On the Transplantability of Lymphoid Tumors, Embryonal Nephromas and Carcinomas of Chickens*

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In previous publications (7, 8) a report has been made of 14 sarcomas and 7 fibromas naturally occurring in chickens and of the successful transplantation of 5 of the sarcomas for an indefinite number of generations. Transplantation of the other sarcomas either failed or was successful for a few passages only. As indicative of causes for these differences our data and those of other workers revealed that the age of the bird bearing the tumor may exert an influence, in the sense that optimum conditions for indefinite transplantability seem to be present in tumors developing in chickens of from about 5 to 12 months of age. Exceptions to this were found and no final conclusions were drawn pending results of other studies. In the present publication we report the results obtained in attempting transplantation of 12 lymphoid tumors, 10 embryonal nephromas, and 2 epithelial tumors.

MATERIALS AND METHODS

Most of the tumor-bearing chickens came from the same farm as had the birds with sarcomas and fibromas (7). The birds were brought alive to the laboratory. Tumor preparations were secured and injected immediately. Cell suspensions consisted of one part of tumor tissue passed through a mincer and 4 cc. of saline solution. Filtrates were made of one part of ground tumor and 19 cc. of saline solution passed through a Berkefeld "N" filter. Chicks of from 1 to 20 days of age, secured from a commercial hatchery, were usually injected with 1 cc. of cell suspension or filtrate in the breast but in some cases with 1 cc. of filtrate in the jugular vein. Adult chickens received 2 cc. of cell suspension into each breast. The results obtained are shown in the tables by the figure of positive inoculations in relation to the total number of animals inoculated.

LYMPHOID TUMORS

Neoplasia of the lymphoid cells is very common in chickens. The literature concerned with the transmission of the disease in its manifold manifestations and the demonstration of causative viruses is most extensive, but the data are often contradictory (19, 26). If in the opinion of some workers the problem is still unsettled, flawless experiments have shown that many of these neoplasms are transmissible by both cells and cell-free preparations as lymphoid neoplasia, or are obviously associated with erythro- and myeloid leukemia, tumors, and osteopetrosis (2, 5, 14, 15, 17, 18, 20).

We have tried to find in the data of other workers whether the age of the donor host influenced the transmissibility of the disease. In the series of Burmester and Prickett (3, 4), 4 of 5 cases from donors between 386 and 421 days old were successfully transplanted, but none of 3 other cases from donors between 428 and 532 days old and another from a donor 72 days old were transplantable. Jungherr (18) transplanted 5 of 6 cases from donors of between 5 and 7 months of age. In the series of Furth (14, 15), strains 2, 5, 11, 12, 13, and 15 were derived from lesions found in chickens that appeared to be under 1 year of age. The original Olson tumor was found in a chicken 5½ months old, but another tumor of the series from a chicken 7 months old was not transplantable (25). The data do not allow a final conclusion but they do not contradict what was observed in the case of chicken sarcomas.

The number of lymphoid tumors in our series is 18, all of them occurring in female Rhode Island Red × Plymouth Rock hybrids on the same farm, from 1943 to 1945. The age of 17 of the affected birds ranged from 5 to 24 months, the other bird was 10 weeks old. They were all typical cases of visceral lymphomatosis without gross involvement of the iris and nervous system. Taking the generalized forms as a whole, tumors were tested from practically all viscera and the skin,

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1 Another case from a donor 24 months old did not result in obvious takes in the first passage, but a second passage from blood showing abnormal lymphoid cells of 1 of the 3 young birds injected gave positive results. The spontaneous incidence of neoplasia in the controls was 1.4 per cent.
2 We are indebted to Drs. Burmester, Prickett, and Furth for supplying us with these data.
bone, and muscle. The solitary tumors measured in their largest diameters from 3 × 3 cm. to 12 × 6 cm. Histologically they showed the usual features, described by many authors, of typical cases of lymphomatosis not associated with other leukoses or tumors. In tumor I 1, there was a large carcinoma of the ovary in addition to the lymphoid tumors.

Twelve of the tumors were tested for transmissibility. The chickens injected were Plymouth Rocks or its cross with Rhode Island Reds. They were observed for a minimum of 30 days, often for 50 and sometimes 90 days after inoculation. Some of the chicks were injected in the peritoneum as well as in the breast. Two of the tumors were also injected into adult chickens and into newborn ducks. As the results in Table I show, in no case was it possible to transplant the tumors, and careful autopsies of the injected chicks did not disclose any suspicious gross change in the tissues.

**Embryonal Nephromas**

These tumors occur rather frequently in chickens. Olson and Bullis (26) found them in 5.5 per cent of their cases, Goss and also Jackson in 3.5 per cent (11). Foulds (13) attempted transplantation of 13 embryonal nephromas without success. Baker (1) carried through at least one passage an embryonal ovarian tumor which produced a sex hormone in the transplants. The tumor contained several types of cells, namely, polygonal or ovoid, with either a non-granular or a vacuolated cytoplasm probably derived from the theca interna islands of columnar-cell tubules and small cysts, and masses and strands of keratinizing squamous cell epithelium. The analogies with embryonal nephromas are obvious. Olson and Bullis also found an embryonal nephroma in a bird affected with a hormonal disturbance (26). We have found 10 of these neoplasms in about 50 chickens bearing tumors. Although about 40 of these chickens, including the 10 bearing these neoplasms, came from the same farm, we cannot state that the real incidence of embryonal nephromas in that flock of chickens was as high as 22 per cent, since chance may have played a part in the selection of the tumor-bearing birds. All the tumors were found in Rhode Island-Plymouth Rock hybrid females. These hybrids and also Plymouth Rock chickens were employed for transplantation. Other characteristics of the tumors and of their hosts, and the results obtained from their study are summarized in Table II.

Cell suspensions of tumors A, N, and Y were also injected into newborn ducks with negative results. Tumor N was grafted into the anterior chamber of the eye of chickens, ducks and guinea pigs. These results will be reported elsewhere.

Seven of the tumors were found as arising from or invading one or both kidneys, while the other 3 were extrarenal. Microscopically, despite pronounced individual differences, they all showed the typical embryonal picture so often described in some mammals and man (Wilms' tumor). The cylindrical cells were arranged in adenomatous and alveolar structures combined with round or spindle cells in indifferent arrangements. We shall refer to the latter as the fibroblastic part. A brief description of the tumors with a more detailed account of the transplantation experiments follows:

**Tumors B 1, F, N, P 1 and N 1**

These tumors ranged in size from 6 × 6 × 4 cm. to 12 × 10 × 10 cm. In general they consisted of a healthy, firm, juicy tissue with a more or less pronounced tendency to form cysts filled with a yellowish, not viscid fluid. They replaced most of the renal tissue, but were well demarcated and showed a capsule of variable thickness. Tumor N, the largest of all, was extrarenal and had caused an extension of small size loosely attached to the intestine. The bird bearing tumor P 1 had lymphomatosis of the liver and of the spleen and the one bearing tumor N 1 had a fibroma 5 × 3 × 3 cm. arising from the lower costal margin. This tumor was included in the group of connective tissue neoplasms as tumor N 1, which proved to be nontransplantable (7).

Microscopically, the tumors varied greatly in cellular structure. In tumor F the greater part of the tissue consisted of a hyaline matrix with collagenous fibers, the opposite being true in tumor N 1. Epithelial structures were most varied and pearls of keratinizing epithelium were observed in tumors B 1, N, and N 1.
TABLE II: TRANSPLANTATION OF EMBRYONAL NEPHROMAS

<table>
<thead>
<tr>
<th>No.</th>
<th>Designation</th>
<th>Age of tumor-bearing chicken, mos.</th>
<th>Metastases; extension; concomitant neoplasia</th>
<th>Cells</th>
<th>Filtrates chicks</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B 1</td>
<td>8</td>
<td>note</td>
<td>0/4</td>
<td>0/2</td>
<td>0/3</td>
</tr>
<tr>
<td>2</td>
<td>F 5</td>
<td></td>
<td></td>
<td>0/2</td>
<td>0/2</td>
<td>0/3</td>
</tr>
<tr>
<td>3</td>
<td>N 24</td>
<td>Extension to intestine</td>
<td></td>
<td>0/7</td>
<td>0/3</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>P 1 8</td>
<td>Lymphomatosis of liver and spleen</td>
<td></td>
<td>0/8</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>5</td>
<td>N 1 8</td>
<td>Fibroma in costal margin</td>
<td></td>
<td>0/9</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>6</td>
<td>A 30</td>
<td>Metastasis in liver</td>
<td></td>
<td>0/4</td>
<td>0/8</td>
<td>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>D 4-5</td>
<td>Metastasis in liver; sarcomas in muscles</td>
<td></td>
<td>0/1</td>
<td>0/3</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>From liver and muscle growths</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Y 5</td>
<td>Hemorrhagic metastases in liver?</td>
<td>1/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>U 1 7</td>
<td>Metastasis in liver</td>
<td>1/4</td>
<td>0/8</td>
<td></td>
<td>&quot;</td>
</tr>
</tbody>
</table>

From kidney growth

10

| E 8 | Sarcomas in muscles | 0/4 |
| E 5 | From sarcomas of muscles | 4/5 | 3/4 | Carried indefinitely as sarcoma |

The fibroblastic part showed cells ranging from the small, round, clear, embryonal type to attenuated spindle cells. In some cases mitoses were frequent and the impression was one of malignancy. Such was the case in tumor N 1, but the fibroma in the same chicken was benign in appearance. Foci of hemorrhage were quite frequent.

**Tumor A**

This tumor was a firm, cystic mass 12 x 10 x 12 cm, filling the abdominal cavity and not involving the kidneys. Several small nodules, extensions of the main tumor, were loosely attached to the intestines while a metastasis 1.5 x 1 cm. was present in the liver. This tumor differed from the ones just described in that it contained large masses of cartilage and keratinizing formations. The liver metastasis consisted, as did the primary tumor itself, of epithelial formations arranged as in an adenocarcinoma but with complete exclusion of cartilage and keratinizing pearls and very few fibroblastic elements. The growth was not infiltrative.

**Tumor D**

A young chicken obviously sick was brought to the laboratory with a much distended abdomen and died shortly afterward. Autopsy disclosed a mass about 10 cm. in diameter looking like a bunch of grapes and attached by a pedicel to the anterior abdominal wall. Each of the many cysts was filled with a dark, serous non-viscid fluid. Cystic viscid nodules ranging in diameter from 1 mm. to 1 cm. were abundant in the mesentery and in the liver. In the breast there was a round cystic mass about 3 cm. in diameter filled with a slightly hemorrhagic, very viscid fluid, and several identical nodules but smaller.

Histologically, the walls of the cysts in the abdominal growth consisted mostly of malignant-looking, large spindle or stellate cells in dense, small whorls which sometimes arranged themselves in such a way as to imitate rudimentary glomeruli. Other areas showed the small, clear, round or oval cells, so frequently seen in most embryonal nephromas. Between these formations there were large tortuous clefts and numerous blood vessels of similar size and shape. Some areas of the growth gave the impression of cavernous hemangiomas.

Only one of the liver metastases was studied histologically, since the others were used for transplantation purposes. It consisted exclusively of the typical small, clear, round or ovoid cells. The picture was the same as in the liver metastasis of tumor U 1, to be described later, but there was no limiting membrane and the growth was infiltrating.
The tumors in the breast muscles were typical sarcomas consisting of large pleomorphic spindle cells in some areas analogous to the malignant-looking cells of the abdominal growth. The tumors showed large cavities in which loose tumor cells were observed as if growing in tissue cultures. Other analogous cavities contained blood. A vigorous fibrous stroma rich in large tortuous vessels would sometimes wall off part of these cavities and would subdivide the tumor tissue of the walls into other smaller cystic units. On the whole, these tumors showed several gross architectural analogies with the abdominal tumor. These have been included in our study of the sarcomas under the designation of tumor D (7).

Concerning the failure to transplant the tumor it must be said first, that sarcoma tissue in the inoculum was rather scanty and, second, that of the 4 chickens inoculated only 1 was young. Conceivably if more sarcoma tissue had been injected into a larger number of chicks, takes would have been obtained.

**Tumor Y**

A soft, not viscid tumor, 5 × 2.5 × 2.5 cm., in the upper part of the right kidney with two large cysts protruding from its surface, of which one looked like a purse filled with blood. Analogous large bloody cysts were found in the muscles of the thigh and several small ones were present in the liver.

The kidney tumor consisted of equal parts of epithelial elements, with some keratinizing pearls, and long attenuated fibroblasts. There were large cavities in which blood was found, and some infiltration by myeloid cells. The liver lesions were cysts filled with blood, and a capsule of spindle cells surrounded by a crown of small, probably lymphoid, cells. These lesions seemed to be metastases of the kidney tumor.

One of the chicks, a 15-day-old Plymouth Rock, inoculated in the breast with cell suspension, developed about 1 month after inoculation a tumor-like lesion beside the xyphoid, a location where transplantable tumors sometimes develop as a result of displacement of the inoculum injected higher in the muscles. The bird was sacrificed 80 days after inoculation and a firm mass 2 × 1 cm. was found which microscopically looked like a typical fibroma composed of long attenuated spindle cells. None of the other birds showed any such lesion and this, together with the relatively long time required for the lesion to develop, seems to favor the interpretation that it was a growth. A passage of the lesion into 4 other chicks by means of cell suspension was attempted but failed.

**Tumor U 1**

A round soft tumor 9 cm. in diameter entirely replaced the right kidney and had invaded and destroyed the gall bladder. The tumor was impregnated by a gelatinous fluid in which blood and bile were obviously present. Such fluid also filled up the abdominal cavity of the host. A metastasis 0.5 × 0.5 cm. was present in the liver.

Histologically, the tumor consisted of a major part of a fibrillar, fibrous matrix and a minor part of either fibroblastic or epithelial cells both typical of embryonal nephroma. The former were clear round cells identical to the fibroblastic cells of the kidney tumor, surrounded by a rather thick fibrous capsule. The tumor was transplanted by cells into 4 chicks and it grew in one. It consisted essentially of the same elements as the renal tumor, but the fibroblastic cells were far more pleomorphic and frankly predominated over the epithelial elements. A new element was a heavy lymphocytic infiltration. Transplantation of that tumor by trocar into 9 chicks failed.

**Tumor E**

A description of this tumor and accompanying sarcomas and of their behavior on transplantation has been given in foregoing publications (7, 8). The chicken was brought to our laboratory at the age of 5 months with several superficial nodules in the muscles of the breast and wings, the largest being 3 × 2 cm. They were firm, well capsulated and non-viscid sarcomas (Fig. 1). Cell suspensions and filtrates of these growths removed by biopsy induced tumors in chicks and pullets, the first generation of an indefinitely transplantable sarcoma which was designated as Tumor E (7). The chicken was killed after another 3 months when it was very ill. Autopsy disclosed the presence of embryonal nephromas in both kidneys and about a dozen ordinary sarcomas, either hard and not viscid or soft and viscid, about 3 × 2 cm. and smaller in the muscles of the lower extremities.

There was in the right kidney a mass 6 × 5 × 4 cm. and other smaller masses either in the same or in the left kidney. Some additional microscopic tumors were discovered. The gross tumors were smooth, firm, non-viscid growths. At least nine-tenths of them consisted of a hyaline material in which could be observed loose bundles of collagen, some fibroblasts and occasional epithelial formations in alveolar arrangement, the lat-
ter increasing at the periphery. A few areas were very cellular with a great majority of rather pleomorphic fibroblastic cells arranged in sheets giving often an impres- sion of malignancy and here and there showing mitoses. The epithelial cells were arranged in rather simple acini and duct-like structures. Hemorrhagic foci and discrete infiltration by myeloid cells were observed (Fig. 2). A capsule around the tumors was either thin or lacking. Cell suspensions of this tumor injected into chicks induced no growths.

The growth had the gross features of a tumor of the Rous sarcoma type and apparently secreted the same viscid material (hyaluronic acid?). Of special interest is the fact that at the second passage a sarcoma developed months after injection in the same site where the carcinoma had been injected, and that this tumor was transplanted and subsequently transmitted by filtrates (13).

Oberling and his co-workers (23) injected a chicken with virus of the MH2 endothelioma. The bird died.

Epithelial Tumors

Feldman and Olson (11) discuss at length the incidence of these neoplasms compared to sarcomas and to other tumors in chickens. In general, epithelial malignancy is far less common than that of mesenchymal tissues. Probably the difference is still apparent even if lymphoid tumors are excluded. Carcinomas seem to occur in adult rather than in young birds.

The only transplantable carcinoma so far known is that described by Foulds (12). The tumor arose from the oviduct of an old Leghorn hen, which 2½ years before had been injected with virus of MH2 endothelioma. The tumor was carried, in many passages, through several breeds of chickens, the original White Leghorn breed being the least satisfactory. Sometimes

5 months after inoculation with primary carcinoma of the liver. Of 2 chickens injected with this material one died with leukosis and retrorenal myeloma. From the latter tumor a strain of leukosis was originated. When infectious blood and tissues were injected in the muscles after storage in glycerol sarcomas resulted. In two cases epithelial neoplasms developed at the side of a sarcomatous mass, which was believed to have been induced by the same virus.

Only 2 epithelial tumors were found. One, in a chicken 2 years old, was a soft round mass 2 cm. in diameter at the tip of the wing. It consisted of large acini formed by cells secreting a mucin-like material with no special traits of malignancy and an abundant myxomatous stroma. It could be diagnosed as a benign

Fig. 1.—Tumor E. One of the sarcoma nodules in the leg. Mag. × 120.
Fig. 2.—Tumor E. The kidney tumor. Mag. × 120.
neoplasm of the skin glands. The other was a large adenocarcinoma of the ovary with abundant fibromatous stroma in the same chicken, aged 8 months, that bore lymphoid tumor I. The 2 epithelial tumors injected into chicks did not induce any growth.

DISCUSSION

In our hands transplantation of lymphoid tumors failed under the same experimental conditions that allowed transmission of some sarcomas and regardless of the age of the donor host. Whether we would have succeeded had we kept the injected animals a longer time we cannot say.

Since other workers have transplanted lymphoid neoplasia and obtained growth shortly after, it is clear that other factors are at play and one of them may well be the genetic susceptibility of the recipient chicks. Thus, Blakemore (2) and Burmester and Prickett (3) employed inbred chicks selected for their relative susceptibility to naturally occurring lymphomatosis (28). Lee and his group (20) noticed considerable differences in the results of injection of White Leghorn chicks with material from a lymphomatosis-leukosis complex depending on whether the birds belonged to “resistant” or “susceptible” flocks. On the other hand, no mention is made of special susceptibility of the recipient chicks in the experiments of Jungherr (18), Olson (25), Johnson (17) and Furth (14, 15), and it may be pertinent to recall that in erythroblastosis-granuloblastic leukoses, as in the case of sarcomas, all breeds of chickens as well as some foreign species can be experimentally infected (24).

But even when special susceptible chicks are employed some cases of spontaneous lymphomatosis are transmissible but others are not (3). The problem is then analogous to that of the transmission of spontaneous sarcomas. One may feel that these neoplasms are easily transplantable because in general only the positive results have been reported. Yet, we have found only 5 of 14 that were so, and Troisier (27) transmitted only 5 of 23. Possibly, the practical issues involved in the case of lymphomatosis is the reason why special attention has been paid to the cases where transmission failed. Also, of the 46 spontaneous cases of erythroblastosis compiled by Engelbreth-Holm (10, page 59) only 13 proved to be transmissible. Whether the age of the donor host played any part in these results is not known.

Results obtained in our studies on the infection of ducks by the virus of the Rous sarcoma and on the accompanying variations of the virus throw considerable light on the present problem (6). It was shown that in ducks transmission was easy with the sarcoma variants; difficult with the intermediate sarcoma-lymphoblastoma (duck tumor E); and impossible with the lymphoblastomas. In other words there was a different capacity in the lesions to permit transmission depending on some inherent quality of the cells, and this quality was determined by the direction in which the original chicken virus had varied.

In the case of embryonal nephromas the main point to consider is whether the sarcomatous growths from which, in one case, a causative virus was demonstrated, were metastases of the fibroblastic part of the embryonal nephroma or concomitant tumors. Since it would be absurd to maintain that the causes of the fibroblastic and epithelial parts of the tumor are different, the issue is important because it would mean that a potential sarcoma virus also can induce epithelial malignancy under certain circumstances.

Tumors B1 and F were solitary. Tumor N had caused an extension to the intestine. Tumors A and U1 had induced liver metastases of the epithelial and fibroblastic parts of the tumor respectively, and lesions in the liver, suspected to be metastatic, were observed in tumor Y. In tumor D fibroblastic metastases were observed in the liver along with several sarcomatous nodules. Finally, in tumor E there were widespread sarcomatous nodules only. These proved to be indefinitely transplantable and a causative virus was demonstrated even in the original tumors.

Moreover, tumor U1, successfully grafted into one chick, grew much as a sarcoma, the epithelial elements being rare. Tumor Y was also grafted and it probably grew like a fibroma, thus posing the problem of whether the fibroma concomitant with tumor N1 was or was not a metastasis of the embryonal nephroma.

In the case reported by Mathews (21) there was a typical primary tumor in which predominated elements “which presented the picture of a rapidly growing sarcoma,” and metastases in the muscles, lung, and opposite kidney “with the same picture of a rapidly growing spindle cell sarcoma” and complete absence of epithelial elements.

The gradation established by these observations certainly suggests that the sarcomas of tumor E were metastases of the embryonal nephroma. If this is the case, one is forced to postulate that a change toward malignancy has occurred in the metastasizing process, for we have seen that embryonal nephromas as such are very difficult to transplant, and functionally these neoplasms in chickens appear to be of only relative malignancy. Whether the age of the tumor-bearing animal has an influence on the transplantability of these tumors is not possible to tell.

Tumor P1 was associated with lymphomatosis of liver and spleen. Olson and Bullis, also, found 6 of their 21 cases associated with lymphomatosis or leukosis (26). Greene (16) studied 4 embryonal nephromas from rabbits and transplanted one, first, through the
eye and, later, through the testes of rabbits. Contrary to what was observed in chickens, only the epithelial parts survived transplantation. Growth was very slow as only relatively malignant and embryonal nephromas of rabbits were considered to what was observed in chickens, only the epithelial eye and, later, through the testes of rabbits. Contrary of Foulds (12) and Oberling (23) tend to support our observations on embryonal nephromas in the sense that mesenchymal and epithelial malignancy can be induced by a single virus or variants of the same.

On analysis of what has been learned on avian cancer from the work of others and from our own (7, 8), it would seem that causative viruses have been demonstrated in indefinitely transplantable tumors from the most representative groups of spontaneous mesenchymal neoplasia, and that one can suspect them, but by no means are they conclusively demonstrated, in mixed and epithelial tumors. Transplantation becomes increasingly difficult starting from sarcomas—and also leukemia—to lymphoid tumors, mixed embryonal tumors and finally carcinomas. Like so many inflammatory viruses some avian neoplastic viruses can vary or mutate and an increasing amount of evidence (6; 22, pages 129-149) suggests that these variations are important in determining the distinctive features of each tumor including the ability to be propagated through autologous, homologous, and also heterologous species; probably factors of age and genetic makeup of the hosts are also involved. In turn, variation of the neoplastic viruses is conditioned by the age (9) and conceivably by the genetic constitution of the hosts.

SUMMARY AND CONCLUSIONS

Transplantation of 12 lymphoid tumors under the same conditions that insured indefinite transplantation of some sarcomas failed entirely. Two epithelial neoplasms could not be transplanted.

Ten embryonal nephromas are described. One of them was carried through a passage in one chick where it grew much as a sarcoma. Another was probably transplanted as a fibroma. The sarcomatous growths accompanying a third case were transplanted indefinitely as sarcomas, and a causative virus was demonstrated even in the original growths.

Since unmistakable metastases of the embryonal nephroma occurred in at least 3 cases and in one of them there were also present sarcomatous growths we must consider the possibility that the transplantable infectious sarcomas may have been metastases of the embryonal nephroma. The factors that seem to govern the transplantability of all spontaneous chicken neoplasms are discussed.

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