Although numerous experiments have shown that erythro- and myeloid leukosis may be transmitted by a filtrable agent (14), only a few reports have suggested that lymphomatosis may be transmitted by a similar agent.

By the use of filtrates of strain 2, Furth (9) produced what was thought to be a rare type of lymphomatosis. It was characterized by the appearance, in the blood and many organs, of large lymphocytes with occasional formation of lymphoid tumor-like nodules, endothelioma and severe anemia. Pentimalli (16) described a readily transplantable lymphoid tumor strain. Based upon results obtained from filtration, desiccation, and glycerination experiments, he concluded that the strain did not contain a filtrable agent. Olson (15) developed a tumor strain having similar characteristics; however, he did not report on attempts to transmit it by cell-free material. The propagation of 4 lymphoid tumor strains by cell transplantation from cases of naturally occurring visceral lymphomatosis was reported by Burmester and Prickett (3). These strains were similar among themselves and to those of Pentimalli (16) and Olson (15) in that the type of involvement was similar, the incidence of tumor takes was high and the rate of tumor growth was rapid. Brewer and Brownstein (2) have also reported on the rapid transmission of visceral lymphomatosis with suspensions of tumor pulp.

Burmester, Prickett, and Belding (4) in a series of 3 experiments demonstrated the presence of a filtrable agent in the lymphoid tumor originally studied by Olson (15) and obtained at this Laboratory in June, 1942 and since designated here as RPL 12. This filtrable agent failed to induce tumors at the site of inoculation in a short period of time (inocula-containing cells produce tumors at the site of inoculation) but produced within 6 months’ time a high incidence of osteopetrosis and lymphomatous tumors of the viscera.

The object of this paper is to report on the propagation of the filtrable agents of the lymphoid tumor strain RPL 12 during 6 serial passages in young chickens and to describe the gross manifestations obtained in the various passages, with different routes of inoculation, and with different types of donors and preparations.

MATERIALS AND METHODS

In the work to be reported here two different types of inoculum were used. The serial passages were made with tumor material or plasma rendered cell-free by centrifugation and filtration. At the same time supplementary inoculations were made in certain instances with tumor cell suspensions or whole blood of the same source as the cell-free preparations.

Preparation of filtrates.—The cell-free extracts of lymphomatous liver tissue were prepared by homogenizing the tumor in 7 parts 0.85 per cent NaCl solution in a Waring Blender for 20 minutes. Examination of samples by direct and dark-field illumination of wet specimens and fixed smears processed with Wright’s stain revealed that after 8 minutes of homogenizing very few intact cells were found, these being mature erythrocytes, but a large number of free nuclei were seen. After 16 minutes no intact cells were found, the number of free nuclei was much less and the innumerable particles seen in the suspension were much smaller than in samples taken after 8 minutes.

For the two inoculations of the second passage Seitz clarifying filters, K1 and K7, were used. The former was more retentive than the latter. Microscopic examination of these filtrates failed to reveal intact cells or free nuclei. Inoculum for the third and fourth passages was prepared by centrifuging the homogenate for 20 minutes at about 3,000 RPM and then filtering through a Seitz S1 pad. The sixth passage was made with material prepared in a similar manner except that 7 parts of
Tyrode’s solution at pH 7.1 was used and the centrifuged supernatant was filtered first through a preliminary and then through a regular Mandler candle.

The plasma was obtained from blood which had been withdrawn from the heart of donors into a syringe containing 0.1 volume of heparin solution having a concentration of 0.4 gm. per 100 ml., in 0.85 per cent NaCl solution. It was separated from the cells by centrifugation and then filtered through a sterilizing Seitz S1 pad for passages 1, 3, and 4. A Jenkins-Fisher sterilizing filter was used for passage 5 inoculum.

Forty-eight hour broth cultures of *Serratia marcescens* were used to test all Seitz S1 filters after their use with the extracts and the regular Mandler candle before and after it was used for liver extract. In all cases 1.0 ml. of the filtered broth cultures failed to seed tryptone broth; whereas the unfiltered portion produced typical growth in all tests.

For the extra inoculation of passage 3 the plasma was diluted with equal parts of sterile distilled water. Part of it was then subjected to high-speed centrifugation. It was spun in an angle centrifuge for 20 minutes at 5,000 RPM and the supernatant transferred to six 14-ml. lusteroid tubes and spun at 19,000 RPM (27,000 times the force of gravity). After centrifuging for 2½ hours the contents of each tube was divided into halves, the upper portions were combined and transferred to other lusteroid tubes and the lower portions were similarly combined. The two fractions were again spun for 2½ hours at 19,000 RPM, after which the upper half of the contents of each tube, containing the first upper fraction, was carefully transferred to a serum bottle for inoculation and the lower portion, containing the first lower fraction, was transferred to a second bottle. Gelatinous pellets or sediments were not obtained in either of the two high speed centrifugations.

Preparation of cellular inoculum.—The tumor cells suspensions were prepared by a method already described (3). The whole blood as used for inoculation was not treated after its collection from the donor.

*Experimental birds.*—The recipient chicks were pedigreed and obtained from matings of an inbred line (line 15) of chickens relatively susceptible to lymphomatosis yet which developed only a few or no cases when maintained under quarantine (17).

The chicks were inoculated at 1 to 3 days of age with 0.25 to 0.5 ml. of the cellular inoculum or 0.5 to 1.0 ml. of the cell-free preparations by the intraperitoneal route except where otherwise indicated. The different inoculation groups of the same passage were reared in the same battery and pens; however, birds of different passages were maintained in separate quarantine pens.

Two groups of non-inoculated control chicks, from the same matings used for the inoculations, were maintained for the first 90 days after hatching in a similar but separate quarantine pen from the inoculated chicks. During the second period of about 90 days, one control group was maintained with birds of the first passage and a second group was kept in the same pen with birds of the extra inoculation of passage 3.

All experimental and control birds were examined after they had died, or were killed to serve as donors, or at the termination of the experiment. All except 2 groups were terminated at 6 months of age. Because of a misunderstanding the birds of the fourth passage were killed at 5 months of age and those of the fifth passage were terminated at 3 months of age. All diagnoses were based on gross alterations observed at necropsy and at periodic clinical examinations. In addition, tissues of all donors were examined microscopically.

**PASSAGES AND RESULTS**

A summary of the transmission data for the various inoculations and passages is presented in Table I. The results of inoculations representing the first passage of this series have already been presented elsewhere (4). They are included here to facilitate comparison with data of subsequent passages. The inoculum used to initiate this series was plasma obtained from 2 chickens which had received an implant of Strain KPL 12 tumor cells 7 days previously. These donors had large intramuscular tumors at the site of inoculation and diffuse involvement of several visceral organs. The filtered plasma produced tumors in 86 per cent of those inoculated by the intravenous and by the intraperitoneal routes. Most of the birds had tumors in the viscera but many also had osteopetrosis.

Two cases from the first passage were used as donors at 190 and 192 days of age. Both had lymphomatous tumors of the viscera but only one showed evidence of osteopetrosis. Filtrates from both donors produced a high incidence of tumors. The Seitz K7 filtrate of the donor having only visceral tumors produced osteopetrosis in 37 per cent of the recipients, while the Seitz K7 filtrate of the donor with osteopetrosis produced this tumor in 53 per cent of those inoculated. However, osteopetrosis did not occur in a group that had received the Seitz K1 filtrate prepared from the latter donor.

For the third serial passage again 2 donors were
used. Both arose in the group of the second passage that had been inoculated with liver tumor filtrate from a bird showing only visceral tumors. The first donor for the third passage had both osteopetrosis and visceral tumors, and was 148 days of age when sacrificed. Filtered plasma and liver homogenates reproduced a high incidence of both pathologic alterations in the recipients. The filtered liver extract developed lymphoid tumors in the viscera; whereas, only 38 per cent of the plasma-inoculated group developed similar tumors.

The fifth passage was made with plasma of a chicken of the previous passage that had been inoculated with plasma and had developed severe lesions of osteopetrosis but showed no evidence of visceral involvement. The recipients were main-

second donor showed evidence of only osteopetrosis at 162 days of age. Since the liver was not tumorous only filtered plasma was used. This inoculum produced a high incidence of visceral tumors and osteopetrosis. A difference in the incidence of the two manifestations between the upper and lower high-speed centrifuged fractions of plasma was not obtained.

The fourth serial passage was made with plasma and lymphomatous liver of a 44 day old bird. This donor had been inoculated with filtered plasma of the 148 day old donor of the third passage. By 5 months of age both groups of this passage developed a high incidence of tumors, although there was a marked difference between the two groups in the percentage with visceral tumors. Seventy-seven per cent of the chickens inoculated with
tained for an experimental period of only 96 days, during which time 3 of 28 birds inoculated had died with tumors and 3 others were found to have tumors when they were killed on the date of termination.

Birds of the sixth serial passage received a Mandler filtrate prepared from the liver of one of the three birds having tumors at termination of the fifth passage. Eighty-three per cent of the chickens that received this inoculum developed osteopetrosis and 95 per cent (all but one) had tumors in the viscera.

The total tumor incidence for all groups that were inoculated with filtrate and were maintained for 6 months was 81.0 per cent. Most of these (88 per cent) had tumors of the viscera and they died on an average of 137 days after inoculation. About

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**Table 2: Transmission Data for the Serial Passage of a Lymphoid Tumor With Filtrates and Comparative Inoculations With Cell Suspensions**

<table>
<thead>
<tr>
<th>Passage No.</th>
<th>Days after inoc.</th>
<th>Diagnosis</th>
<th>Inoculum</th>
<th>Source</th>
<th>Filter used</th>
<th>No. chicks inoc.</th>
<th>% with tumors</th>
<th>Total % pos.</th>
<th>Average survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>P, V, 0</td>
<td>Whole blood</td>
<td>None</td>
<td>4*</td>
<td>100 0 100</td>
<td>100 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P, V</td>
<td>Whole blood</td>
<td>None</td>
<td>4</td>
<td>100 100 0</td>
<td>100</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>189</td>
<td>V</td>
<td>Liver tumor cells</td>
<td>None</td>
<td>14</td>
<td>43 57 7</td>
<td>86 132</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(extra)</td>
<td></td>
<td>Liver homogenate</td>
<td>Seitz K7</td>
<td>14</td>
<td>53 87 7</td>
<td>87 143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>145</td>
<td>V, 0</td>
<td>Liver homogenate</td>
<td>Seitz K1</td>
<td>14</td>
<td>50 65 7</td>
<td>65 137</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3159</td>
<td>0</td>
<td>Liver homogenate</td>
<td>Seitz S1</td>
<td>14</td>
<td>44 69 6</td>
<td>69 135</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasma Centrif.</td>
<td>Upper fract.</td>
<td>14</td>
<td>50 70 6</td>
<td>85 136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>43</td>
<td>V, 0</td>
<td>Liver homogenate</td>
<td>Seitz S1</td>
<td>14</td>
<td>53 77 7</td>
<td>77 104</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>96</td>
<td>V</td>
<td>Plasma Jenkins</td>
<td></td>
<td></td>
<td>83 95 0</td>
<td>95 140</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Inoculated by intravenous route, all others intraperitoneal route.
†P = Intramuscular tumors. V = Visceral tumors, O = Osteopetrosis.
‡Passages 4 and 5 were terminated at 5 and 3 months, respectively, all others were terminated at 6 months of age.

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- **Table 1:** Transmission Data for the Serial Passage of a Lymphoid Tumor With Filtrates and Comparative Inoculations With Cell Suspensions

- **Table 3:** Total and average percentages for cell-free preparations, exclusive of passages 4 and 5

*Inoculated by intravenous route, all others intraperitoneal route.
†P = Intramuscular tumors. V = Visceral tumors, O = Osteopetrosis.
‡Passages 4 and 5 were terminated at 5 and 3 months, respectively, all others were terminated at 6 months of age.
one-half (55 per cent) had osteopetrosis and only a few (6 per cent) had neurolymphomatosis.

None of the chickens of the two non-inoculated control groups showed any evidence of tumors or other manifestation of lymphomatosis.

The pathological manifestations obtained with filtrate inoculations of the 6 passages were similar to the visceral tumors and osteopetrosis previously described as the result of inoculations with cell-free preparations of this tumor strain (4). Massive lymphomatous tumors of the viscera occurred in all groups, and osteopetrosis occurred in all except 2 groups inoculated with filtered material. One to 3 cases typical of neurolymphomatosis were observed in 8 of the total of 15 groups inoculated with cell-free material.

Almost half of the positive cases had more than one type of involvement. Of the 153 cases obtained in the filtrate-inoculated birds held for 6 months, 67 had a combination of osteopetrosis and visceral tumors, 66 had visceral tumors without osteopetrosis and only 17 had osteopetrosis without gross evidence of visceral tumors. Of the 9 cases which had nerve involvement all but 3 also had osteopetrosis or visceral tumors.

| Table II: Gross Involvement of Visceral Organs After Inoculation With Cell-Free Material |
|------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Passage No. | No. of | Lesions among organs of lymphomatous birds | Percentage distribution |                      |
|             | cases  | Liver | Spleen | Kidney | Gonad | Heart | Prov. | Perit. |
| 1           | 11     | 100   | 82     | 73     | 18    | 9     | 9     | 9     |
| 2           | 28     | 97    | 82     | 53     | 25    | 7     | 4     | 0     |
| 3           | 51     | 100   | 73     | 39     | 24    | 4     | 4     | 0     |
| 4           | 15     | 100   | 80     | 80     | 27    | 20    | 0     | 0     |
| 5           | 5      | 100   | 80     | 80     | 20    | 0     | 0     | 0     |
| 6           | 17     | 100   | 88     | 76     | 12    | 18    | 0     | 6     |
| Total       | 127    | 99    | 79     | 57     | 22    | 9     | 3     | 2     |

Gross tumor involvement of the various visceral organs in chickens that died after inoculation with cell-free material is summarized in Table II. Cases showing any visceral involvement almost invariably had lymphomatous livers. Most of them also had spleen and kidney involvement, followed by tumors of the gonad, heart, proventriculus and peritoneum in frequency. Other organs were occasionally involved. No apparent difference in the frequency of involvement of any organ was noted between the various passages, between different routes of inoculation, types of donors used or preparation of inoculum.

Study of tissues from all the donors and a limited number of recipients showed that the microscopic alterations found were uniformly typical of visceral lymphomatosis (8, 11) and osteopetrosis (1, 4, 10).

**DISCUSSION**

It is apparent that no significant trend or change occurred in the manifestations of this agent during the 6 passages. The type of lesions remained the same, and the incidence of osteopetrosis, visceral tumors, and neural involvement remained at about the same level, although there was a suggestion of an increase in activity since the lowest incidence of osteopetrosis and of visceral tumors occurred in the first and second passages, while the highest incidence occurred in the last or sixth passage. The average age at death was also remarkably similar for the 11 filtrate inoculated groups maintained 6 months.

Data presented in Table I indicate that the rate of tumor growth from transplants of tumors induced by filtrates was much slower than that from transplants of tumors that had been propagated in series with tumor cells, i.e., in serial passage with cellular inoculum. In inoculations of the first 3 passages, groups of chicks were also injected with whole blood or tumor cell suspensions from the same source as the filtrate preparations. Whole blood of birds with 7 day intramuscular transplants produced visceral tumors and death of all birds in an average of 7 (intravenous route) to 13 (intraperitoneal route) days' time with no evidence of osteopetrosis (Table I, passage 1). In contrast, chicks injected with whole blood from lymphomatous and osteopetrotic cases produced by filtrates of the second passage, developed an incidence of 63 and 69 per cent visceral tumors, respectively, and the age at death was prolonged to an average of 43 and 119 days, respectively. Two cases of osteopetrosis occurred in the latter group. Cellular suspensions of lymphomatous livers were also used in inoculation of the second and third passages. The incidence of visceral tumors was high and cases of osteopetrosis appeared in the 3 inoculated groups. The birds died on an average of 84, 71, and 75 days after inoculation.

When birds were inoculated with cell suspensions, prepared from tumors that had been induced by a cellular inoculum, tumors were produced in all birds, the average survival was 7 and 13 days for 2 routes of inoculation (passage 1) and osteopetrosis was not evident; whereas birds in-

1This is typical of results obtained at this Laboratory during 55 serial passages of this tumor strain with cellular preparations. All of the 548 birds used in these passages developed tumors, the average survival time was 9.52 days and gross evidence of osteopetrosis was not observed. Microscopic alterations indicative of osteopetrosis were observed in one case (a donor for passage 1 herein described). The lack of appearance of gross bone involvement may have been due to the short survival period, which did not allow sufficient time for grossly visible bone alterations.
occulated with cell suspensions prepared from tumors that had been induced by a cell-free inoculum developed similar tumors, but the incidence was lower, the average survival time was much longer (43 to 119 days) and osteopetrosis was present in all but one group. This longer survival time, which is directly related to the rate of tumor growth and malignancy may be related to the fact that the filtrate-induced tumors used in the transplants took much longer to develop (average of 137 days) than did tumors grown in serial passage with cell transplants (average of 10 days).

A similar difference in results between cell-free preparations of tumors induced by cellular inoculum and cell-free preparations of tumors induced by filtrates was not obtained in these passages. Actually, the filtrate used for the sixth passage produced the highest incidence of tumors and the third passage filtrate group had the lowest age at death (excluding passage 4). However, the differences between these values are small and insignificant. Since the apparent activity of the filtrates remained at about the same level, whereas the apparent malignancy of the tumor cells was much greater in the first than in subsequent passages, one may infer that a positive relation between the malignancy of the tumor cells and virulence of the tumor agent was not obtained in the present experiment.

Tumors in birds that received filtrates were presumably due to a filtrable agent or agents contained therein; however, tumors in birds that were injected with cell suspensions may have been due to direct multiplication of the transplanted cells, or due to an agent within the cells injected or a combination of both. It is significant that except for the first passage (from transplanted tumor) the cellular inocula were no more effective in producing lymphomatous tumors and osteopetrosis than filtrates prepared from the same source.

Although cases of neurolymphomatosis occurred only in inoculated groups the incidence is low and of doubtful significance. Its occurrence may or may not be due to factors other than the inoculum.

During the course of the serial passage inoculations a limited number of transmission variables were tested in a preliminary manner. In the first and fifth passages the intravenous route of inoculation was compared with the intraperitoneal route. The differences obtained were small and not consistent. In the first test the intravenous route caused death in a shorter time but the intraperitoneal route produced the higher incidence of visceral tumors. In the second test a higher incidence in a 3 months’ period was obtained with the intravenous route. No difference was obtained in either test in the occurrence of osteopetrosis.

In passages 3 and 4 filtered plasma was compared with the liver filtrate from the same donor. No differences were obtained in the passage 3 test with respect to incidence of osteopetrosis, visceral tumors, or to length of survival time. In passage 4, which was terminated at 5 months, the incidence of visceral tumors in the liver filtrate group was almost twice that in the plasma-inoculated group; however, there was but little difference in the total incidence of tumors. It is thus apparent that an active agent was present in both the lymphomatous liver and in the blood plasma. Differences in the concentration of the active agent could not be estimated because the experimental design does not lend itself to such analyses.

An attempt was made to concentrate the agent in plasma by two centrifugal runs at 19,000 RPM (27,000 times gravity). No difference in transmission was obtained between the upper and lower one-half of the contents of the centrifuge tubes. This result was to be expected since no pellet or other evidence of separation was obtained during this centrifugation. In later experiments, working with muscle tumors, Burmester (5) obtained evidence of sedimentation of the same agent or agents by centrifugation at 19,000 RPM and at 40,000 RPM.

Homogenized lymphomatous liver tissue filtered through a Seitz sterilizing filter in the third passage produced as many tumors as similar material filtered through Seitz clarifying K1 and K7 filters for inoculation in the second passage. Although comparisons of these filters must be made with results obtained with different donors, it would appear that under the conditions of these inoculations the fine filters did not remove much more agent than the coarser ones.

There were variations in the pathological alterations of the donor which were not correlated with similar variation in the recipients. The donors used for passages 2 and 6 had massive lymphomatous involvement of the viscera but no gross or microscopic evidence of osteopetrosis; however, 37 per cent of the birds of passage 2 and 83 per cent of those in passage 6 developed osteopetrosis. This incidence was as high as, or higher than, other passages in which the donor had osteopetrosis.

Donors used for the extra passage 3 and for passage 5 had osteopetrosis without gross or microscopic evidence of lymphomatous involvement of the viscera, yet a high percentage of the recipients developed visceral tumors. Although no attempt was made to separate the two manifesta-
tions during several serial passages, there was no indication of a tendency for one manifestation or the other to become predominant when a donor with only one type was used.

Two explanations may be presented: (a) the two manifestations are due to one and the same agent, and tissue resistance or other similar factors determine the type of involvement obtained, or (b) two separate agents are responsible for the two different manifestations and the alterations obtained are due to the relative activity of each agent. It has already been suggested that osteopetrosis and lymphomatosis may be due to different agents (4). Further evidence of a separate etiology was obtained by differential centrifugation studies (5). The "masked" or "latent" nature of the agent of osteopetrosis was noted by several investigators (1, 7, 10). A similar phenomenon has been demonstrated for Rous tumor virus (13), the Shope papilloma virus (12), and has been suggested for other agents of the avian leukosis complex (7). Thus, there is some evidence suggesting that osteopetrosis and lymphomas of the viscera are produced by different agents, and that either may remain latent in recipients and become overt in subsequent passages.

The results of 8 different inoculations and 2 control groups furnish conclusive evidence that an agent or agents passing through bacteria-retaining filters will induce the formation of osteopetrosis and lymphoid tumors of the viscera. The incidence of grossly visible tumors was high (69 to 95 per cent) in all groups inoculated with the filtrates and maintained for 6 months; whereas no evidence of tumors appeared in two control groups.

Sterilizing Seitz S1 filters were used for the preparation of 7 filtered inocula tested. All filters when tested after filtration of the inocula were found to retain Serratia marcescens completely. An 8-pound Mandler candle filter was used for another inoculation. This filter was tested before filtration of the inoculum and again after it was cleaned and resterilized. In all cases, filtrates from 48 hour broth cultures of Serratia marcescens were sterile. A Jenkins-Fisher filter was used for the ninth filtrate. This particular filter was not tested for its retention of bacteria; however, 6 filters of the same type and chosen at random were found to completely retain Serratia marcescens.

Since all passages after the first were made with filtrates from donors that had received only filtered material, it may be assumed that the agent was propagated in the host as a result of the action of the agent or agents.

In working with visceral tumors from cases of naturally occurring lymphomatosis, Burmester and Denington (6) were able to produce a high incidence of lymphomatous tumors with cell-free preparations from 5 of 10 of the original tumors. One of these preparations also produced osteopetrosis. Four tumors were propagated in serial passage with cellular preparations (7). Filtrates prepared from three propagated tumors produced a high incidence of lymphoid tumors within a period of 200 days. The transmission and pathological characteristics of these three strains (RPL 18, 20, and 21) appear to be similar (except for a variation in the incidence of osteopetrosis) to the tumor strain used for experiments reported herein (RPL 12).

SUMMARY AND CONCLUSIONS

1. The filtrable agent or agents inducing osteopetrosis and lymphoid tumors of the viscera were propagated through 6 serial passages in chickens.

2. The incidence of tumors and average survival time were quite uniform for the several filtrate inoculations and passages. An average of 81 per cent of all birds inoculated showed some gross involvement and they died on the average in 137 days. Of the total positive cases 55 per cent had osteopetrosis, 87 per cent had visceral tumors, and 6 per cent had neurolymphomatosis.

3. Results obtained from inoculation of chicks with tumor cell suspensions and filtrates prepared from the same tissue suggest that there was no relation between the malignancy of the tumor cells and the virulence of the agent.

4. After the first passage, filtrates were as effective as cell suspension in producing visceral tumors, and the filtrates invariably produced a higher incidence of osteopetrosis.

5. Filtrates appeared to be about as effective by the intraperitoneal route as by the intravenous route.

6. Filtered plasma of tumor-bearing birds produced about as high an incidence of tumors in recipients as did filtrates of lymphomatous livers.

7. Neurolymphomatosis appeared in 8 of 15 groups inoculated with filtrates but the incidence was not significant.

8. Donors showing only osteopetrosis produced about the same incidence of visceral tumors and osteopetrosis in recipients as donors with only lymphomatous visceral tumors or those with a combination of the two manifestations.

9. Conclusive evidence is presented that this lymphoid tumor contains a propagative agent or agents that will pass through bacteria-retaining
filters and will induce a high incidence of osteopetrosis and visceral tumors in chickens within 6 months' time. The latter tumors have thus far been indistinguishable from the tumors seen in cases of naturally occurring visceral lymphomatosis.

REFERENCES


The Propagation of Filtrable Agents Producing Lymphoid Tumors and Osteopetrosis by Serial Passage in Chickens

B. R. Burmester and G. E. Cottral