A Study of Five Transplantable Chicken Sarcomas Induced by Viruses*

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In a preceding paper (7) we described the main features of 21 connective tissue tumors of chickens which may serve to indicate the influence of the age of the host and other factors on the transplantability and on the demonstration of causative viruses in these types of tumors. Five of the tumors, all sarcomas, proved to be indefinitely transplantable and to be produced by filtrable viruses. These tumors are described in some detail in the present publication.

METHODS

As a routine the tumors were transplanted by means of cell suspensions. The tumor tissue was first cut in small pieces with scissors, passed through a mincer, and suspended in four parts of saline solution. Chicks and pullets were injected with 0.5 cc. of this suspension into each breast, while adult chickens received 2 cc.

Filtrates were obtained by passing tumor extracts diluted 1:20 in saline through Berkefeld "N" candles, and 1 cc. was injected into the jugular vein or in the breast. Desiccates were obtained in a vacuum with calcium chloride and the powder was kept in sealed tubes in the refrigerator. When needed they were resuspended in the minimum amount of saline which made the mixtures easy to inject. In this way the components of the tumor were always more concentrated in desiccates than in filtrates or extracts. One cubic centimeter of the desiccate preparation was injected into the breast.

Tissue cultures were obtained of one of the tumors by using rabbit plasma, chick embryo juice, human placenta serum, and Tyrode's solution in a Carrel flask.

Sections were stained with the hematoxilin-eosin, and occasionally by the Masson method.

The conventional signs used for the expression of results are represented in Fig. 1. Giving definite ages to what we call chicks, pullets, chickens, and hens is arbitrary but it simplifies analysis of the results.

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Chicken Tumor B

Description and behavior on transplantation.—This sarcoma was observed in March 1941, in a Plymouth Rock hen 6 to 10 months of age, obviously ill. The bird died and the autopsy disclosed the presence of a round, reddish, viscid mass, 8 cm. in diameter filling the abdominal cavity, and many smaller nodules attached to the peritoneum and mesentery. Possibly the tumor had originated in the ovary.

Results on transplantation by cell suspensions in 7 representative passages are given in Fig. 2. The tumor did very well in chicks of the first passage but very poorly in the 2 following ones, only one chick of the fifth passage dying of generalized tumor in 5 months. This tumor grew so slowly that 2 months after inoculation no local growth was detected. From the lung metastases of this bird (the primary tumor was wholly necrotic) the tumor was successfully transplanted into 25 adult chickens for another 9 passages. At the 12th passage the tumor was carried through 3 generations of tissue culture. It was then reinoculated into chicks and successfully passed through 14 chicks or older birds in another 5 passages. Through transplantation, the tumor kept the main gross traits of the original growth. In young hosts it was more soft, viscid and infiltrating than in adult ones. It grew in White Leghorns just as well as in Plymouth Rocks.
Generalized lesions.—As shown in Fig. 2, metastases were frequently observed in the chicks of the first 3 passages. Besides lung, liver, and spleen they were characteristically present in the intestinal tract, pancreas, and in the skin, as well as in the bones as periosteal and endosteal tumors. Analogous bone tumors and also lung metastases were also present in the only chick of the fifth passage which responded to inoculation with tumors. Several chicks of the tissue culture strain also developed metastases but never in the skin or intestinal tract. Metastases in adult chickens were only found in the lungs.

Hemorrhagic lesions were first observed in a chick of the second passage and in practically all the chicks of the tissue culture lines, but never in adult chickens. They were commonly found in spleen, liver, pancreas, intestines and bone-marrow, and occasionally in the meninges. The lesions and the evolution of the disease were of the same sort as described for the Rous and Fujinami viruses (4).

Effect of cell-free preparations.—Free virus in the tumor was detected in the second passage coincidently with the appearance of hemorrhagic lesions in another chick of the series. Filtrates were again tested intra-venously with positive results from tumors of the sixth and 10th passage and also from one of the third passage of the tissue culture strain, not represented in the chart. Filtrates from the tissue culture strain yielded periosteal and endosteal tumors, while the other filtrates induced in the spleen and liver both tumors and blood blebs. These lesions were exactly like those resulting from generalization of primary tumors.

Desiccates from 10 young tumors from the first to the 13th passages were tested in young hosts. Only 4 of the desiccates proved to have some activity, never very great. The desiccates in these cases were never older than 75 days. Because we relied too much on the activity of older desiccates, this tumor strain was lost.

Histological data.—The original tumor was a fibromyxosarcoma riddled with blood sinuses giving to it a hemorrhagic appearance. There were areas of long, attenuated, fibroblasts alternating with other areas of blunt or round cells. These elements were often very loosely arranged, and the abundant intercellular matrix was very rich in collagen.

In the course of the passages both by cell suspensions and filtrates the tumor retained for two years the same polymorphism shown by the spontaneous tumor, but

Fig. 2.—Diagrammatic representation of chicken tumor B passages.
it became much more cellular; collagen production became scant and the blood sinuses disappeared. Growth was always infiltrating and mitoses were rare.

The periosteal and endosteal tumors consisted of either packed, attenuated fibroblasts or of loose, blunt, round cells, probably depending on the surrounding pressure. In chicks the endosteal tumors were sometimes combined with hemorrhagic lesions, while in other cases only the latter were present. In no case was there a secondary formation of osteoid tissue, the initial stage of osteosarcoma (5).

Summary and comments.—Tumor B, a fast growing fibromyxosarcoma, did very well in young hosts in the first 3 passages. During this period free virus was present and generalization was frequent, involving characteristically the skin, digestive tract, and bones. Then, at the fourth and fifth passages the tumor did very poorly and the strain would have been lost had not one of the chicks developed late growths. From that time on, as though the virus had become adapted to transplantation, the tumor acquired a new vigor and grew in 100 per cent of hosts of all ages. Generalization was also frequent, especially in chicks, but the skin and the digestive tract were not involved and bones less frequently.

During the first three passages the tumor was very much like some of the duck variants of the Rous virus, e.g., strains H.C. and H.V. (5), for in these variants the virus showed a characteristic affinity for the digestive tract, skin, and bones. Further, it would not grow in adult chickens, nor serially even in chicks. Final adaptation to chicken transplantation of tumor B seems to have taken place in the growths of the one bird that developed them in the fifth passage.

From the fifth passage on, the tumor behaved much as the ordinary Rous sarcoma, with the minor exceptions that with chicken tumor B, the lungs of adult birds were the organs exclusively affected by metastases and the virus did not survive desiccation to the same extent as the Rous agent.

**Chicken Tumor E**

Description and behavior on transplantation.—A Plymouth Rock × Rhode Island Red hybrid was brought to us in March, 1943, at the age of 5 months with several nodules 3 × 2 cm. and smaller, in the wings and breast. They were firm, well encapsulated, and not viscid. Under anesthesia, they were removed, cell suspensions and filtrates obtained and injected into chicks and chickens. Both preparations induced tumors and by passing these a stable line was obtained. These tumors proved to be sarcomas. The chicken lived another 3 months and when very sick, was killed. Autopsy disclosed the presence of large, very firm, resilient tumors in both kidneys. These tumors proved to be embryonal nephromas. Other nodules 3 × 2 cm. and smaller were present in the muscles of the legs. These tumors proved to be sarcomas. The sarcomas removed 3 months before had recurred. Cell suspensions from the kidney tumors were injected into chicks but no growth ever resulted.

The line from the tumors secured by biopsies was carried through 17 passages within a period of 11 months by means of cell suspensions. The number of takes in birds of all ages was 77 per cent, but when the tumor was passed exclusively through chicks the percentage rose to 92 per cent. From the second passage on, growth was fast and survival after inoculation was short. Frequently in adult chickens tumors did not grow or regressed after a period of initial growth. The tumors were generally soft, viscid and infiltrative. The behavior on transplantation during the first 7 passages, quite representative of the whole line, may be seen in Fig. 6.

**Generalized lesions.**—Metastases were present in 26 out of 191 chickens of all ages showing tumors (13 per cent), but were rare in adult chickens. When present they were found in the liver, spleen and lungs and showed no unusual characteristics. In one chick each of the first, second, and seventh passages periosteal and endosteal tumors of the long bones were observed. Hemorrhagic lesions were not clearly manifest until the fifth passage as a result of generalization from tumors induced by desiccates. These lesions were observed in liver, spleen and lungs in 10 per cent of the chickens of the following passages.

Effect of cell-free preparations.—As shown in Fig. 3 an interesting feature of the tumor is that free virus was present in the original growth as shown by the effectiveness of its filtrates, which induced tumors in 4 of the 7 chicks injected. Also, in another chick the injection of cell suspension in the breast was ineffective but tumors did develop in the spleen. In the course of the passages, filtrates from 9 young tumors grown in chicks were tested and they were found active in 6 cases.

Desiccates from 3 chick-grown tumors were tested with positive results in all. One of the preparations was very effective 27 months after desiccation.

The tumors induced by these cell-free preparations injected into the breast were like those induced by the inoculation of cells. The lesions induced by the intravenous injection of filtrates were generalized tumors or hemorrhagic blebs like those following generalization of primary tumors. In only one chick of the second passage was there a widespread generalization with involvement of the skin, digestive tract, muscles and bones (periosteal and endosteal tumors) besides the other commonly affected organs.
Histological data.—There were present in the kidney, along with the gross tumors, microscopic growths in the middle of relatively healthy renal tissue. All these growths showed a picture of embryonal nephromas. They will be described in a forthcoming paper.

The breast and wing tumors secured by biopsy were sarcomas, probably rhabdomyosarcomas, with numerous giant cells, few or no mitoses, and a moderate amount of collagen. The leg tumors secured at autopsy were sarcomas as were those in the breast and wing, but without giant cells. The fibroblastic cells of all these tumors were large, elongated and arranged in whorls but sometimes they were small and round as in the kidney tumors.

In the course of the transplantation this picture kept on repeating itself, one or another type of cell predominating in the different random tumors.

The periosteal and endosteal tumors consisted of packed bundles of attenuated fibroblasts. Some sections were extremely suggestive of a secondary formation of osteoid tissue such as were observed in the bone nephromas and the latter sarcomas. Only the sarcomas proved to be transplantable and a causative virus could easily be demonstrated not only in the transplants but also in the original tumors. They thus behaved like the two sarcomas studied by Foulds, about which no details are given (8) and strain 7 of Jungherr (10).

The sarcoma line behaved as if the virus was well adapted from the very beginning. It grew steadily in most of the chicks and pullets inoculated, and in this property, as in its morphology and in the stability of the virus in desiccated tissue, it resembled very much the tumors of the Rous sarcoma type. It differed from tumors induced in chicks by viruses of some of the duck variants of the Rous sarcoma, but not by the original Rous virus (5).

Summary and comments.—The original host, when killed at the age of 8 months, showed the largest tumors in the kidney and many smaller ones in the muscles of the extremities and breast. Although the first impression was that of primary kidney tumors with muscle metastases, the former were embryonal

![Diagram of chicken tumor E passages](image-url)
them in the paucity of generalized lesions, in its more pronounced inability to grow steadily in adult hosts, and possibly by some features shown by the bone tumors. Further comments on tumor E will appear in a following publication.

Passages I 2 3 4 5 ...... 8 ...... II

Chicken Tumor D 1

Description and behavior on transplantation.—The original chicken was a Plymouth Rock × Rhode Island Red hybrid hen 8 months old brought to our laboratory in December, 1944, showing in the breast 4 main confluent tumors about 5 × 5 × 3 cm. and others of a smaller size. The bird was killed and autopsy showed the tumor to consist of a medium firm or soft viscid tissue with some necrosis in the deeper parts. A metastasis measuring 5 × 5 mm. was found in the lung.

Over a period of 10 months the tumor has been carried by means of cell suspensions through 15 passages of which 7, the most representative, are expressed in Fig. 4. It is clear that the tumor did very poorly at the beginning and it was almost by chance that a stable line was secured. No growth was obtained in

FIG. 4.—Diagrammatic representation of chicken tumor D 1 passages.
adult chickens and regressions even in young hosts were frequent. However, the tumor became progressively adapted to transplantation and from the sixth passage on no more regressions took place. The incidence of takes, at least in young hosts, was nearly 100 per cent with a survival period shorter than in the earlier passages. Viscidity seemed to be a feature of the fast growing tumors. In all the passages the tumor grew in hybrids as well as in Plymouth Rocks.

Generalized lesions.—During the first 6 passages only 2 chicks showed metastases. In the next 10 passages metastases developed in 8 of 73 chicks and pullets showing tumors; in 2 cases these were combined with hemorrhagic lesions. The metastases generally discrete, were located in the liver and lungs, and in one case in the ovary.

Hemorrhagic lesions were first observed in chicks at the seventh passage, the number of cases showing them in the next 10 passages was 8; in 2 cases these were combined with gross tumors. The blebs were located in the liver and spleen, and showed the usual characteristics described for tumor viruses.

In some chicks there were abundant small areas of necrosis in the liver, in addition to tumors, to hemorrhagic lesions or to both.

Effect of cell-free preparations.—Filtrates were tested from the original tumor, and from tumors of the third, fifth, and eighth passages with negative results, while those from the fourth, ninth, tenth, 11th, 12th and 13th induced tumors. Of 13 chicks injected with filtrates of the growths from the fifth and tenth passages only 2 developed lesions while infection was manifest in 22 of 33 chicks injected with filtrates of tumors from later passages. The number of generalized lesions—neoplastic and hemorrhagic—induced by filtrates from 20 primary breast tumors was 8, a higher incidence than when cells were used for inoculation material. The lesions induced by filtrates were of the same sort as those induced by cells. Focal necrosis in the liver was also observed.

Histological data.—The original tumor was a fibromyxosarcoma, rather pleomorphic, somewhat infiltrated by mononuclear cells, showing few mitoses, and abundantly vascularized. The most common pattern in the course of the passages consisted of packed bundles of fibroblasts, often in mitotic division, alternating with loose areas of large distorted cells and occasional giant cells.

The random sections available of hemorrhagic lesions in the liver show absence of neoplasia. Necrosis in this organ was either scattered in small groups of cells or in larger foci sometimes in a periportal location. These lesions were absent in the liver of the original chicken.

Summary and comments.—This is a tumor of the Rous type which did not become adapted to transplantation until carried through 5 passages within a period of from 3 to 4 months. The history of the transplantation illustrates the part chance can play in the establishment of a stable tumor line, for had the only chick of the first passage that developed tumors died accidentally the neoplasm would have been considered as nontransplantable.

It is worth noting that hemorrhagic lesions did not appear until the eighth passage, 6 months after the line was started, when free virus was abundant in the tumors, as observed with the Rous sarcoma. With the latter it took 15 months to reach the eighth passage, but adult chickens were used (11, 13).

Study of chicken tumor D 1 is still in progress, special attention being paid to the nature of the necrotic lesions in the liver.

Chicken Tumor V 1

Description and behavior on transplantation.—A normal Plymouth Rock hen was purchased at the age of 12 months and kept in the general animal room. In February, 1945, the bird died at the age of 18 months and the autopsy disclosed the presence of a tumor 10 cm. in diameter in the ovary. The growth consisted of many nodules in a grape-like arrangement, some composed of a very viscid tumor tissue, others just sacs of blood. The derivation of these nodules from normal ova could clearly be observed. A large blood clot surrounded the neoplasm. The mesentery of the adjacent intestine was studded with small metastatic tumors.

As is shown in Fig. 5, the tumor grew well at once but some regressions occurred later. It is now at the eighth passage, 7 months after the first transplant. The tumors grew moderately fast and retained the gross characteristics of the original tumor, but the growths themselves were not hemorrhagic. Plymouth Rock, Rhode Island Reds and their hybrids were used, all with the same success.

Generalized lesions.—Metastases appeared from the first passage. Those in the adult chicken occurred in the ovary and were hemorrhagic, thus reproducing the gross features of the original tumor. Those occurring in chicks from the first or other passages were mostly located in the liver and in the lungs.

Hemorrhagic lesions also developed at the first passage, this being the only tumor where such event was observed. They were present mostly in the liver, spleen and lungs, the organs often being studded with blebs.

Effect of cell-free preparations.—Filtrates tested at the fifth and seventh passages gave positive results in 10 of the 19 chicks injected.
Histological data.—The original tumor was a rather loose fibromyxosarcoma consisting of bundles of rather small cells, generally fusiform, but sometimes round and rarely in mitosis. Some giant cells and nodules of infiltrating round cells were observed. This picture was reproduced throughout the transplants with the only difference that the tumor became more dense and was frequently arranged in packed whorls. Giant cells were constantly present in both the primary tumor and the spread neoplastic and hemorrhagic lesions, even at the first passage. This makes it probable that free virus would have been found in the tumors of the first passage and even in the original tumor if it had been looked for. Indeed, the tumor behaved from the beginning as others did after several transfers. Yet one can rule out the possibility of a contamination—an event never observed in chicken tumors—since the tumor showed very definite morphological features which easily distinguished it from other chicken tumors. A more detailed study of this neoplasm is now under way.

**Chicken Tumor C**

Description and behavior on transplantation.—A Rhode Island Red hen, from 5 to 10 months of age, was brought to our laboratory in March, 1942, bearing a tumor 9 x 4 x 4 cm. in size in the lower part of the breast. Two much smaller tumors were found attached to the breast bone and in the skin around the anus. The bird was killed and the autopsy dis-
closed in the abdomen an extension of the primary
tumor (the abdominal tumor measured $8 \times 4 \times 4$
\text{cm.}) plus two metastases in the liver, another in a ves-
sel around the spleen, and still another in the thoracic
wall compressing the lung. All tumors were extremely
firm, generally non-viscid, devoid of necrosis, and
days after inoculation of cells and death occurred in a
few weeks. In adult chickens growth was very slow
and death did not occur until sometime more than 1
year after inoculation when the tumor had reached an
enormous size. The incidence of takes was 82 per cent
if tumors from young hosts were used, but only 38

![Diagram of Chicken Tumor C](image)

**Fig. 6**—Diagrammatic representation of chicken tumor C passages.

showed cavities full of a yellowish serous fluid. They
were fibrosarcomas.

The results of transplantation during the first 7 pas-
sages are shown in Fig. 6. The adult chicken and some
chicks of the first passage were Plymouth Rocks while
the pullets were Rhode Island Reds. Growth was
obtained in all of these. In the course of almost 4 years
the tumor has been carried through 20 passages, in
Rhode Island Reds, Plymouth Rock chickens, or their
hybrids. In chicks, growth was manifest from a few
per cent with tumors from older hosts. The gross
characteristics of the tumor in the original host were
reproduced in the passages.

**Generalized lesions.**—Metastases were observed in
only 5 per cent of the chickens showing tumors and
never were widespread. They were located in the
liver and lung and in one case in intestines and ovary.
They showed the same gross traits as the primary
tumors. Hemorrhagic lesions were never observed.

**Effects of cell-free preparations.**—Filtrates were
tested at the second, sixth, eighth, ninth, 16th, and 18th passages with negative results and at the seventh, 15th, 17th, and 19th passages with positive results. There were indications that filtrates from tumors grown in young hosts were more effective than those grown in old ones. The filtrates that gave positive results were far from being very active, since only 9 of the 28 chicks inoculated developed tumors and results were far from being very active, since only 9 of the 28 chicks inoculated developed tumors and these were solitary in most cases even when the filtrate was injected intravenously. The organs affected in the latter case were the liver and spleen.

Desiccates 48 days old were tried once at the seventh passage on 2 chicks with positive results. The tumors induced by both filtrates and desiccates were of the same morphology as those induced by cells.

**Histological data.**—The original tumor was a very collagenous fibrosarcoma consisting of bundles of very attenuated fibroblasts densely packed and with rare mitoses. Some other tumors were frankly fibromatous. This picture was faithfully reproduced throughout the passages in both primary tumors and metastases. Growth was sometimes infiltrating.

**Summary and comments.**—The tumor was entirely different from those of the Rous sarcoma type. It was a very firm, slowly growing tumor specially in adult hosts, metastasizing but rarely, and never inducing hemorrhagic lesions. Paralleling this relative benignity free virus was found only in occasional tumors. The tumor was well adapted to transplantation through chickens from the very beginning and it grew steadily in them regardless of their age. In its histology and behavior it is much like Mill Hill 1 of Begg (1), but contrary to the latter, which became more malignant at the seventh passage, chicken tumor C has kept its initial characteristics throughout 20 passages.

**DISCUSSION**

It is evident from our studies and those of others that chicken tumors may be classified according to certain common properties. However, differences do occur that show that these growths are not identical. The situation is very much like that observed with the several tumor strains obtained experimentally by adapting the Rous virus to foreign species. It was shown in the latter case that the plurality of strains involved variation of the tumor virus in much the same way as inflammatory viruses may vary (5, 6).

The fast growing chicken tumors E and V1 behaved as if they were adapted to transplantation through chickens from the first passages and the same was true of the slowly growing tumor C and of the osteochondrosarcoma of Rous and his associates (12) and sarcomas Mill Hill 1 and 14 (1, 9). On the other hand, fast growing tumors B and D 1 did not become fully adapted to transplantation to chickens until carried through a number of passages, and the same was true for chicken tumors 1, 18 and 43 of Rous and his group, and many other tumors. Therefore, speed of growth is not an indication of adaptability to transplantation.

On trying to correlate the presence and abundance of a free virus active for chickens with the behavior of the tumor one notes that in tumor E, and probably in V1, free virus was present in the original tumors and in the tumors of the first few passages. On the other hand, in tumors B and D 1 free virus, active for chickens, was scanty or absent before the tumor became adapted to transplantation, but plentiful later. The same is probably true for the tumors studied by others in which adaptation was not achieved until after several passages. In slowly growing tumor C, virus was demonstrated with irregularity throughout transplantation and the same was probably true for the analogous tumors described by others.

In our fast growing tumors the presence of free virus in a given passage was accompanied by the appearance of hemorrhagic disease, although in the case of tumor E there might have been a delay in the appearance of these lesions. The virus of slow-growing tumor C never induced blebs but in our experience the virus of the rapid growing Mill Hill 2 endothelioma of Begg did not induce hemorrhagic disease either, despite its affinity for the vascular system (4). With rare exceptions hemorrhagic disease was only induced in young hosts, as was the case with the Rous and Fujinami viruses. That young hosts are more susceptible than are old ones has been fully shown with our tumors, and some of the lines would probably have been lost had not chicks and pullets been employed. Another factor of importance in maintaining the tumor lines is the stability of the virus in desiccates. That of tumor E was very stable; that of tumor B was not.

The breed of the inoculated chickens played no part in determining the results in the transplantation of our tumors and of the tumors studied in this respect by others. Moreover, 4 of our tumors grew well when transplanted into ducklings. However, that by genetic selection it is possible to develop lines of chickens resistant to transplantable chicken sarcomas is shown by the work of Cole (3) and Carr (2).

**SUMMARY**

Five sarcomas spontaneously developing in chickens have been carried through this species in a number of passages by inoculation of cells, and they may be considered as indefinitely transplantable. Four of them were found in chickens of from 5 to 10 months of age; the fifth, in a chicken 18 months old. All had induced metastases in the original host. A causative virus has
been demonstrated in each of the tumors, in one case in the original growth itself.

In 4 of the lines hemorrhagic disease developed at the same time that free virus was demonstrated in cell-free preparations. In the fifth tumor, a slow-growing fibrosarcoma, the virus never induced hemorrhagic lesions although not infrequently metastases were present. Although the tumors can be classified as to certain common properties, each of them has typical features that justify the statement that no two of them are identical. Each of them raises different problems, which are discussed.

REFERENCES

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