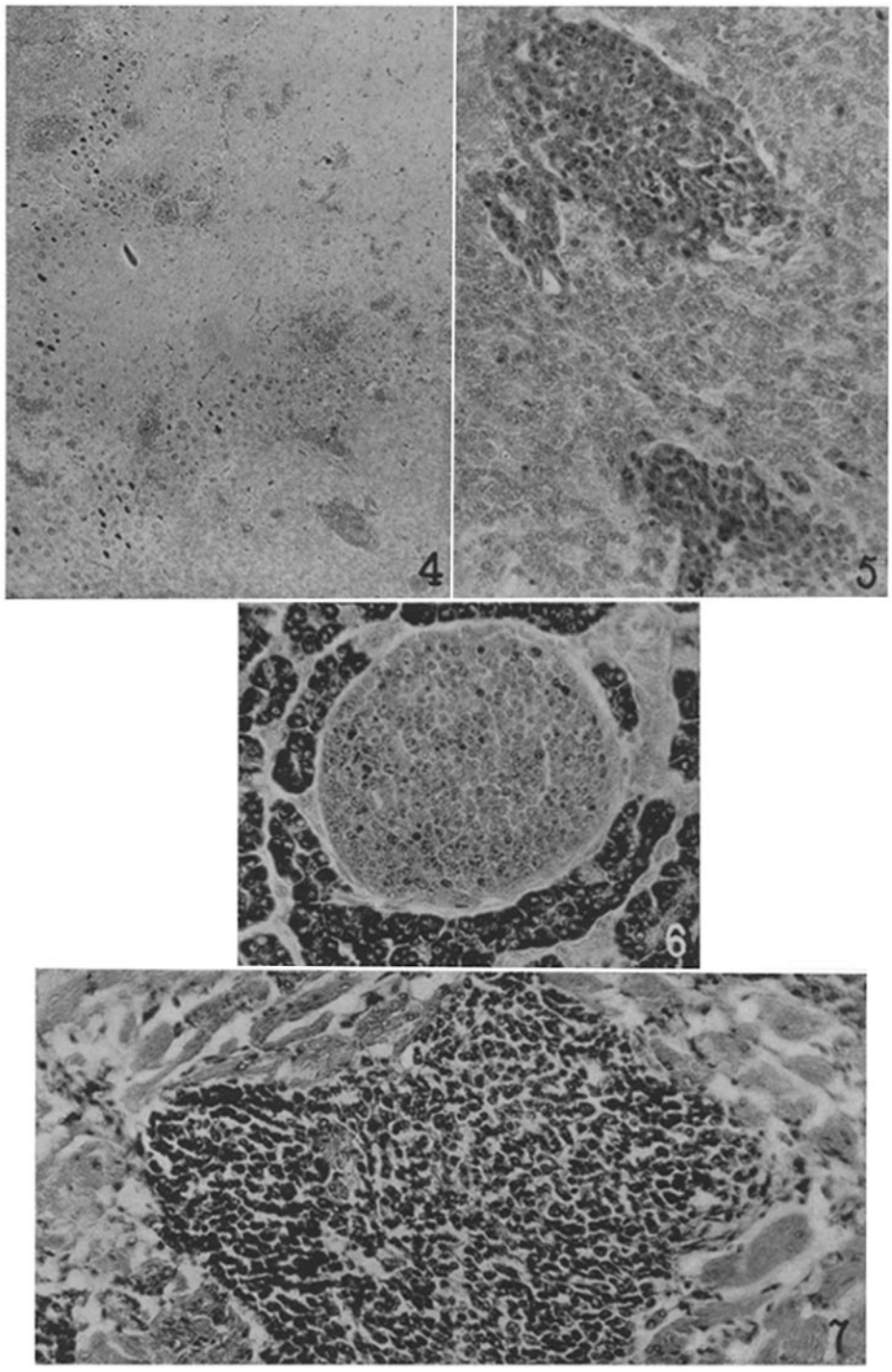
FIG. 5. Lesion in cerebellum of inoculated bird. Eosin-methylene blue stain. × 300.

FIG. 6. Pancreas of bird (spontaneous case) showing rounded collection of lymphoid cells. Eosin-methylene blue stain. × 300.

FIG. 7. Heart of bird (spontaneous case) showing area of infiltration with lymphoid cells. Hematoxylin and eosin stain. × 300.



(E. Elizabeth Jones: Epidemic tremor in young chickens)



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