The Transmission of Avian Visceral Lymphomatosis by Contact

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The neoplastic disease, visceral lymphomatosis, is unique, not because the causative agent is a filtrable virus, but because it is contagious and may be spread from bird to bird by contact. It is this characteristic, together with the fact that it is malignant, which makes it one of the most destructive diseases affecting poultry.

Although a large number of reports on the natural transmission of lymphomatosis have appeared, the precise avenues of entrance and the methods responsible for the spread of the disease are still not clearly defined. The slow progress experienced in this field of investigation has been due in large part, no doubt, to (a) the multiplicity of forms or types of the disease which may or may not be related etiologically, (b) the long incubation period and often low incidence of the disease, and (c) the presence of inapparent or latent infection.

The literature on contact transmission of lymphomatosis may be divided into two categories—those reporting primarily on fowl paralysis or neurolymphomatosis and those dealing with all forms, i.e., ocular, neural, and visceral lymphomatosis. In many cases only the total lymphomatosis mortality was given, thus preventing an assessment of the relative importance of the various forms and an accurate interpretation of results, inasmuch as it is quite possible that some forms are more easily transmitted by contact than others. Of these three, only the visceral form is of significance in experiments to be reported; therefore, only the literature including this form will be cited here. Most of these investigators believed that all forms of the disease are the result of a single agent; however, it should not be implied that the present authors, because of the selection of literature, also adhere to this theory.

Patterson (9) in 1936 indicated that all forms of lymphomatosis mortality was given, thus preventing an assessment of the relative importance of the various forms and an accurate interpretation of results, inasmuch as it is quite possible that some forms are more easily transmitted by contact than others. Of these three, only the visceral form is of significance in experiments to be reported; therefore, only the literature including this form will be cited here. Most of these investigators believed that all forms of the disease are the result of a single agent; however, it should not be implied that the present authors, because of the selection of literature, also adhere to this theory.

Patterson (9) in 1936 indicated that all forms of lymphomatosis are transmitted directly by bird to bird contact and indirectly by contact with infected litter. Barber (1), using chicks from a farm relatively free from infection, obtained highly significant transmission when the chicks were raised at the experiment station where infection was present. His results show an inverse relation between the age at which exposure began and the incidence of the disease. Hutt et al. (19) found that chickens raised close to, but not with, infected adult chickens developed a higher incidence of the disease than those raised at a greater distance, thus indicating transmission by indirect contact.

Waters and co-workers (38—37) in a series of experiments showed that when progeny from infected stock were raised in clean, isolated quarters they had much less lymphomatosis than when maintained with other infected stock. They also showed that when the progeny of comparatively disease-free stock were placed, at various ages, with infected chickens, the incidence in the former test chicks was roughly inversely proportional to the age of the chicks when exposed. Similar results were reported by Hamilton and Bearse (18), and by Cole and Hutt (13).

Transmission from inoculated birds to non-inoculated penmates has been reported by several investigators (3, 4, 26, 30, 31). Lee et al. (25) found that younger chicks were more susceptible to infection by inoculation or by direct contact and that transmission did not take place between pens.

Experiments to determine whether the infectious agent is present in feces have been inconclusive. Thorp and Graham (32) and Barber (3), using adequate numbers of birds per group, obtained no evidence for the presence of the agent in the feces of birds affected with lymphomatosis when it was fed fresh to chicks. Jungherr (22), using only a few chickens in each of several groups, concluded that fresh or desiccated feces occasionally contained the transmitting agent which was effective by the oral route.

On the basis of experiments reported to date, it would appear that lymphomatosis, including the visceral form, is transmitted to susceptible chicks...
by contact with chickens infected either by natural means or by inoculation. This is in contrast to results obtained with chicken leukemias, erythroleukemia, and sarcoma of the Rous type (11, 12, 16). These neoplastic diseases are caused by highly infectious viruses, yet no evidence of direct or indirect contact transmission has been recorded.

The purpose of this report is to present experimental data indicating variations in the contagiousness of propagated strains of visceral lymphomatosis and the influence of age at exposure, and exposure dosage, on the rate of contact transmission. Preliminary results of the first experiment on contact transmission were presented earlier (7).

SUMMARY OF PRELIMINARY EXPERIMENTS

During the past several years, numerous transmission experiments have been conducted with several strains of visceral lymphomatosis. These strains were all obtained from naturally occurring cases, and with one exception they were from the breeding flock of the Laboratory and have been described elsewhere (5, 9). The one exception has been identified in this report and at this Laboratory as strain RPL 12; it originated at the Massachusetts Agricultural Experiment Station and was first described by Olson (27, 28). The filtrability of this tumor was demonstrated at this Laboratory (Regional Poultry Research Laboratory [8, 10]).

Some of these experiments included contact controls (noninoculated chickens in direct contact with inoculated ones) and isolated controls (chickens of the same parentage raised in isolated pens). Results of most of these experiments relative to contact transmission will be included here. Two criteria were used in selecting the experiments for this report: (a) a high proportion of the chicks must have been inoculated with a cell-free preparation of lymphomatous tumors and (b) noninoculated chicks must have been in direct contact with inoculated chicks beginning with the day of inoculation.

Strain RPL 12 was used for most of the experiments to be reported here—it was obtained at the 138th serial transplant passage (a suspension of tumor was used as the inoculum) from Dr. Carl Olson.1 Since that time some 150 similar passages have been made at this Laboratory. At the 85th RPL transplant passage, successful transmission was obtained with cell-free preparations (10). Thus far, a total of fifteen serial transfers have been made with cell-free centrifugates or filtrates.

1 Dr. Carl Olson was located at the Massachusetts Agricultural Experiment Station, Amherst, Massachusetts, at the time the tumor was obtained.

The source of inoculum for the first three experiments listed in Table 1 was intramuscular tumor of the 65th and 146th transplant passages. For the remaining experiments with strain 12, the inocula were prepared from lymphomatous livers obtained in serial passage with cell-free preparations. Cell-free passage 7 supplied inoculum for experiment 4; passage 9 supplied inoculum for experiment 5; and passage 14 supplied inoculum for experiments 6 and 7.

In the experiment with strain RPL 18, the inoculum was prepared from a pool of tumors of the 1st, 5th, and 4th passages with cellular preparations. In the experiment with strain RPL 18 tumors of the 16th serial passage were used; again, in this instance, the tumor was induced by cell implants. Strain RPL 20 was employed in the last two experiments listed. In the first of these, lymphomatous liver of the fifth transplant passage was used as the source of inoculum. Livers of several positive cases of this first experiment were used in the preparation of the inoculum for the second experiment with this strain.

The general procedure for the preparation of the various inocula was to homogenize the tumor in a Waring Blender and suspend in 9—10 parts of a phosphate buffer at pH 7.2. The homogenate was then rendered cell-free by either double centrifugation or centrifugation and filtration through a filter which had previously been shown to prevent the passage of Serratia marcescens organisms. The inoculum was either used within a few hours of its preparation or sealed in glass and stored1 in a CO2 ice chest until used. The inocula stored served as the source of inoculum except in experiment 6 where serial tenfold dilutions were used.

The chicks for these experiments came from an inbred line of S.C. White Leghorns selected for susceptibility to lymphomatosis and which had been raised in isolation for several generations to reduce natural infection with the disease (56, 57). After inoculation the chicks were placed in batteries with the noninoculated contact chickens until about 6 weeks of age. At this time they were transferred to pens, where they remained until the experiment was terminated.

The pen-isolated stock served both as the source of chicks and as an index of the extent of egg-borne infection (14, 54) present in the stock.

Results obtained in these inoculated and contact exposure chickens have been summarized in Table 1. The incidence of tumors among the inoculated chickens varied considerably between the various strains and experiments. Strain 16 had the lowest incidence with 33.8 per cent visceral lymphomatosis and strain 18 produced the highest incidence of 95.0 per cent. There was also a considerable variation in the incidence of this form of the disease among the noninoculated contact chickens;
however, it did not coincide with the variation in the incidence obtained in the inoculated chickens. A significant incidence of the disease was not obtained in birds in contact with those inoculated with strains other than RPL 12. Contact transmission did not occur with strains 16, 18, and 20, even though in three of the tests the incidence of tumors in inoculated chickens was very high and the contacts were maintained for a period of 300 days.

The first and seventh experiments with strain 12 gave no indication of contact transmission. There was moderate transmission to contact chickens in experiments 2, 3, and 6 and a comparatively high rate in experiments 4 and 5. In experiment 4, in which five serial tenfold dilutions were used, a disease occurred to a variable extent in the inoculated groups, but none occurred among the contact chickens. A few cases of neural lymphomatosis also appeared. The over-all average incidence of this form among 858 inoculated chickens was 1.37 per cent, 1.90 per cent among the 263 contacts, and 1.84 per cent among 298 isolated controls; hence, this form of lymphomatosis is of very little significance in these transmission experiments.

The cause for the variation in the amount of contact transmission is not apparent from observations of these experiments. The lack of correlation between incidence of tumors in inoculated and contact birds of different experiments indicates that factors controlling the incidence of gross tumors upon inoculation do not appear to be the most important influencing factors of contact transmission, although, as will be shown in a latter experiment, the exposure dosage has an important influence when other factors such as the inoculum are held constant.

The various inoculations were made over a period of 8 years with the same strain (RPL 12), yet contact transmission did not occur in the first and last of these experiments. Similar management procedures and equipment were used in taking care of the chickens throughout this period. Variation in source of inoculum, that is, whether from cell-transplant or virus-induced tumors, does not coincide with variation in contact transmission, although the two highest rates of contact transmission did occur in experiments where inoculum prepared from filtrate-induced tumors was used. Variations which may have been due to serial passages cannot alone account for differences in contagion. Birds of experiment 6 were inoculated with a filtrate of lymphomatous livers of the same cases.

### TABLE 1

<table>
<thead>
<tr>
<th>RPL strain</th>
<th>Exp. no.</th>
<th>Tumors</th>
<th>Osteopetrosis</th>
<th>Exp. no.</th>
<th>Tumors</th>
<th>Osteopetrosis</th>
<th>Exp. no.</th>
<th>Tumors</th>
</tr>
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<tbody>
<tr>
<td>12</td>
<td>1</td>
<td>45</td>
<td>81.3</td>
<td>12</td>
<td>15</td>
<td>6.7</td>
<td>16</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>32</td>
<td>50.0</td>
<td>3</td>
<td>44</td>
<td>68.2</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>166</td>
<td>49.4</td>
<td>5</td>
<td>280</td>
<td>59.3</td>
<td>6</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>65</td>
<td>86.7</td>
<td>7</td>
<td>35</td>
<td>90.1</td>
<td>10</td>
<td>35</td>
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<tr>
<td></td>
<td>10</td>
<td>19</td>
<td>95.0</td>
<td>10</td>
<td>71</td>
<td>55.8</td>
<td>11</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>45</td>
<td>71.1</td>
<td>18</td>
<td>19</td>
<td>95.0</td>
<td>12</td>
<td>35</td>
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<td>20</td>
<td>12</td>
<td>35</td>
<td>77.1</td>
<td>20</td>
<td>45</td>
<td>71.1</td>
<td>12</td>
<td>35</td>
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</tbody>
</table>

* Osteopetrosis occurred only in inoculated birds.
† Maintained in cubicle isolation for 90 days only.
‡ Experimental period 300 days; for all others it was about 200 days.
that supplied the filtered plasma used to inoculate chickens of experiment 7. The inoculated and contact chickens of both experiments were maintained under the same environment and management conditions during the same period. Yet, birds in contact with the liver filtrate-inoculated chickens had about 3 times the incidence of tumors of the chickens in contact with plasma-inoculated birds. Of the 96 sibling noninoculated chickens maintained by themselves in adjacent cubicles, none developed gross lymphomatosis even though they were mixed at 90 days of age with the inoculated birds and held for 200 days.

These preliminary experimental observations indicated that visceral lymphomatosis is transmitted by contact with inoculated chickens, but the extent of this transmission is subject to variation which at present cannot be explained.

**CONTACT TRANSMISSION EXPERIMENTS**

In view of the transmission that took place between chickens inoculated intraperitoneally with the agent of visceral lymphomatosis and noninoculated penmates, it is probable that similar transmission could occur between various inoculated groups; this could reduce or obliterate effects of any particular inoculum. The most obvious procedure for the prevention of such transmission is to maintain each lot in complete isolation until the termination of the experiment. This is difficult when facilities are limited because of the long experimental period required and the large size of the experimental animal. Under the circumstances it seemed advisable to explore further the extent of contact transmission and some of the factors which influence it.

The prevention of direct contact by the use of cubicles and variations in the length of isolation periods was tested in the first two experiments to be described. The exposure dosage as a factor in influencing the amount of contact transmission was studied in a third experiment.

**MATERIALS AND METHODS**

**General.**—Cubicles 3 feet high and 4 feet square were built with 1/4-inch plywood to provide limited isolation for these experiments. Five of these cubicles were placed in a pen 12 × 16 feet in size. This left only enough room for disinfecting equipment and a narrow service aisle. Dehydrated sugar cane was used as litter and was placed on a concrete floor. Water was supplied in an ordinary fountain and fed in a trough-type feeder. Electric lamps were used to provide supplementary heat for brooding chicks. After the chicks were about 8 weeks of age a wire frame was placed over the top of each cubicle to prevent chickens from flying from one cubicle to another. All service in any one cubicle was done by the assigned care taker while wearing long rubber gloves or using grapples. The gloves and equipment were immersed in disinfectant before being used in a cubicle or coming in contact with anything in the cubicle. The same procedure was followed after servicing each cubicle and before going to the next. Thus, procedures were followed which would prevent the direct transfer of infectious material from one cubicle to another. The disinfectant was a 5 per cent aqueous lye solution.

The chicks, of the same source as previously described, were removed from the incubator, banded, and placed in assigned cubicles at 1 day of age. Inoculations were all by the intraperitoneal route in doses of 0.2 ml. and were made just prior to placing the chicks in cubicles. At the maximum age of 90 days the chickens from several cubicles were combined and moved to pens with a capacity for 100-125 birds.

All birds that died during the experimental period or the survivors which were killed at the termination of the experiment were examined, and diagnosis was based on clinical and gross findings except in unusual or questionable cases, when the final diagnosis was based on microscopic findings. The experimental period for experiments 1 and 2 was 250 days and that for the third experiment was 300 days.

**Experiment No. 1.**—Twenty-four inoculated chicks were placed with eight noninoculated chicks in each of four cubicles of two pens. Thirty-two noninoculated chicks occupied the fifth cubicle of each pen. At 30 days of age all chicks in the cubicles of one of the pens were moved to a similar pen, but without cubicles, and allowed to intermix. This same procedure was followed at 60 days of age for the chicks of the second pen. The inoculum for this experiment was prepared from the livers of three lymphomatous birds from the tenth cell-free passage of strain RPL 12. It was prepared by homogenizing the tumorous tissue in a Waring Blender with 9 parts of an isotonic saline-phosphate buffer solution adjusted to pH 7.2. The suspension was centrifuged for 20 minutes at 4,000 × g. The resulting supernatant was transferred to lueroid tubes and spun for 5 minutes at 30,000 × g. The supernatant was transferred to serum tubes, sealed, and stored in a CO₂ ice chest until used for inoculation. The storage periods for tubes used in this experiment were 7 and 21 days for tests of pens 1 and 2, respectively.

**Experiment No. 2.**—Forty-eight chicks were inoculated on the day they were removed from the incubator and placed in two cubicles, and groups of eighteen noninoculated chicks were placed in three other cubicles. At 30 days of age one-half of the inoculated chicks were placed with one lot of eighteen chicks and at 80 days of age the remaining inoculated chicks were placed with a second lot of noninoculated chicks. At 90 days all chicks were moved to an adjoining pen without cubicles so that there was a mixing of all groups after 90 days of age. Thus, this experiment provided contact exposure to different lots at 30, 60, and 90 days of age. The inoculum used was prepared from the livers of three lymphomatous cases which developed as a result of inoculation in experiment 1. The inoculum was prepared in a similar manner to that for the first experiment, except that 10 parts of buffered saline were used, and the second centrifugation was for 20 minutes at 4,500 × g.

**Experiment No. 3.**—The primary purpose of this experiment was to determine the influence of the exposure dosage (the proportion of chicks inoculated) on the incidence of disease in the contact chickens. The size of some of the cubicles used in this experiment was increased so that the floor area per chick was approximately the same for all lots. Ninety-two inoculated and 30 noninoculated chicks were placed in a large cubicle 9 feet square. A second pen contained one cubicle 4 × 9 feet, and three cubicles 4 × 9 feet in another. The large cubicle in this pen received 30 inoculated chicks and 30 noninoculated chicks. In one of the small cubicles were placed ten inoculated and 30 noninoculated chicks, and in the second were placed four inoculated and 30 noninoculated chicks.
Thirty-six noninoculated chicks were placed in the third cubicle.

The inoculum for this experiment was prepared from tumorous livers and serum of several chickens from the seventh cell-free serial passage of strain RPL 12. The tumor tissue was suspended with a Waring Blender in 4 parts of saline-phosphate buffer and then serum from the same donors was added in the proportion of 2 parts serum to 1 part of original tumor tissue. The mixture was centrifuged 10 minutes at 30,000 X g. The supernatant was sealed in serum tubes and stored in a CO₂ ice chest until used. The storage period for tubes used in this experiment was 1,259 days.

RESULTS AND DISCUSSION

Early in the course of the first experiment, a number of chicks died as the result of an outbreak of infectious bronchitis. These chicks were removed from the experiment and are not included in the data presented, inasmuch as there was no significant variation between lots in the occurrence of this disease. A summary of the results of the first two experiments is presented in Table 2.

The inoculum in the first experiment produced a high incidence of disease in both the inoculated and contact birds of both pens. The incidence of visceral lymphomatosis was somewhat higher in pen 1 (70 per cent) than in pen 2 (48 per cent), and this was supplemented by the occurrence of many cases of osteopetrosis in the inoculated chickens. The incidence of the latter was higher in the inoculated chickens of pen 2 than in those of pen 1, with the result that the percentage of total positives among the inoculated chickens was about the same in the two pens. The incidence of visceral lymphomatosis among chickens that were in direct contact with inoculated animals during the entire period was also high. In fact, the incidence was actually higher in the contacts than in their respective inoculated penmates. However, the average age at death for those that died of the disease was much greater for the contact birds (186 and 174 days) than for the inoculated birds (104 and 100 days). Part of the deaths among the inoculated birds were due to osteopetrosis and occurred at an early age; this may in part account for the lower incidence of visceral tumors among the inoculated chickens than in the contacts.

Noninoculated birds maintained by themselves in a cubicle but in the same pen as inoculated birds for the first 30 days of life and then mixed with the other chicks of the pen for the duration of the experiment had a lower incidence of lymphomatosis (56.7 per cent) than those in contact for the entire period (71.5 per cent). When cubicle isolation was extended to the first 60 days of life (pen 2) the incidence was further greatly reduced to 20.7 per cent. Not a single case of osteopetrosis appeared among the 57 contact chickens, even though it occurred at a high rate in the inoculated animals.

Among the 51 chickens of the same stock that were maintained in an isolated pen, only one case of lymphomatosis developed during the period of this experiment. The incidence in the lot isolated in a cubicle for 60 days, although much lower than for those in contact the entire period or for those exposed after 30 days, was still much higher than that in the pen of isolated chickens. This difference in incidences between the pen of isolated chickens and those isolated for 60 days in cubicles could, at least in part, be owing to an indirect transmission occurring between cubicles or to a direct transmission between chickens after the 60-day isolation period. A test of the latter possibility was planned for the next experiment.

The results of experiment 2 are presented in the

### TABLE 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Per cent</th>
<th>Per cent</th>
<th>Per cent</th>
<th>Av. age at death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per</td>
<td>visceral</td>
<td>osteo-</td>
<td>visc. lym.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lot</td>
<td>lympho.</td>
<td>petrosis</td>
<td>and osteo.</td>
<td>(days)</td>
</tr>
<tr>
<td>Experiment 1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>70</td>
<td>70.0</td>
<td>25.0</td>
<td>91.5</td>
<td>104</td>
</tr>
<tr>
<td>Pen 1: Contact—entire period</td>
<td>28</td>
<td>71.5</td>
<td>0.0</td>
<td>71.5</td>
<td>186</td>
</tr>
<tr>
<td>Cubicle-isolated (1st 30 days)</td>
<td>30</td>
<td>50.7</td>
<td>0.0</td>
<td>56.5</td>
<td>203</td>
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<tr>
<td>Inoculated</td>
<td>59</td>
<td>47.5</td>
<td>37.3</td>
<td>76.8</td>
<td>100</td>
</tr>
<tr>
<td>Pen 2: Contact—entire period</td>
<td>29</td>
<td>49.0</td>
<td>0.0</td>
<td>49.0</td>
<td>174</td>
</tr>
<tr>
<td>Cubicle-isolated (1st 60 days)</td>
<td>29</td>
<td>20.7</td>
<td>0.0</td>
<td>20.7</td>
<td>189</td>
</tr>
<tr>
<td>Pen-isolated controls</td>
<td>51</td>
<td>1.9</td>
<td>0.0</td>
<td>1.9</td>
<td>155</td>
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<tr>
<td>Experiment 2:</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>47</td>
<td>74.5</td>
<td>40.4</td>
<td>91.5</td>
<td>77</td>
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<tr>
<td>Cubicle-isolated (1st 30 days)</td>
<td>17</td>
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<td>5.8</td>
<td>250</td>
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<td>&quot; (60&quot;&quot;)</td>
<td>17</td>
<td>5.8</td>
<td>0.0</td>
<td>5.8</td>
<td>250</td>
</tr>
<tr>
<td>&quot; (84&quot;&quot;)</td>
<td>16</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Pen-isolated controls</td>
<td>120</td>
<td>0.0</td>
<td>0.0</td>
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</table>
lower part of Table 2. The 47 inoculated chicks developed a high incidence of visceral lymphomatosis and osteopetrosis, with an average age at death of only 77 days. However, none of the three contact groups developed a significant incidence of the disease. On the basis of the previous experiment a marked reduction in the proportion of inoculated chickens, significant transmission by contact still may result. In spite of the fact that the inoculum was obtained directly from several lymphomatous cases that had developed in the previous experiment, it is conceivable that variations had oc-

### Table 3

<table>
<thead>
<tr>
<th>Cubicle</th>
<th>Population inoculated</th>
<th>Treatment</th>
<th>No. of chickens</th>
<th>Per cent visceral lympho.</th>
<th>Per cent osteo. petrosis</th>
<th>Per cent visc. lym. and osteo.</th>
<th>Av. age at death (days)</th>
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<tr>
<td>1</td>
<td>76</td>
<td>Inoculated</td>
<td>90</td>
<td>53.3</td>
<td>60.0</td>
<td>81.1</td>
<td>212</td>
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<tr>
<td></td>
<td></td>
<td>Contact</td>
<td>29</td>
<td>65.5</td>
<td>0.0</td>
<td>65.5</td>
<td>224</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>Inoculated</td>
<td>51</td>
<td>45.2</td>
<td>48.4</td>
<td>64.5</td>
<td>174</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contact</td>
<td>30</td>
<td>56.7</td>
<td>0.0</td>
<td>56.7</td>
<td>222</td>
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<tr>
<td>4</td>
<td>26</td>
<td>Inoculated</td>
<td>10</td>
<td>60.0</td>
<td>50.0</td>
<td>70.0</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contact</td>
<td>29</td>
<td>41.4</td>
<td>0.0</td>
<td>41.4</td>
<td>215</td>
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<tr>
<td>5</td>
<td>10</td>
<td>Inoculated</td>
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<td>25.0</td>
<td>50.0</td>
<td>75.0</td>
<td>209</td>
</tr>
<tr>
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<td>224</td>
</tr>
<tr>
<td>3</td>
<td>Isolated cubicle 90 days</td>
<td>37</td>
<td>Isolated pen entire period</td>
<td>181</td>
<td>10.8</td>
<td>0.0</td>
<td>10.8</td>
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</table>

Experimental period 300 days.

The results of the transmission in the third experiment are given in Table 3. The incidence of visceral lymphomatosis in the various inoculated groups was moderately high, and the incidence of osteopetrosis was unusually high. Sixty per cent of the birds in cubicle 1 developed osteopetrosis with or without visceral lymphomatosis. The non-inoculated chickens in direct contact with the inoculated also developed a high incidence of visceral lymphomatosis. This incidence was directly proportional to the percentage of the population which had been inoculated. Thus, in cubicle 1, where 76 per cent of the population had been inoculated, 65.5 per cent of the noninoculated chicks developed visceral lymphomatosis. This rate is actually higher than that obtained in the inoculated penmates (53.3 per cent). Fifty-one per cent of the chickens in cubicle 2 had been inoculated, and 56.7 per cent of the noninoculated birds developed tumors. This, again, was a higher rate than among the inoculated penmates. These results, i.e., higher incidences in contact than in inoculated chicks, were similar to those obtained in the first experiment of this series. The explanation, no doubt, is in the high incidence of osteopetrosis occurring in the inoculated groups and the lack of it in contact groups. The relation between the exposure dosage in terms of percentage of the population inoculated and the response of the contact chickens is graphically presented in Chart 1. When the percentage of

![Chart 1](chart_1.png)

**Chart 1**—Relationship between the proportion of chickens inoculated and the incidence of visceral lymphomatosis among the contact chickens.
birds inoculated was plotted against the response in terms of percentage of contact birds that develop tumors, the points fell on a smooth curve. When the exposure dose was converted to logarithms and the percentage response to probits, a straight line relationship was obtained.

Although osteopetrosis occurred at a high rate maintained in cubicle isolation for the first 90 days adjacent to cubicles containing inoculated chickens. After this time they were mixed in one large pen and held until 300 days of age. The average incidence of lymphomatosis was 51 per cent in the inoculated birds, 46 per cent in contact, and 11 per cent in cubicle-isolated chickens. The latter was a
gest that spread of infection through the air at a distance of 5 feet or more was not important in the spread of this disease, even where much dust was present. It is possible that the agent soon becomes inactivated when dispersed in the air, and this route becomes important only when infected birds are in close proximity to uninfected ones.

Osteopetrosis gallinarum (24) is a distinct feature of strain RPL 19 in that it occurs to a variable extent in almost all groups inoculated with filtrates of this strain. It is noteworthy that, although the inoculated chickens of the three experiments had a rather high incidence of osteopetrosis (ranging from 23 to 60 per cent), not a single case of it appeared among 290 chickens of the eleven contact groups. On the basis of the high incidence of visceral lymphomatosis in most of the contact chickens (up to 65.5 per cent) it is quite evident that the causative agent of this disease, present in the inoculum and producing the disease in inoculated chickens, was transmitted to birds in contact at a rate sufficiently high to produce a high incidence of visceral lymphomatosis. If osteopetrosis was produced by the same agent, then some cases of this disease should have occurred in the contact groups. Since this did not occur, one is forced, at least tentatively, to conclude that the agent producing visceral lymphomatosis is different from that causing osteopetrosis. It may be suggested that the induction of osteopetrosis requires a much greater dosage of the same agent than is necessary for visceral lymphomatosis, and for this reason the latter was obtained by contact and not the former. However, the available evidence does not lend support to this view. If the dosage of a single agent were the controlling factor and the differential were due only to difference in dosage threshold, then the incidence of osteopetrosis should be closely correlated with that of visceral lymphomatosis. An examination of results obtained with inoculated groups reveals a lack of such a correlation. Thus, the results obtained in two of the contact experiments reported here remain favorable to the hypothesis that visceral lymphomatosis and osteopetrosis are caused by two different filtrable agents. The data indicate that visceral lymphomatosis is contagious, whereas osteopetrosis although infectious is not contagious. Earlier results (6) suggested another possible differential. Biological tests indicated that the osteopetrotic agent was more completely sedimented under certain conditions of centrifugation than was the agent causing visceral lymphomatosis.

Neural lymphomatosis has been classified on the basis of pathology along with visceral lymphomatosis under the general term “avian leukemia complex” (Jungherr et al. [23]) and many investigators (13, 19, 25, 26, and others) believe that the two forms are caused by the same agent. It is, therefore, of interest that neural lymphomatosis did not occur to a significant extent in any inoculated, contact, or isolated group of any of the experiments reported herein. In the eleven transmission experiments, results of which are presented in Table 1, the average incidence of the neural form in the inoculated, contact, and isolated groups was 1.37, 1.90, and 1.34 per cent, respectively. A similar result was obtained with chickens of the contact experiments presented in Tables 2 and 3.

These low rates of infection with no differences between inoculated, contact, and isolated groups, indicate that all birds, irrespective of purposeful experimental treatment, were exposed to a low-grade infection with the causative agent of neural lymphomatosis. Since the inoculated groups were no different from the controls, it would appear that the neural agent was not present, at least in an active form, in any of the four strains used in these investigations. Furthermore, the fact that visceral lymphomatosis was transmitted at a high rate by these tumor strains, and neural lymphomatosis was not, would indicate that the two forms are not caused by the same virus. Similar results were obtained by Burmester (5) and Davis and Doyle (15) with inoculum of naturally occurring cases of visceral lymphomatosis and by Cottral, Burmester, and Waters (14) who inoculated chicks with preparations of normal liver tissue of embryos from apparently healthy hens and in many inoculations obtained a significant transmission of only visceral lymphomatosis.

Thus, the data presented confirm results of other investigators and are in support of the hypothesis that various forms of lymphomatosis and osteopetrosis are caused by distinctly different viruses.

**SUMMARY AND CONCLUSIONS**

1. Data were collected on the incidence of neural and visceral lymphomatosis and of osteopetrosis in 1,926 chickens inoculated with the filtrable agent of visceral lymphomatosis, 508 chickens in direct contact with inoculated sibs, and 687 isolated chickens.

2. The incidence of visceral lymphomatosis among inoculated groups varied from 38.8 to 95.0 per cent and osteopetrosis from zero to 60 per cent. In the various contact groups visceral lymphomatosis varied from 5.4 to 71.5 per cent. Osteopetrosis did not occur in any contact or isolated group and neural lymphomatosis was present to the extent of less than 8 per cent in any major group.

3. Of the four strains of lymphomatosis, RPL
contact transmission, and, even with this strain, it did not occur in all inoculations. The reasons for such variations were not determined.

4. High rates of contact transmission that occurred under experimental conditions were reduced to insignificant levels by confining inoculated and noninoculated lots to separate cubicles with solid walls but open tops.

5. Cubicle isolation to 60 days of age did not prevent all contact transmission, whereas cubicle isolation to 90 days in two other experiments resulted in no apparent contact transmission as measured by incidence of gross tumors to 800 days of age.

6. The extent of contact transmission in probits was found to have a direct linear relation to the exposure dosage when in terms of the logarithm of the percentage of the population inoculated.

7. Transmission results indicate that the causative agent of visceral lymphomatosis is different from that causing osteopetrosis and that causing neural lymphomatosis.

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The Transmission of Avian Visceral Lymphomatosis by Contact

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