STUDIES ON FOWL PARALYSIS (NEUROLYMPHOMATOSIS GALLINARUM).*

II. Transmission Experiments.

By ALWIN M. PAPPENHEIMER, M.D., LESLIE C. DUNN, Ph.D., and S. M. SEIDLIN, M.D.

(From the Storrs Agricultural Experiment Station, Storrs, and the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.)

(Received for publication, August 23, 1928.)

In a foregoing article, a brief description was given of the symptoms and pathology of the disease known as fowl paralysis. Although efforts to demonstrate a causative organism in the tissues or by cultural methods were fruitless, it seemed fundamentally important to determine whether or not the disease was transmissible from one chicken to another under experimental conditions. The present article presents, in condensed form, experiments which have been carried out toward this end.

Previous workers have made efforts to establish the transmissibility of this disease. Kaupp (1), May, Tittsler and Goodner (2) and Doyle (3) all report negative experiments—using a variety of methods and material for inoculation. Van der Walle and Winkler-Junius (4), however, report the following experiment which is regarded by them as establishing the infectious nature of the disease. A hen fed with maize and barley and given water out of the well of the farm where paralysis occurred, showed symptoms of paralysis after 2 months. Another hen, fed with muscles from an affected hen, became atactic after 5 months. The blood of this hen, kept anaerobically for 18 days at 37° was injected into a healthy bird. A culture of bouillon was made from the blood of the latter, filtered through a Chamberland filter and injected intravenously into another hen. This showed

* This work was aided by a generous grant from the Mount Hope Farm, Williamstown, Mass. The writers wish also to express their grateful acknowledgment to the Scientific Director, Dr. D. H. Goodale, for material assistance and valuable suggestions. We have also received material and interested cooperation from numerous other sources. Although it is impossible here to make individual acknowledgments, the writers wish to express their thanks for this assistance.

symptoms of paralysis after 2 months, but recovered after 2 weeks. Another hen injected intraperitoneally with blood of a diseased bird, developed paralysis after 46 days. Five birds kept in the same pen with the affected birds, failed to develop the disease. The authors concluded from the foregoing experiment—in our opinion from insufficient evidence—that the disease is an infection due to a filtrable virus.

Our preliminary experiments were begun in June, 1925. At that time, we did not realize how frequently lesions of the central nervous system may be found in apparently normal chickens, nor were we aware of the long period which may elapse between the inoculation and the appearance of symptoms. In spite of these difficulties, a careful histological examination of the first twenty inoculated birds (Experiments I, II, III, IV) showed a suggestively higher incidence of lesions in brain, cord, spinal ganglia and more particularly in the peripheral nerves than was found in a series of uninoculated, non-paralysed controls. Thus, infiltrations of the sciatics or brachial plexus were present in 40 per cent of the 20 experimental animals, and in but one (3 per cent) of the 32 controls.

In subsequent experiments, inoculations were made by preference into newly hatched chicks, in the hope that spontaneous infection during the long developmental period of the disease might be avoided, and that the chicks might be less refractory at an early age.

Experiment V.—August 7, 1925. Eight 10 day old chicks were inoculated *subdurally* with unfiltered brain suspension of a typical advanced paralysis case. Eight chicks of the same hatching were kept as controls under identical experimental conditions. They were raised indoors on pine shavings; cod liver oil was included in the diet to prevent leg weakness.

One of the inoculated chickens was found dead and discarded without examination. The remaining seven were killed, 10, 14, 24, 34, 53, 62 and 120 days after inoculation, a control being sacrificed at the same time. One chick was also killed for examination at the beginning of the experiment.

The results of our histological studies in this series are summarized in Table I.

Lesions of the nervous system were found in all but one of the inoculated birds, and in but one of the controls. Only one inoculated chick was permitted to survive to an age at which clinical paralysis may be expected to appear. This chicken developed weakness of the legs 2 weeks before death, which did not improve with cod liver oil;

it was killed on the 120th day. There were found infiltrations of the brain, cord, ganglia and peripheral nerves, slight in intensity, but of the usual character, and this may be regarded as a positive case.

Experiment VI.—In this experiment, the eggs were obtained from a farm in New Hampshire where in a flock of 6000, no clinical case of paralysis had been observed. The eggs were hatched in the laboratory of the Storrs Experiment Station.

One hundred and twenty 5 day old chicks were divided into four lots of 30 each.

October 27, 1925. Lot 1 was injected with 0.1 cc. of unfiltered brain suspension, in 0.85 per cent NaCl, of a typical paralysed chicken.

Lot 2 received 0.1 cc. of brain suspension of a normal 5 day old chick.

Lot 3 received 0.1 cc. of a 24 hour old anaerobic "culture" of brain and cord of typical paralysed fowl (F-301), with Hartley broth used according to the technique of Gye for the demonstration of a filtrable agent in avian and mammalian tumors. Anaerobiosis in the hydrogen jar was not complete, the test solution of methylene blue failing to be completely reduced.

TABLE I.

10 day old chicks inoculated subdurally with saline suspension of brain of paralysed chicken. Occurrence of lesions compared with controls from same hatch. Killed at intervals from 10 to 122 days.

	Lesions			
	Brain	Cord	Ganglia	Nerves
Inoculated.....7	6	4	2	1
Controls.....8	1	1	2	0

Lot 4 was kept as controls, and was not inoculated.

The chicks were raised indoors on a table covered with pine shavings and sand. The different lots were separated by wire netting partitions. Drinking cups and feed troughs were kept separate, and disinfected daily. Cod liver oil was incorporated in the ration to prevent leg weakness. Weekly weighings were continued until the loss of a large number of chicks through an epidemic of bronchitis during the latter part of December and early January made the weight data of the survivors statistically valueless.

It may be stated here that the mean weight of each of the four groups during the first 9 weeks, before the onset of the epidemic, did not differ significantly, and was approximately normal for the conditions and breed. In other words, the inoculation *per se* had no effect upon the general development and nutrition of the birds.

The chickens that died spontaneously or that were killed for examination were subjected to a careful gross and histologic study. Blocks were taken from all parts of the brain; the entire spinal cord was removed, divided into segments and longitudinal sections of each block studied, together with some of the attached spinal ganglia. Sections also were taken from each brachial plexus, from the sciatic nerves at various levels and from lung, liver, spleen, myocardium, kidneys, pancreas, duodenum, ceca, adrenals and gonads.

100 birds from this experiment were examined after appropriate intervals, distributed as follows: Lot 1, 28; Lot 2, 24; Lot 3, 21; Lot 4, 27.

The results may be briefly summarized as follows:

Lot 1.—Seven chickens of this group (25 per cent) inoculated with suspension of paralysis brain and cord developed characteristic lesions of the disease, being sacrificed or dying spontaneously after 64, 71, 73, 75, 132, 190 and 276 days after injection.

Lot 2.—Inoculated with brains of healthy 5 day chicks. Of the 24 birds examined, two presented, after 156 and 176 days, the typical anatomical picture of paralysis, in the case of one chicken associated with visceral lymphomatosis. A third showed atypical lesions, not involving the peripheral nerves.

Lot 3.—Inoculated with 24 hour anaerobic Hartley broth "culture." Out of 21 birds examined, only one, killed after 150 days, developed symptoms and lesions of paralysis.

Lot 4.—Uninoculated controls. Two definitely positive cases occurred in this group, one on the 65th day, the other on the 86th day after the beginning of the experiment. A third apparently healthy cock, killed 175 days after inoculation, showed minimal infiltration of the peripheral nerves and was regarded as a doubtful case.

There is thus a definitely higher incidence of the disease in Lot 1, inoculated with material from the paralysed bird, than in the three other control groups. Expressed percentally upon the basis of the pathological findings, there are 25 per cent of positives in Lot 1, as against 8.3, 4.8 and 7.5 per cent in Lots 2, 3 and 4. This difference suggests, but does not convincingly establish the transmissibility of the disease.

Under ideal conditions, each individual bird should have been strictly segregated throughout the long experimental period, so that any possibility of secondary infection, either from other inoculated

birds of the same group, or from extraneous sources, would be so far as possible excluded. It was not practicable to carry out a large scale experiment under these rigid conditions. While the effort was made to segregate the different groups, it must be admitted that the possibility of infection from bird to bird or through intermediary sources was by no means adequately controlled.

Under these circumstances, the occurrence of a certain number of cases in the control groups is not surprising. In spite of the technical limitations in the conduct of the experiment, the considerably higher incidence of the disease in Group 1, in which the number of positive cases surpassed that in the other groups combined, must be taken to mean that the inoculation of material from paralysed birds favored the subsequent development of the disease.

It is clear, however, that only a certain proportion of newly hatched chicks are susceptible; in this experiment, only one in four later manifested the disease in its typical form.

Assuming the existence of a specific virus, failure to acquire the disease may theoretically be explained in one of several ways. There may have been an uneven distribution of the infective material in the inoculum. Resistance to the graver form of the disease may have been acquired through a mild spontaneous infection picked up during the early stages of the experiment. There may be inherited factors influencing resistance or susceptibility.

One cannot profitably discuss these possibilities with the facts at hand. But another question arises which bears even more closely upon the interpretation of the experiment. What is the significance of the slight infiltrations of the brain and cord, found in so large a proportion of both inoculated and control chickens? Do they signify the existence of the disease in a mild form, undisclosed by clinical symptoms? If so, are we justified in regarding as positive only those cases which develop pronounced and intense lesions of the peripheral nerves?

Excluding the frankly typical positive cases in each group, we find the infiltrations of the brain and cord distributed amongst the four groups as shown in Table II.

The slightly higher incidence of brain lesions in Group 1 is counterbalanced by the lower incidence of cord lesions. With the small

numbers involved, and the possibility of overlooking isolated lesions in routine examination, it seems likely that the differences are accidental, and that the lesions are fairly uniformly distributed throughout the four groups.

Table III shows the distribution when not only birds with pronounced lesions involving the peripheral nerves, but also those showing slight infiltrations limited to the brain and cord, are included amongst the positive cases.

Although there is again a higher incidence of lesions in Group 1, the

TABLE II.

	Brain + or ±	Brain −	Cord + or ±	Cord −	Brain Per cent +	Cord Per cent +
Lot 1	13	6	5	14	68	26
Lot 2	10	11	8	13	47	38
Lot 3	10	9	8	10	52	44
Lot 4	11	14	9	16	44	36

TABLE III.

	Positive	Negative	Percentage positive
Group 1	21	6	78
Group 2	16	8	66
Group 3	14	7	66
Group 4	15	12	55

difference does not appear to be very striking, nor perhaps beyond the range of chance distribution; so that if we regard the chickens with mild lesions as being infected with a mild form of the disease, it would be impossible to ascribe any effect to the inoculation other than a slightly increased susceptibility to the graver form of the disease. In other words, from 55 to 65 per cent of the chickens acquired the disease naturally during the experimental period; and an additional 10 per cent acquired it as a result of inoculation. This is indeed a puzzling situation.

It may be suggested that the slight lesions found in so large a proportion of the birds represent a reaction of the tissues to a different

agent from that causing the more intense lesions found in typical paralysis cases. This possibility cannot be controverted so long as we are forced to depend upon the histological lesions for a recognition of the disease. But our material includes an unbroken series of transitions between the slightest lesions and those which approach true neoplasms in the intensity of the cell proliferations, so that it seems logical, for the present, to assume that we are dealing with the same inciting cause acting upon organisms of varying reactivity.

Experiment VII.—In the hope of eliminating so far as practicable, the possibility of extraneous infection during the long experimental period, another experiment was carried out under the following, more rigid, conditions.

Fertile eggs were obtained from the same source as those used in the preceding experiment. They were hatched at Storrs in a Blue Hen incubator in a separate disinfected compartment. The date of hatching was April 28, 1926. They were kept in the incubator until April 30, on which day 18 were inoculated subdurally and 15 intramuscularly into the left leg with material from a typically paralysed bird. The cord, enlarged cervical ganglia and left sciatic nerves were asceptically removed, ground in 10 cc. of 0.85 per cent salt solution, centrifuged and the turbid supernatant fluid used for injection. Of this fluid, 0.1 cc. was injected subdurally, and 0.2 cc. intramuscularly.

After injection, the chicks were replaced in the incubator, and on the following day removed to a farm situated at a distance of a mile from the Experiment Station. At this time there were no chickens on this farm, nor within a radius of about a quarter of a mile. The experimental chicks were placed in charge of Mr. Milton Moore, with the arrangement that he was scrupulously to avoid contact with other chickens throughout the course of the experiment.

The brooders were not ready until May 3, so that for 3 days the chicks were kept in the house in a separate room from the controls. On that day, they were transferred to a specially constructed outdoor brooder house, with electric hovers. These were so designed that food and water could be given and bedding changed without handling the birds. A sun porch screened with fine mesh wire was attached to the end of the brooder house, and covered with burlap to avoid possible contamination by the droppings of wild birds. Freshly distilled water was used throughout the entire course of the experiment. The food was that used in the previous experiment, and peat moss was used for bedding. For a brief period during the 1st month, fresh grass was added as a supplement to the diet. This unfortunately introduced an unknown factor and a theoretically possible source of outside infection, but was considered necessary, because several chicks had died with extensive urate deposits in the kidneys. After the addition of grass to the food, no further trouble from this condition was experienced.

34 chicks of the same hatching were used as controls, and were kept under identical experimental conditions in a brooder house about 30 feet away.

It was deemed inadvisable to subject the chicks to the handling involved in weekly weighings. On August 23, a sample of 15 chicks of the inoculated group, and 17 of the controls were weighed. The average weight of the inoculated was 1060 gm.; of the controls, 1147 gm. This difference is probably without statistical significance.

The experiment was thus planned to exclude infection by direct contact, by contact with possible human carriers, by insects of a size larger than the fine meshed wire gauze used and by drinking water.

Results.—Of the 31 inoculated birds, three developed the disease in a pronounced and typical form, one dying spontaneously on the 78th day, and two being killed on the 68th and 117th days after having exhibited unmistakable symptoms of paralysis. At least four other chickens of the inoculated group presented lesions of sufficient gravity to warrant their inclusion amongst the positive cases. The total incidence—seven out of 31, or 22 per cent—agrees well with the incidence of positive cases in Group 1 of the previous experiment.

The control group, unfortunately, did not remain wholly free of the disease, although there were no outstanding cases comparable in severity to the three above mentioned, and but two cases which could reasonably be regarded as comparable to the four mildly positive cases amongst the inoculated birds. The incidence in the control group may thus be reckoned as two out of 32, or 6.2 per cent.

Table IV brings out the comparative incidence and severity of the nervous lesions in the two groups.

There can be no question that in this experiment there is a significantly higher incidence of infiltrations in all parts of the nervous system, and particularly in the peripheral nerves in the inoculated group. One cannot escape the conclusion that the inoculation of the supposedly infective material is in some way correlated with this higher incidence, inasmuch as the inoculation was the only known variable in the experiment.

Several points require comment. The long period which elapsed between the inoculation and the first appearance of symptoms or the development of manifest lesions is striking. In one chicken paralysis was first noted 68 days after inoculation; another chicken died on the 78th day, and the third positive case first presented symptoms on the 115th day, and was killed 2 days later. We have seen in discussing the age incidence of the spontaneous cases, that there is a refractory period of approximately 3 months after hatching, during which, in our experience, the disease does not become manifest. This freedom of young growing chicks from the clinical evidence of the disease is apparent also in our experimental cases.

Another point of interest is that in at least one of the three positive cases, the disease developed after intramuscular injection. In two of the mildly positive cases, also, the inoculation was by the intramuscular route. We may conclude, therefore, that it is not essential to introduce the supposedly infective material directly into the nervous tissue in order to produce the characteristic effect.

Experiment VIII.—The purpose of this experiment was to determine whether the disease could be transmitted by the inoculation of a cell-free Berkefeld N filtrate alone, or by a filtrate "activated" by the addition of embryonic extract.

April 5, 1927. Brain and cervical cord with attached ganglia of a paralysed White Leghorn hen (Storrs 12–014) were removed aseptically, ground in sterile Tyrode's solution (pH 7.8), centrifuged at low speed and the supernatant filtered through a tested Berkefeld N filter. Inoculations were made into 6 day Spangled

TABLE IV.

	Brain			Cords			Nerves		
	Marked	Moderate or slight	Negative	Marked	Moderate or slight	Negative	Marked	Moderate or slight	Negative
Inoculated	0	24	7	2	23	6	3	14	14
Controls	0	8	24	1	15	16	0	5	27

Hamburg chicks obtained from a commercial hatchery in Ohio. This strain was selected because it had exhibited an unusually high mortality from the spontaneous disease during the previous season. 15 chicks were inoculated subdurally with 0.1 cc. of the clear filtrate; 16 chicks received 0.1 cc. of filtrate diluted with an equal volume of freshly prepared chick embryo extract; 18 chicks were injected with 0.1 cc. of embryo extract alone, to control the possible presence of the "virus" in the embryos used to prepare the extract. The two groups receiving the injection of material from the paralysed birds were reared in a separate room at the College of Physicians and Surgeons; the control group was strictly segregated in another room of the animal house.

Two of the chickens died spontaneously after 70 and 97 days respectively, both showing a severe coccidial infection, but no symptoms or lesions of paralysis.

The remaining members of the three groups were sacrificed during the period from 115 to 124 days after inoculation. No clinical symptoms of paralysis were observed, and a very careful gross and microscopic study failed to disclose typical lesions of paralysis.

This experiment therefore offered no support for the view that the disease is carried by a "filtrable" virus. We attach only limited importance to this negative experiment, however. Apart from the complex factors which affect the filtrability of ultramicroscopic viruses, the importance of this particular experiment is limited by the choice of an alien strain of chicks for inoculation. Possibly also the dosage of "virus" was insufficient.

The addition of embryonic extract to the inoculum failed to exert an activating influence.

Experimental IX.—The material for inoculation in this experiment was obtained from a chicken hatched in the laboratory at the College of Physicians and Surgeons; injected on the 2nd day into muscle of right leg with suspension of nerve tissues and arsenious acid (1–150,000), from a typical spontaneous case of paralysis. The bird had been isolated with four other chicks of the same hatching. On the 82nd day, it was found to have definite paralysis of the right leg, with drooping of the wings. 4 days later, it was etherized and bled from the heart. Brain, cord and peripheral nerves were removed asceptically and mashed in 50 per cent glycerol. The material was kept in the ice box for 9 days.

Thirteen 4 day old chicks were inoculated into right thigh with 0.2 cc. of the thick glycerolated suspension. Twelve chicks of the same lot were kept as controls in the same room with the inoculated birds, and allowed to mingle with them.

Results.—Amongst the thirteen inoculated birds, three positive cases developed one of which was of a very severe character, with clinical paralysis and extreme gross and microscopic lesions; the other two presented fairly marked microscopic changes in the peripheral nerves, and were undoubtedly positive although neither obvious clinical symptoms nor gross lesions were present. These birds were sacrificed on the 106th, 125th and 126th days respectively. Three of the thirteen of this group died of an acute infection 7, 8 and 20 days after inoculation—too early for the possible development of paralysis lesions. The incidence may thus be reckoned as three out of ten, or 30 per cent.

In the control group of twelve uninoculated chicks, three died from acute infection on the 18th, 34th and 34th days and may be eliminated from consideration. Of the remaining nine, eight were undoubtedly negative; one only showed a sparse and scattering infiltration of lymphoid cells in one of the bundles of the brachial plexus, and may be classed as a questionably positive case.

In this experiment therefore, which will be repeated on a larger scale, the glycerolated extract of paralysis material proved to be effective in about the same percentage of cases as in the experiments with saline or Tyrode suspension. In the control group there was one doubtful case.

Experiment X.—The following experiment was suggested by the interesting reports of Carrel (5) and White (6) upon the activating influence of dilute arsenious acid in the production of sarcoma in fowl. It was hoped that the introduction of this chemical agent in appropriate concentration in combination with suspensions of nerve tissues from paralysed fowl might induce a multiplication of mononuclear cells and at least reproduce the histopathological features of the disease.

October 21, 1927. Under ether anesthesia[1] and strict asepsis, the sciatic nerves of three healthy White Leghorn cockerels of known paralysis-free stock (Waldo) were exposed. The right sciatic was injected with 0.05 cc. of heavy suspension in Tyrode's (pH 7.4) of ground cord, ganglia, nerve roots and brain from a typical paralysis case, to which had been added dilute arsenious acid to make a final concentration of 1–150,000. The left sciatic was injected in a corresponding place with dilute arsenious acid alone, to determine whether this chemical agent by itself was capable of evoking a cellular reaction.

Three cockerels of the same lot were injected into the right pectoral muscle with 1 cc. of suspension and arsenious acid (final dilution 1–150,000); into the left with arsenious acid alone.

The three chickens which received the intraneural injections were killed after 6, 21 and 28 days. They showed no limping or other evidence of illness during this period. Although there was found, on histological examination, a fairly marked local infiltration of mononuclears which in one of the birds was more marked upon the side injected with suspension and arsenious acid than upon the side injected with arsenious acid alone, the lesions showed no tendency to extend centrally along the nerve trunk, and could perhaps be accounted for by the trauma and introduction of foreign material. The experiment was regarded as inconclusive.

The three birds injected into the pectoral muscle were killed 19, 38 and 50 days after inoculation and were free from symptoms and lesions of paralysis. In the first of these, however, there was found a circumscribed oval tumor mass locally in the muscle between the internal border of the right scapula and the vertebræ. Histologically, this proved to be a sarcoma, composed of large spindle cells, usually bipolar with vesicular nuclei, and surface fibrils; mitoses were numerous. An attempt to transplant fragments with and without the addition of arsenious acid into a cockerel of the same stock, was not successful.

[1] In this and subsequent experiments involving operative procedures, ether anesthesia was used.

The histology of the tumor, its location at a distance from the site of inoculation and the fact that it was found within less than 3 weeks after the injection, make it probable that we were dealing with a spontaneous neoplasm, not evoked by the experimental procedures.

Experiment XI.—November 2, 1927. The previous experiment was repeated on three cockerels of the same stock (Waldo); the right sciatic was injected with suspension of ground sciatic nerve from a paralysed fowl plus arsenious acid to a final dilution of 1–150,000; the left sciatic was injected with the suspension alone.

Results.—Chicken 11–3: 9 days after inoculation a cutaneous nodule 2 mm. in size appeared on the right leg, 2 cm. from the lower border of the incision. This increased in size during the next 4 days; was removed and proved to be a lymphoblastoma. The bird became anemic, lost weight and was killed December 13, 41 days after inoculation.

Autopsy: Large tumor masses were found in thoracic and abdominal cavities, apparently originating in the bursa of Fabricius which was thickened to a cm. or more by tumor tissue, forming an oval mass 3 cm. in length. The center of this was occupied by a sharply sequestrated mass of necrotic tissue. The liver was enormously enlarged, and filled with large and small tumor nodules. Other metastases were present in the kidneys and retroperitoneal cellular tissue. The ovary and oviduct were not involved.

The sciatics, as well as the other portions of the peripheral and central nervous system, showed no significant gross or microscopic lesions.

The development of this extensive visceral lymphomatosis in a chicken inoculated with paralysis material and dilute arsenious acid, is an interesting coincidence in view of the not infrequent association of the two conditions. We do not feel justified, however, in attributing the development of the tumor to the injection, and regard the association as an accidental one.

The two remaining birds of this experiment (Nos. 11–8, 11–2) showed no clinical evidences of paralysis or characteristic microscopic lesions after 48 and 75 days respectively. Two other chickens of the same group (Nos. 11–4, 11–0), inoculated subdurally with 0.15 cc. of the same suspension plus arsenious acid, were negative after 40 and 57 days.

Experiment XII.—November 22, 1927. Three 5 month White Leghorn cockerels were inoculated into the exposed sciatic nerves with suspension of sciatic nerves from a typical paralysis case. Into the right sciatic was injected 0.1 cc. of suspension plus arsenious acid; into the left sciatic, 0.1 cc. of suspension alone.

Results.—Chicken 24–6: Killed after 18 days, having developed fowl-pox. No clinical signs of paralysis. Slight and atypical local lesions at site of injection in right sciatic nerve. Left sciatic negative.

Chicken 29–0: Killed 44 days after inoculation. No clinical or pathological evidence of paralysis.

Chicken 26–2: On February 10 (70 days after inoculation) paralysis suddenly appeared; the right leg was completely helpless, the left leg flexed; general condition good. The chicken was kept under observation until February 20, on which day both legs and wings were paralysed.

Autopsy: Sciatics. Small nodules were found at the site of injection together with diffuse thickening of some of the bundles and disappearance of the normal wrinkling. Characteristic gross changes were found in the brachial plexuses, the vagus and sympathetic nerves and the dorsal root ganglia. There were no significant visceral lesions. Histologically, there were characteristic infiltrations in all blocks examined. But there was no indication that the site of injection was the starting point of the lesions. The nodular swellings noted grossly were due to inflammatory thickening of the perineurium. The cellular infiltrations were more pronounced in the brachial plexuses than in the sciatics.

Experiment XIII.—November 20, 1927. In the following experiment, five 2 day old Rhode Island Red chicks of unknown stock, hatched in the laboratory, were inoculated intramuscularly in the calf or thigh with 0.3 cc. of cord and nerve suspension of a paralysed White Leghorn fowl, plus arsenious acid to a concentration of 1–150,000.

Results.—Three of the five chicks, examined 100, 106 and 180 days after inoculation were negative clinically and pathologically. One chick was lost.

One chicken developed typical paralysis on the 82nd day after inoculation and was found to have characteristic gross and microscopic lesions of ganglia, sciatics and bracheal and lumbosacral plexuses.

In another experiment, weekly injections of dilute arsenious acid (1–150,000) were given over a period of 14 weeks following the inoculation of paralysis suspension. None of the four birds thus treated developed paralysis.

Experiment XIV.—It was thought that the resistance to the hypothetical agent of the disease might be inhibited by a preliminary "blockade" of the reticulo-endothelial system. The report of Roskin (7) indicated that the resistance of birds to the implantation of mammalian neoplasms could be broken down by the intravenous injection of saccharated ferrous oxide.

A group of ten White Leghorn cockerels of known paralysis-free stock was given 5 cc. of saccharated ferrous oxide intravenously; on the following day they were injected by various routes—intracutaneous, intramuscular, intraneural and subdural—with suspension in Tyrode's solution of a typical paralysis case. The chickens were kept under observation for periods of from 58 to 161 days. None of the group showed clinical, gross or microscopic evidence of paralysis, with the exception of one chicken, killed on the 161st day, which showed definite but sparse infiltrations of the sciatic nerves. Preliminary blockades of the reticulo-endothelial system therefore failed to induce an increased susceptibility to inoculation.

DISCUSSION.

The difficulties in the interpretation of these experiments will have become obvious. The insidious development of the disease, the long

latent period between the inoculation and the development of symptoms or frank lesions, the resistance of many individual birds, the occurrence of lesions of every grade of intensity, ranging from insignificant histological infiltrations to massive tumor-like growths, the difficulty of obtaining control material free from lesions—all these factors render the experimental study of the disease laborious and conclusions hazardous. The line between the normal and abnormal as regards the presence of lymphoid tissue about the vessels of the nervous tissue, cannot be sharply drawn, and there is some room for subjective bias. So also with the visceral infiltrations. In the fowl, true lymph glands are not found, but lymphoid tissue is widely disseminated not only through the areolar connective tissue, but within the substance of the viscera. Thus, in routine sections of the heart, collections of lymphoid cells as well as eosinophil myelocytes and polynuclears are found in practically every case. The lymphoid cells are loosely scattered amongst the muscle fibers or collected into follicle-like masses. In the liver also, one finds regularly collections of myeloid or lymphoid elements in the vicinity of the portal spaces, but also definite nodules of lymphoid cells within the substance of the lobules. That such infiltrations may and often do transcend the normal in extent, even to the degree of simulating a pseudoleucemia cannot be denied, but the transition between the normal and the pathological is not defined.

In spite of these various difficulties, one general conclusion seems firmly established by our experiments and that is the significantly higher incidence of typical neurolymphomatous lesions in the inoculated birds as compared with the controls. This result was consistently obtained in all the experiments in which unfiltered suspension of nerve tissue from paralysed fowl was used. Taking only the unmistakable and characteristic positive cases, we find in 89 inoculated birds, 24 positive cases, an incidence of 26+ per cent; while in the control groups comprising in all 104 birds kept under comparable conditions, only 7 cases of paralysis developed—an incidence of less than 7 per cent. This proportion was approximately maintained in each of the several experiments—a fact which lends added significance to the data.

We feel justified therefore in drawing the conclusion that the

inoculation of the material from paralysed fowl definitely favored the subsequent development of the disease.

The mere fact that the disease may be experimentally transmitted to a certain proportion of inoculated chickens hardly warrants one in drawing inferences as to the nature of the causative agent or agents, and speculation at this time seems unprofitable. The definitely neoplastic character of the infiltrating tissue in some cases brings the disease in line with the transplantable sarcomata of chickens. In Experiment XVIII, evidence was secured that the active agent may survive glycerolation; and several other considerations make it unlikely that transmission is accomplished by the implantation of viable cells. The long period elapsing between inoculation and the development of characteristic lesions is against this possibility, as is the fact that there is no local accumulation of cells at the site of inoculation. In the limited number of experiments we have not been able to demonstrate the filtrability of the agent but further work is needed to reach a conclusion on this point.

Although the evidence, epidemiological and experimental, points to an infective agent, we are totally ignorant as to its nature, and as to the manner in which the disease is conveyed under natural conditions. It is in the hope of stimulating further research in this interesting and important problem that these experiments are submitted.

CONCLUSIONS.

1. Inoculation of suspensions of brain, cord, ganglia or nerves of chickens with neurolymphomatous lesions, into newly hatched chicks, is followed by the development of typical lesions in approximately 25 per cent of cases.

2. In control chickens kept under laboratory conditions the incidence of the disease is about 7 per cent.

3. The disease does not become manifest until at least 2 months after inoculation; symptoms may not appear until after 4 months.

4. The active agent is not destroyed by 50 per cent glycerol in 9 days at ice box temperature.

REFERENCES.

1. Kaupp, B. F., *J. Am. Assn. Instructors and Inv. Poultry Husbandry*, 1921, vii, 25.
2. May, H. C., Tittsler, R. P., and Goodner, K., *Rhode Island Agric. Exp. Station, Bull. 202*, December, 1925.
3. Doyle, L. P., *J. Am. Vet. Med. Assn.*, 1926, lxviii, 622.
4. Van der Walle and Winkler-Junius, *Tijdschr. vergelijk. Geneesk. Enz.*, 1924, x, 34.
5. Carrel, A., *Compt. rend. Soc. biol.*, 1925, xciii, 1083.
6. White, A. W. M., *J. Cancer Research*, 1927, xi, 111.
7. Roskin, G., *Z. Krebsforsch.*, 1927, xxiv, 515.