TRANSPLANTABLE LYMPHOSARCOMA IN MICE.¹

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Among the 100 tumors that have been induced in this laboratory during the past year by the injection of 1: 2: 5: 6-dibenzanthracene into mice were three lymphosarcomata. Lesions of the lymphatic system, lymphoid leukemia and lymphosarcoma have not occurred as a genetic factor in the untreated mice of the pure inbred strains used, although apparently such strains are not uncommon or difficult to obtain by selective breeding. Slye, Korteweg, Mercier and Gosselin, MacDowell and Richter, Dobrovolskaia-Zavadskai'a, Furth and Strumia, and Furth, Seibold and Rathbone have described strains of mice with a genetic tendency to develop lymphadenosis.

Furth, Seibold and Rathbone (1933), in studies on five lymphatic neoplasms occurring in the mice of their strains, have reviewed the literature on lymphadenosis in mice. The results of our own investigations add little to the knowledge of diseases of the lymphatic system that has already been acquired through previous investigations (Slye, Richter and MacDowell, Potter and Richter, Furth, Seibold and Rathbone, Furth and Strumia, Mercier and Gosselin, Korteweg, Krebs and Faber), but since the three lymphosarcomata were induced tumors that arose at the site of an injection of dibenzanthracene and were studied as transplanted sarcomata and not as blood diseases, it is believed that a description of them is important.

The first of these tumors was discovered in a mouse of the C3H strain that had been injected 75 days previously. Owing to the illness of the animal, the cyst which had formed around the injected oil and dibenzanthracene was removed and transplanted into a normal mouse of the same strain. Thirty-two days later the implanted cyst had grown into a large tumor which, when studied in tissue cultures and in stained preparations, proved to be composed of lymphocytes. The number of this tumor was 172 and it has been transplanted through twelve generations into C3H strain mice during a period of 152 days. In every instance the transplanted tumor retained the characteristics of the primary lymphosarcoma.

Tiny fragments of the tumor transplanted subcutaneously into normal young C3H strain mice grew to the size used for transplantation, 25 \times 15 \times 5 \text{ mm.}, within two weeks. From that time on the tumors rapidly increased in size (40 \times 20 \times 5 \text{ mm.}) and led to the death of the host about a week later. The tumor proved to be strain specific; while it grew in every one of the Bar Harbor C3H strain mice into which it was transplanted, it failed to grow when implanted into mice of the A, BA or C₃7 strains.

Macroscopically the tumor had a peculiar glistening appearance, quite unlike that of the majority of induced sarcomata. It was composed of rather

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soft, pearly gray material, and when implanted subcutaneously it proliferated through the subdermal and subcutaneous tissue of the entire side of the mouse, instead of growing out as a lump into the space between the skin and body wall, as is usual with transplanted tumors. Its appearance may be roughly described as resembling that of an oyster drawn under the transparent layer of the subcutaneous tissue of the skin (Fig. 1).

**Fig. 1. C3H Strain Mouse with a Seventeen-Day Growth of Lymphosarcoma 172 in the 4th Generation, Transplanted Subcutaneously on Each Side**

The tumors extend through the subdermal and subcutaneous layers of the entire two sides. The enlarged axillary and inguinal lymph nodes can be seen incorporated in the tumor. The spleen is enlarged and extends posteriorly.

In the animals bearing the transplanted tumors the adjacent lymph nodes of the axilla and groin enlarged and became more or less incorporated in the neoplasm (Fig. 1). The spleen, which in normal mice weighs from 90 to 150 mg., increased in size as the tumor grew until it attained a weight of 300 to 600 mg., and in a few instances as much as 1000 mg.

The blood of the mice bearing the transplanted lymphosarcoma did not exhibit the neutrophilia characteristic of the majority of transplanted dibenzanthracene tumors (Lewis, 1937). On the other hand, the blood picture was not that of a lymphatic leukemia. It was difficult to identify the malignant
lymphocytes in the stained spreads of peripheral blood, although they must have been present, since normal mice injected subcutaneously with 0.3 to 0.5 c.c. of whole blood drawn from the heart of a mouse bearing the lymphosarcoma later developed tumors at the injection site. The tumors that originated from the implanted blood were indistinguishable from those arising from transplanted fragments of the tumor itself.

![Image of stained section of the original induced lymphosarcoma showing invasion of the muscles by the malignant lymphocytes.](image)

**Fig. 2. Stained Section of the Original Induced Lymphosarcoma 172 Showing Invasion of the Muscles by the Malignant Lymphocytes**

The malignant lymphocytes were present in great numbers in the spleen and in the lymph nodes of the axilla and groin, and to a lesser extent in the other organs. Fragments of spleen and of axillary and inguinal lymph nodes, implanted into normal mice, brought about tumors in about the same length of time as did pieces of tumor. The tumors resulting from implanted fragments of the lumbar lymph node, kidney, lungs and liver developed more slowly, but eventually reached the same size as the neoplasms that arose from pieces of the tumor. This was not a virus tumor, for although it could be passed from many tissues of the host, the resulting tumor growth proved to be dependent upon the presence of malignant cells in the implant. Injections of plasma from the blood of mice bearing the tumor and of the supernatant fluid from centrifugalized extracts of tumor did not result in the growth of tumors, as occurred when the plasma and cell-free extracts were used as the tumor-producing agent in studies on chicken sarcoma No. 1 (Lewis and Andervont, 1926).

Stained preparations of the primary tumor and of the transplanted neoplasms showed that, regardless of whether they arose from implanted pieces of spleen, blood, lymph node, or tumor, they were composed of closely packed lymphocytes that invaded the neighboring subcutaneous tissue, body fat, and muscles.

In tissue cultures of the tumor the stroma was greatly stimulated to growth; the multiplication and outgrowth of stroma cells that took place within twenty-four hours were as extensive as were reached after four days growth in cultures of normal lymph nodes (Figs. 3, 4 and 5). On the other
The monocyte is larger than the lymphocytes, and contains in the upper right part a group of stained neutral red granules.

hand, the rapid migration and outpouring of lymphoblasts and lymphocytes that take place within two to three hours in cultures of normal lymph nodes did not occur in cultures of the lymphosarcoma; there were noted, instead, abundant multiplication (by mitosis) and outgrowth of lymphocytes along with that of stroma cells. In appearance and locomotion the malignant lymphoid cells resembled greatly enlarged lymphocytes rather than lymphoblasts. A comparison of the malignant lymphocytes in growths from the lymphosarcoma with lymphocytes in growths of a normal lymph node showed that the former were considerably larger than the latter (Figs. 3 and 4).

Permanent stained preparations of the tissues of the mouse in which the primary tumor developed, i.e., the cyst-bearing mouse, disclosed extensive damage to all the organs. Areas of invading lymphocytes were present in the kidneys, lungs, liver, and spleen. The spleen was only slightly enlarged, weighing 200 mg. A study of the organs of the hosts bearing the transplanted tumors showed that in most instances only one or two small areas of lymphocytes were present in the lungs and kidneys and small scattered areas of infiltrating leukocytes were noted in the liver. On the other hand, the spleens of these animals had become greatly enlarged and contained large areas of actively multiplying lymphocytes. In a few instances the splenic pulp had been largely replaced by tumor tissue. The lymph nodes in the neighborhood of the tumor were hypertrophied and the normal lymphoid tissue of these glands was extensively invaded by tumor tissue. The lymph nodes of the peritoneal and pleural cavities, while in some instances slightly enlarged, retained their normal histologic structure.

After tumor No. 172 was discovered, two other lymphosarcomata were found. One of these (No. 226) arose in a C3H strain mouse that had received a small amount of dibenzanthracene into the pleural cavity 115 days pre-
These lymphocytes are larger than normal ones. The upper right lymphocyte in the upper picture is undergoing mitotic division.

The lower picture shows the same cells as the upper, eleven minutes later. The dividing cell has completed division and the cell below it is now in early prophase. The lymphocytes have changed their shape and position but the stroma cells are about the same. The black granules shown in the stroma cells and the dividing lymphocyte are highly refractive fat globules.

Previously. This tumor developed from the thymus gland and filled the upper part of the pleural cavity. It extended into the lungs along the trachea and bronchi and into the auricles of the heart, almost destroying the muscular structure of the auricles. It grew as a thin layer over the entire surface of the ventricle, between the epicardium and the muscles, and in some regions
small areas of lymphosarcoma cells extended into and between the muscle fibers.

The mouse died before the tumor was discovered, so it was not transplanted. Autopsy showed that about two-thirds of the lung tissue was filled with tumor cells. There were some areas of lymphoid tissue in the liver and kidneys. The spleen and lymph nodes were not enlarged. The pulp of the spleen was hemorrhagic and partly replaced by lymphoid tissue.

A third lymphosarcoma developed from the lymph node at the site of the dibenzanthracene injection in the right axilla of a BA strain mouse and spread through the muscles and subcutaneous tissue of that region.

This mouse had received two injections of dibenzanthracene in olive oil 237 days previously. One injection made into the right axilla resulted in the lymphosarcoma (No. 233), the other made on the back at the base of the tail gave rise to a large spindle-cell sarcoma (No. 234). Autopsy of this mouse showed areas of lymphoid cells in the lungs, liver, spleen, and kidneys. There were also a few small areas of infiltrating myeloid cells in the liver and some increase in the number of polymorphonuclear cells present in the blood stream. The myeloid cells were probably due to the presence of sarcoma No. 234, as such myeloid changes are frequently found in mice bearing tumors induced by means of dibenzanthracene.

**DISCUSSION**

Furth and Strumia (1931) and Seibold, Rathbone and Furth (1932) studied two types of lymphoid diseases in mice, and later Furth, Seibold and Rathbone (1933) studied five lymphatic lesions in mice; one a lymphatic leukemia, one an aleukemic lymphomatosis with tumor formation, two subleukemic lymphomatoses without tumors, and one a systemic lymphomatosis without tumors that produced large growths when implanted subcutaneously into normal mice. When the cells of the subcutaneous tumors were injected intravenously into healthy mice they brought about lymphatic leukemia in the hosts. These investigators, therefore, consider the original lesions as identical processes. In our studies on the induced lymphosarcomata comparable experiments were not carried out, and it is therefore impossible to surmise whether intravenous injection of the malignant cells of tumors Nos. 172, 226 and 233 would have brought about a lymphatic leukemia.

The finding that the malignant cell of the induced lymphosarcomata was an enlarged lymphocyte agrees with the observation of Furth, Seibold and Rathbone, who concluded that the malignant cell in each of their five strains was a lymphocyte and not a lymphoblast. These investigators also found that the disease could be transmitted only by material containing viable lymphocytes. The fact that some of the lymphatic neoplasms described by these observers were transplantable into more than one strain of mice suggests that their strain may have been less inbred (brother-to-sister matings) than those used by MacDowell and Richter and by ourselves.

Krebs and Faber (1932) studied the cells of a transplanted mouse lymphosarcoma in tissue culture. These observers noted the active proliferation of stroma cells, lymphocytes, and large monocytes. They suggest a possible
relationship between the round-cell and spindle-cell sarcoma. In our cultures of the induced lymphosarcoma the growth of the stroma cells, although more rapid and luxuriant, was quite like that of the stroma of normal lymph nodes growing in cultures, and not like that of the spindle-shaped tumor cells growing in cultures of sarcomata. On the other hand, the lymphoid cells that grew in cultures of the lymphosarcoma were distinctly different from those in cultures of normal lymph nodes.

The systemic effect of a lymphosarcoma is quite different from that of a spindle-cell sarcoma. The former is accompanied by an increase in lymphoid cells and invasion of the organs by these cells, while the latter is characterized by an increase in number and invasion by the myeloid cells. In a mouse bearing a lymphosarcoma and also a spindle-cell sarcoma, such as the host of tumor 233 and tumor 234, there takes place some increase of myeloid cells in the peripheral blood, and in those organs largely invaded by lymphoid cells there are also to be found areas of infiltrating myeloid cells.

In our experiments it was evident that the usual drainage from a region injected with dibenzanthracene without direct injury to the lymphoid tissue did not result in the development of malignant lymphoid tissue. Over 250 spindle-cell tumors have developed in the axillae of mice injected at this site with dibenzanthracene, without causing lesions of the lymphatic system. A number of the enlarged axillary lymph nodes adjacent to spindle-cell sarcomata were implanted into healthy mice of the same strain, but so far none of them has resulted in a tumor.

On the other hand, the injection of carcinogenic material into the spleen of the mouse did not give rise to lymphosarcomata, as hoped. This was probably because in the rodents the spleen has a wider hemopoietic function than in other mammals. In rats and mice megalocaryocytes, myeloblasts and, in many instances, some myelocytes are present in the outer layers of the spleens. Barnes and Furth describe a transplantable leukemia that was induced in the spleen of a mouse by the injection into it of benzpyrene. These investigators found that the malignant cells in this lesion were not typically lymphoid, myeloid, or monocytic in type. The cell they describe seems to resemble the small megalocaryocyte frequently observed in areas of infiltrating leucocytes in the livers of mice injected intraperitoneally with dibenzanthracene dissolved in olive oil. On the other hand, the cell shown in certain of their figures resembles the large polymorphonuclear cell, designated as a giant neutrophil by Musser and Wintrobe, and described by Parsons (1935, 1936) and by Lewis (1937, a, b) as present in the severe myeloid hyperplasia that develops in the host coincident with the growth of certain tumors.

**Summary**

Three induced lymphosarcomata (Carnegie 172, 226 and 233) developed in mice that were injected with 0.8 mg. 1: 2: 5: 6-dibenzanthracene dissolved in 0.4 c.c. of sterile olive oil. These tumors arose at the site of the injection 75, 115, and 237 days later respectively. They were composed of malignant lymphocytes. Two of them arose from the axillary lymph nodes and the third from the thymus.
One of the lymphosarcomata (No. 172) was transplanted through 12 generations in mice of the strain in which it originated. It was found to be 100 per cent transplantable in mice of the same strain but not transplantable in mice of other strains. The tumor was passed by implantation of whole blood or pieces of lung, liver, spleen; kidney, lymph node or tumor, but not by the implantation of blood plasma or the supernatant fluid of centrifugalized extracts of the tumor.

Note: Since this paper went to press another transplantable lymphatic tumor (C3H 284) has been studied. The tumor arose from a mediastinal lymph node, invaded the lungs and heart, and partly filled the pleural cavity. It was transplanted through many generations in mice of the C3H strain but did not grow in other strains. In some instances it exhibited characteristics of a lymphatic leukemia. The malignant cells appeared to be lymphoblasts. In some generations many blast cells were present in the blood stream of the host, while in other generations it was difficult to identify the malignant cell in the blood spreads. The tumor was transmissible by means of subcutaneous implantations of whole blood and of fragments of spleen, liver, thymus, lymph nodes, and tumor, but not by means of cell-free extracts of the tumor or by blood plasma.

Literature