THE CYTOLOGY OF THE 1,2,5,6-DIBENZANTHRACENE MOUSE TUMORS

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With the production of carcinogenic tars (10), the isolation (4) of 3,4-benzpyrene, and the synthesis of 1,2,5,6-dibenzanthracene (2) and other carcinogenic or sarcogenic chemical compounds, a new era in cancer studies was initiated. British (3, 4) and American (6) chemists have been responsible for the synthesis of most of the carcinogenic compounds. With their biologist collaborators, these chemists have tested their products biologically, and have reviewed the important literature on the chemically induced tumors up to 1938, presenting a complete and concise survey of this rapidly developing phase of the cancer problem. The cytological aspects of these chemically induced tumors, however, except those produced by tar (11), have not been studied.

A comparison of the cell physiology of the 1,2,5,6-dibenzanthracene tumors in the mouse with the better known neoplasia in these and other laboratory animals seemed to be of interest. The comparative ease with which these tumors may be transplanted made it further desirable to study their cellular behavior through a series of transplant generations to determine the effects, if any, that may be exerted by the host on the growth of the transplant. Since these chemical tumors arise de novo, it seemed of interest also to trace, if possible, certain cellular constituents, such as giant tumor cells, from transplant to transplant and to attempt a correlation of the tumor age with the appearance of these cells. The effects of filtrates of Pseudomonas tumefaciens, a tumor-producing organism of plants, on the induced mouse tumors also seemed worthy of investigation. A large part of this report is concerned with the unexpected appearance of liposarcoma tissue in the 7th transplant generation of a fibrosarcoma originally induced with 1,2,5,6-dibenzanthracene. In another series of transplants through 21 passages of a 1,2,5,6-dibenzanthracene tumor, no change in the nature of the neoplastic tissue was observed.

McDonald and Woodhouse (12) studied the histology of transplants from a strain of 1,2,5,6-dibenzanthracene spindle-cell mouse sarcoma for 85 generations extending over a period of thirty-seven months. Their purpose was to determine the variation in the histologic structure of homoiografts, the spread of the tumor cells, and the nature of the vascular reaction of the host. The initial tumors were induced with 0.5 per cent of the hydrocarbon dissolved in hog lard. Succeeding injections of 0.5 c.c. of the mixture were made at monthly intervals. Tumors developed nine to ten months after the injections. The transplants were made in a single strain of stock mice, fragments from the periphery of tumors not more than twelve days old being minced in saline and injected into the musculature of the hind leg. Animals with transplanted tumors were sacrificed after one to thirty-six days for histologic study. A large

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variety of carefully selected stains was used. The authors found little deviation in the transplants from the cell type characteristic of the original tumor. No giant cells or grossly aberrant nuclear structures were observed.

Hval (9) also studied the development of 1,2,5,6-dibenzanthracene tumors in mice. The chemical effects preceding malignant growth were observed in 235 animals. Lard was used as a medium and 2 mg. of the agent were injected subcutaneously. In 40 of the mice three injections were given in twenty-five days. In another series neosalvarsan and lard were injected and the tissue reactions were studied after 120 days. Some of the animals were intravitaly stained with trypan blue.

One injection of dibenzanthracene, Hval states, induced vascular reactions similar to those produced by neosalvarsan. Telangiectasis appeared which lasted for more than six weeks but finally disappeared. The tissue reactions differed from those induced by the lard or neosalvarsan. They were characterized by connective-tissue degeneration, infiltration of macrophages, fibroblasts, and many giant cells of foreign-body type. Degeneration of the tissue resulted in the formation of a cavity lined with syncytial cells and surrounded by fibrous connective tissue. With repeated injections of dibenzanthracene telangiectasia persisted until sarcomas developed. The latter arose from small foci of atypical cells resembling macrophages and fibroblasts of mesenchymal origin. Several types of sarcomata are described by Hval, including fibrosarcoma, fibroliposarcoma, lymphosarcoma, and rhabdomyosarcoma.

Haagensen and Krehbiel (7) studied the effect in mice, rats, and rabbits of 1,2,5,6-dibenzanthracene injected subcutaneously suspended in paraffin. One gram of dibenzanthracene was used in 100 gm. of paraffin, m.p. 48° C. Fifty-two tumors were induced in animals which survived more than four months, of which 14 occurred in 14 mice. These tumors appeared in 132 up to 275 days, or in an average period of six months. Thirty-six tumors were obtained in 26 rats and 2 tumors in 2 rabbits. The length of time required for tumor production in the rat varied from 214 to 500 days, in the rabbit from 308 to 379 days. Of the 52 tumors, 11 were fibrosarcomas, 10 rhabdomyosarcomas, 5 leiomyosarcomas, 3 squamous-cell carcinomas, and a large number were unclassified. The fibrosarcomas showed fibroblastic origin.

In a later paper (8) Haagensen and Krehbiel described liposarcomas produced by 3,4-benzpyrene in a mouse and 4 guinea-pigs. These workers claim that liposarcomata are unusual neoplasms, since fat is an inactive tissue with little growth capacity. Hval (9) reported one fibroliposarcoma among 18 tumors. The studies reported below seem to confirm these observations. Since the primary purpose of this study was to determine the cytological behavior of a given tumor type in subsequent transplants, precautions were taken to include the actively growing tumor tissue in the grafts. Yet it has been noted here that lipomatous tissue may, under certain technical conditions, appear only in later tumor transplants and not be detected in the primary tumor.

In studying the effects of applications of 0.3 per cent cholanthrene and methylcholanthrene in benzene, Page (13) observed an increase in size of the cells, nuclei, and nucleoli, as evidence of cancerization of the treated tissues. He emphasizes especially the nucleolar changes, which he considers of sig-
significance. The applications consisted of 8 to 10 drops of the solutions, three times weekly. Carcinoma was induced by methylcholanthrene in seven weeks, while cholanthrene required approximately eighteen weeks. The solvent used alone proved ineffective.

Weil (14) attempted to produce brain tumors in rats with 1,2,5,6-dibenzanthracene, with methylcholanthrene dissolved in lard or cholesterol, and with styryl 430. He obtained benign growths with lard injections as well as with a mixture of lard with 1,2,5,6-dibenzanthracene. These granulomatous reactions consisted of small masses characterized by slit-like empty spaces lined by elongated multinucleate cells. An epidermoid brain tumor was found in one rat seven months and a half after injection. This seems to have been induced by 1 c.c. of 1,2,5,6-dibenzanthracene lard suspension.

**Experimental Methods and Results**

The animals used in these experiments were of an inbred stock of white mice that has not been known to develop spontaneous tumors in the past twenty years. Ten mice, males and females, weighing 15 gm. were injected (Nov. 20, 1935) subcutaneously in the interscapular region with 0.25 c.c. of a solution of 0.2 gm. of 1,2,5,6-dibenzanthracene (yellow) in 6 c.c. of olive oil. The hydrocarbon was rubbed up in the oil so that the suspended crystals passed through a fine hypodermic needle. This was repeated before each injection to insure an equal distribution of the material through the medium. Similar injections were made, in approximately the same area, weekly, for as many as eleven weeks in some animals. The animals were sacrificed at various intervals beginning three months after the first injection. Parts of the suspected tumor tissue were transplanted as described below. The remaining tissue about the injected area, together with all the parenchymatous organs, was prepared for microscopic examination.

In another series of 10 animals a single injection was made consisting of 0.1 c.c. of a mixture of 0.2 gm. of 1,2,5,6-dibenzanthracene in 6 c.c. of olive oil. In the two series of animals studied 7 tumors developed. All were transplanted at least two or three times. Tumor No. 1 and tumor No. 28 were carried through 14 and 21 passages, respectively, for intensive cytologic study.

The tumors were aseptically removed. A medium slice was excised and fixed in Bouin’s solution for the purpose of a general histological survey of the tissue. The periphery was then separated from the rest of the tumor and cut into fragments, approximately 1 mm. cubes. These were thoroughly mixed and kept moist by the addition of a drop or two of sterile physiological salt solution. Two to three fragments of this tissue selected at random were injected by the trocar method into 6 to 12 young mice, five to six weeks old, the inoculations being made in the right flank. The remaining fragments were fixed in Meves’ agent or Flemming’s medium solution, for cytological study. The tissues were imbedded in paraffin and sectioned serially 5.0 to 7.5 μ in thickness. Heidenhain’s iron haematoxylin and Flemming’s triple stain were principally used. Delafield’s haematoxylin, with eosin as a counter stain, was employed for survey studies.

Tumor transplants were generally made at regular intervals of eighteen to
twenty-two days. The animal with the most rapidly growing transplant was sacrificed. The tumor tissue was treated as described above for study and further transplantation into another series of mice. A microscopic study of the transplanted tumor was made. All the parenchymatous organs were fixed and studied. The remaining animals with tumors of the same transplant generation were sacrificed at various intervals after inoculation. Their tumors and organs were likewise studied microscopically. All animals showed positive takes three days after the implantation. Of these, approximately 66 per cent progressed and formed malignant tumors. The others began to regress after five to twelve days. Regressions were generally evident after the seventh or eighth day. The prolonged growth of the tumor transplants insured tumor maturity with abundant viable active peripheral tissues for transplantation. Central necrosis of these tumors was frequently observed.

Animals in which the transplant had regressed were isolated and reinoculated at a later date. These animals as a group showed about the same percentage of takes as any group of the stock mice selected for inoculation. In a small number of animals with well developed tumors a filtrate of a pure culture of *Pseudomonas tumefaciens* grown in bean broth for twenty-one days was injected subcutaneously. The animals so treated died or were sacrificed. The tumors and organs were removed and fixed for cytological study.

*Cysts and Giant Cells with Acicular Clefts:* Of 10 mice injected weekly for seven to eleven weeks with comparatively large doses of dibenzanthracene in olive oil, 3 died more than four months after the injections were begun; the others were sacrificed in the third, fifth, seventh, and tenth months. The first reaction to the injection of oil and dibenzanthracene in all animals consisted in the development of soft, pliant, definitely isolated cysts. Epilation of the injected area followed. The oil cysts increased in size, became firm, and at various periods after the third or fourth week frequently discharged some oil in which crystals of the dibenzanthracene were observed. About these open areas keratinized wart-like structures were found in 3 animals, thirteen weeks after the first injection. The injection of the chemical was discontinued when the skin began to break down. Sizable tumor masses were found in these three animals from seven to ten months after the last injection. No transplantable tumors were found in less than seven months. In animals sacrificed at three months after the injections were begun, several organized masses of foreign-body giant cells were found. These were associated with small, oval, cyst-like bodies which had apparently been filled with oil, although the fluid was lost in preparation of the tissue for sectioning. The walls of the cyst-like bodies (Fig. 1) were made up of two to three layers of foreign-body giant cells. Some of the cells showed the characteristics of typical fat cells with alveolate cytoplasm in which one or two well differentiated nuclei were present. The giant cells in the cyst walls are characterized in section by a small number of clefts, pointed at both ends. These traverse the cell so as to divide the cytoplasm into segments, some bearing no nucleus while others contain one or more nuclei. Round cells and some polymorphonucleate cells are also seen scattered in small clusters through the wall.

Associated with the cyst-like bodies are collections of foreign-body giant cells. These cells have characteristic small nuclei, arranged about the periph-
FIG. 1. SECTION OF CYST SHOWING FOREIGN-BODY GIANT CELLS WITH ACICULAR CLEFTS INDUCED IN A WHITE MOUSE BY 11 WEEKLY INJECTIONS OF 1,2,5,6-DIBENZANTHRACENE IN OLIVE OIL.
Injections begun Nov. 20, 1935; animal sacrificed Feb. 11, 1936. × 28.

ery of the cytoplasm. The nuclei are sparsely filled with chromatin gathered about the nuclear membrane. Cells considerably larger than the ordinary foreign-body giant cells are found here. They have many long spindle-shaped clefts running through them. Sections of giant cells with these clefts in their early development show them partially divided by a cleavage vacuole (Fig. 2). This cleft seems to arise near the cell membrane and to extend through the cytoplasm. In section, these cleavage vacuoles take the form of acicular spaces. They increase in number and appear to stretch the cell in a plane parallel with their long axes. The boundary of a cell containing many of these clefts is not definitely recognizable. The slits, in a single section of the cell, appear to be disoriented, but when an attempt is made to reconstruct them from serial sections, it seems that they form first through the center of the cell. Succeeding clefts cause the cytoplasm to shred and form a central lacuna, as shown in Figs. 2 and 3. Clefts are then formed along the periphery of the cell, cutting off segments of cytoplasm, nucleate or non-nucleate (Fig. 3). These strands of cytoplasm with their spindle-shaped clefts appear to arise in a single cell. The pressure exerted on the surrounding cells by these swollen giant cells makes a definite determination of this point difficult, but the unity of each cleft precludes the hypothesis that it extends through more than one
cell. The formation of the cleft is apparently due to the imbibition of oil, which as it increases in amount stretches the cytoplasm. No oil or crystals of dibenzanthracene were found in the spaces. It seems probable, however, that they are the products of oil injection. Their final stages have not been determined. Tissues bearing parts of cysts have been transplanted without success. Giant cells with the characteristic clefts were not found in sections of tumors nor in the surrounding tissues of animals sacrificed seven months after the injections began. The figures reproduced here are similar to those shown by Weil (14), and designated by him as granulomas induced by lard.

In another study a single injection of 0.1 c.c. of a suspension of 0.2 gm. of 1,2,5,6-dibenzanthracene in 6 c.c. of olive oil was made into the interscapular region of 10 mice. These injections induced tumors in animals which survived five months, and the tumors developed into sizable masses in seven to ten months. The conception, which is becoming prevalent, that repeated injections of chemical irritants are necessary to induce tumor formation, does not seem to be sustained by these observations.

Of the 7 tumors which developed in these studies, all were transplanted for two or three tumor generations; only tumor No. 1 and tumor No. 28 were transplanted regularly through many transplant generations—14 and 22 respectively, and from material thus obtained the cellular studies were made. All the tumors were fibrosarcomas with the exception of one possible leio-
myosarcoma. All the tumor giant cells which appeared in these studies showed aberrant chromosomal behavior in division. Cells of normal size also appeared in large numbers. They divided normally and showed the normal chromosome counts for somatic tissue of the mouse.

**Tumor No. 1**

Tumor No. 1 was induced in a mouse receiving eight injections of 1,2,5,6-dibenzanthracene in olive oil. Twenty-two weeks after the last injection, a tumor approximately the size of a large hickory nut was discovered in the interscapular region. The tumor was slightly adherent to the muscles, but readily separable from the overlying skin. A median slice, 5 to 7 mm. in thickness, was removed, showing a clear solid mass of grayish-white tissue free from necrosis. A section was prepared as described above for microscopic examination. The periphery of the tumor was aseptically removed, fragmented, mixed with a small quantity of saline, and injected by trocar into 8 white mice of the inbred tumor-free stock. The unused fragments of tissue were fixed for cytological study. This tissue, which is described below, was propagated through 14 generations, after which it failed to take in three separate trials with 25 young mice. Up to this time the implantations took in 50 to 100 per cent of the animals inoculated. The average for the 14 generations was 64 per cent. The transplantations were made at intervals of twenty to twenty-two days. Approximately the same results were obtained by McDonald and Woodhouse (12).
Fig. 6. Tumor No. 1: Multinucleate Giant Cells in Prophase Stage, from a Fourth Transplant Generation, Sixty-six Days after Inoculation. X 600

Fig. 7. Tumor No. 1: Giant Cells in Division, Showing Chromosomes in Polar View, from a Tumor of the Third Transplant Generation, Thirty-four Days after Implantation. X 600

The histology of the primary tumor No. 1 is that of a fibrosarcoma, as shown in Fig. 4. The tissue is made up, characteristically, of fibroblasts with only a few round cells and a sparse distribution of leukocytes. Sections of the second transplant generation show no variation in early stages, i.e., about twenty-one days after the inoculations, but in mice sacrificed fifty-five days (Fig. 5) after implantation the tumor tissue is characterized by multinucleate tumor giant cells. Large binucleate cells are present, and occasionally large cells with pale-staining cytoplasm can be seen, though these are not characteristic. Monocytes and leukocytes are present in small numbers. Normal division figures in this tissue indicate apparently normal behavior. The large cells in division are quite abundant, with aberrant chromosome number exceeding a tetraploid count.

In the compact peripheral tissue of this tumor, the cells vary in size. The cytoplasm in all cells appears uniformly of a fine granular reticulate structure, which stains beautifully with the orange G of Flemming's triple stain. The nuclei are crudely globular in shape with two or three nucleoli which frequently show the presence of adherent chromatin granules. The nuclear structure consists of a somewhat coarse linin network with accumulation of faintly staining chromatin material about the nuclear membrane.

In large multinucleate giant cells such as the one shown in Fig. 6 (taken from a section of a tumor in the fourth transplant generation), the cytoplasm tends to be delicately granular with many peripheral vacuoles. The nuclei are large with numerous nucleoli and smaller clusters of chromatin granules staining with gentian violet. These cells are quite common in old tumors; the section shown here was taken from a tumor sixty-six days after implantation of the inoculum. In Fig. 7 a giant cell is shown, taken from a third generation tumor transplant thirty-four days after inoculation. Here a large
number of chromosomes may be counted in a single large mass in polar view. A smaller mass of chromosomes, out of focus in the photograph, contains more than 40 chromosome-like bodies. The chromosomes lie free in the nucleoplasm with no indication of spindle fibers or nuclear membrane. This figure represents one of a series of four sections of this cell. A similar picture has been observed in mouse tumor 180 and in the mouse tar tumor (11). The cytoplasm consists of a finely granular reticulate structure. Small vacuoles and deeply stained gentian violet granular bodies, which are probably extruded chromatic substance, are present.

Apparently normal division figures are found in which the thickened rod-shaped chromosomes appear in metaphase. The spindle usually forms a bipolar structure with single centrosomes. Occasionally multipolar spindles, with two to three well defined centrosomes, are seen in the same plane. Fig. 8 is a photograph of a cell in metaphase stage. The surrounding cells are of the fibroblast type. This section was made from a tumor in the sixth transplant generation, twenty-one days after the implantation of the tissue. Aberrant karyokinetic phenomena may be seen in young transplants. Fig. 9 shows a tripolar spindle in early telophase stage with lagging chromosomes. Small cells are seen in this photograph with segmented spiremes. This section was made from a tumor of the third transplant generation, thirty-four days after inoculation.

It is clear from the figures presented here that the cellular behavior in this tumor is identical with that observed in malignant growths of man, rat, mouse, and bird. It appears, in general, that the older the tumor the more abundant are the aberrant cellular structures. These abnormal cells seem not to be directly related to disintegration, although they may occur simultaneously with necrosis. These observations and those made on other animal tumors (11) seem to indicate that the aberrant nuclear phenomena in cancer tissue are an expression of tumor maturity rather than the result of tumor degeneration.

**Late Appearance of Liposarcoma:** As mentioned above, tumor No. 1, a fibrosarcoma, was transplanted for 14 tumor generations. Without making serial sections of a complete tumor of this size, it is impossible to determine accurately all the cell types that may be present. The cellular structures of the common laboratory tumors are so well known that only a section or two of a transplant is necessary to reaffirm its cellular nature. With these large tumors induced de novo by chemical means, more careful exploration is necessary. In view of these facts the tumor tissue used for transplants was taken from the periphery of the tumor, only two to three fragments were inoculated in each of 6 to 12 mice, and the remaining portions were sectioned serially. A cross section of the tumor 5 mm. to 7 mm. thick was also sectioned serially, selected portions being mounted and stained for microscopic examination. In such preparations of tumor No. 1 no evidence of cell types other than the fibroblast cells shown in Fig. 4 was observed in the first six transplant generations, though tumors from 30 mice were studied. In the seventh transplant generation, a new type of cell appeared among the fibroblasts. This cell (Fig. 10) was rounded, containing a single, centrally placed nucleus surrounded by foam-like or alveolate cytoplasm. It was apparently carried over in the next
FIG. 8. Tumor No. 1: Giant cell in sixth transplant generation, twenty-one days after implantation, showing division in metaphase stage. × 900

FIG. 9. Tumor No. 1: Giant cell in tripolar division, showing early telophase stage, from third transplant generation fixed thirty-four days after inoculation. × 900
generation of transplants. Figs. 11, 12, and 13 show a portion of a section of the tumor under different magnifications in which all the cells are of this alveolate or fat-cell type. These photographs were made from a section taken twenty-two days after the implantation of the eighth tumor generation. The cells are uninucleate or binucleate (Fig. 12) with a densely staining cell membrane. The cytoplasm had a distinct foam-like appearance with coarse granular bodies outlining the coarser cytoplasmic structures, and a delicate reticulum of fine granules filling them. The cells stain very lightly as contrasted with the fibrosarcoma cells. The nuclei are well differentiated, each bearing a single nucleolus which stains a ruby red with safranin, while the chromatin takes a gentian-violet color with Flemming's triple stain. Leukocytes and round cells seem to be more abundant in the photograph made under low magnification (Fig. 11) than in those parts of the tissue selected for high-power magnification. Occasional spindle cells, deeply stained, are seen among the fat cells. The delicately staining fat tissue is in sharp contrast with the more densely stained tissue of the fibrosarcoma with which it was associated in this tumor.

Division figures are not abundant, but cells occur with a tetraploid chromosome number, having apparently arisen from the simultaneous division of a binucleate cell. In Fig. 14 chromosomes in metaphase stage are shown. The cytoplasm in this cell is densely granular, yet its alveolate structure is evident toward the periphery. Here the chromosomes are arranged in a uniform band on a delicate spindle. This photograph represents one of three sections of this cell. The surrounding cells are composed characteristically of the foamy cytoplasm. This tissue seems to be similar, if not identical, with that of the liposarcoma induced by 3,4-benzpyrene by Haagensen and Krehbiel (8) in the mouse.

The senior writer is not convinced that cellular transformations have occurred here, though fibroblasts are known to be transformed into fat cells. It seems from the study of subsequent transplant generations of this fatty tumor, that certain hosts favor different tissues. In these transplant generations 6 mice were inoculated with the tissue; one of them developed liposarcoma; in 3 the transplants failed to take. The other two animals died shortly after transplantation. On the other hand, we may be dealing with a fortuitous transplant. A section of the transplant in the ninth or succeeding generation is shown in Fig. 15. Only a few fat cells now appear scattered among the fibrosarcoma cells. This tumor was fixed twenty-one days after transplantation. In subsequent generations, including the fourteenth, no fat cells appeared. The tumor showed poor development and failed to grow, as indicated above. It thus seems that few hosts favor the liposarcoma type of tissue. This type of tumor could be used to great advantage in the study of the graft-host relationship.

Tumor No. 28

Mice receiving a single injection of approximately 3 mg. of 1,2,5,6-dibenzanthracene in olive oil produced tumors from five to ten months afterward. The tumors proved to be fibrosarcomas with one possible leiomyosarcoma.
FIG. 10. TUMOR NO. 1: SECTION OF SEVENTH TRANSPLANT GENERATION, SHOWING APPEARANCE OF SPARSELY SCATTERED FAT CELLS THROUGH THE FIBROSARCOMA TWENTY DAYS AFTER IMPLANTATION.  × 375

FIG. 11. TUMOR NO. 1: SECTION THROUGH EIGHTH TRANSPLANT GENERATION, SHOWING A TYPICAL LIPOSARCOMA, TWENTY-TWO DAYS AFTER IMPLANTATION.  × 187.5
FIG. 12. PART OF TUMOR SHOWN IN Fig. 11. × 375

FIG. 13. ANOTHER SECTION OF TUMOR ILLUSTRATED IN Figs. 11 AND 12, SHOWING BINUCLEATE GIANT CELLS. × 375
The mouse with tumor No. 28 was sacrificed nine months after injection. As already stated, a single injection bearing a penetrating threshold value is sufficient for tumor production. The repeated injection of the hydrocarbon in our experiments caused greater injury than a single injection but no larger number of tumors. It has been shown recently by Dobrovolskaia-Zavadskaia (5) that a single injection of 2.5 gamma of 1,2,5,6-dibenzanthracene is capable of producing tumors in mice. It has been suggested that doses as small as 1.5 gamma may possibly be similarly effective; at any rate the limit of tumor-producing power of dibenzanthracene is reached with these small quantities of the agent.

![Image](https://example.com/image.png)

**FIG. 14. FAT CELL IN METAPHASE STAGE FROM THE LIPOSARCOMA SHOWN IN FIGS. 11-13**

Note the densely stained cell membrane and dense cytoplasm in contrast with the cytoplasm of surrounding cells in resting stage. × 800.

The original tumor No. 28 was not a typical fibrosarcoma. The cell processes were short, especially in the compact area of the tumor away from the periphery. This tumor was transplanted through 22 tumor generations, with an average of 55 per cent takes. The takes varied from generation to generation from 100 per cent to as low as 16 per cent. Fig. 16 shows a section through the periphery of the twenty-first tumor transplant generation, twenty-one days after implantation. Fibroblasts form the type cells which under slightly different conditions vary somewhat morphologically but are within the range of fibrosarcoma tissue.
As stated above, all organs of the injected and implanted mice were studied microscopically. Tumors were found in the lungs of three animals. One of these, which had been given 9 weekly injections beginning Nov. 20, 1935, died on April 8, 1936. The tissues were fixed within a few minutes after death. No tumor was found in the region of the injection. One lung showed a large sarcomatous mass. Lung tumors were found in a second animal in the eleventh and in a third in the twelfth generation of tumor No. 1. These animals were sacrificed twenty-six and twenty-five days, respectively, after implantation. The tumors seemed to be extensive peribronchiolar proliferations of a sarcomatous nature. That they were of metastatic origin has not been determined. Spontaneous tumors, however, are unknown in the strain of mice which was used in these studies. No tumors were found in the other organs.

**FILTRATE EFFECTS**

The effects of filtrates of various pathogenic organisms have been studied on tumors. The absence of any effect of filtrates of the plant pathogene *Pseudomonas tumefaciens* on plant tissue suggested a study of their effects on these dibenzanthracene tumor transplants.

Six animals bearing old tumors from the tenth transplant generation of tumor No. 1 were injected with 0.5 to 1.0 c.c. of a filtrate obtained by passing...
100 c.c. of bean broth, in which the organism had been grown for twenty-one days, through a Seitz filter. The filtrate was tested bacteriologically on bean broth agar and found negative for the organism. The tumors were implanted in the right flank on Nov. 4, 1936, and injected with the filtrate in the left flank on Nov. 26, 1936. The tumors appeared discolored and hemorrhagic in eighteen hours and two of the mice died in twenty-four hours. Two animals which survived were sacrificed forty-eight hours after the injection. The remaining two were in fair condition five days after the injection. On excision

The nature of the filtrate precludes a possible specific relation to these tumors. *P. tumefaciens* is foreign to the mammalian organism and has no known carcinogenic or sarcogenic properties when injected into animals. Yet its exogenous products together with the protein content of the bean broth induce hemorrhages in these tumors similar to those described by Andervont (1) for *B. coli* in similar tumors. No regression of the well established tumors was observed in the limited number of experiments reported here. It would seem that other factors than the bacterial products are involved in this complex reaction.
A cytological study of 1,2,5,6-dibenzanthracene tumor is reported. Tumor tissue was obtained by injecting two series of mice with relatively large doses of the hydrocarbon suspended in olive oil. One series was given multiple injections and another series a single injection. Seven malignant growths developed from the two series five to ten months after injection.

Tumor No. 1 described here arose from multiple injections of 1,2,5,6-dibenzanthracene in olive oil and was transplanted through 14 transplant generations. Tumor No. 28 was induced with a single injection of the same agent and was propagated through 22 transplant generations. Tumor No. 1 was a fibrosarcoma in which isolated fat cells appeared in the seventh transplant generation. In the eighth transplant generation, the fat cell type became dominant, forming a liposarcoma. The following generations showed a regression of the liposarcoma and a return to fibrosarcoma type. Primary tumor No. 28 was microscopically a fibrosarcoma, which persisted throughout the 22 transplant generations.

These primary tumors and their transplants show normal cells which divide normally. Aberrant giant-cell types also appear. Cell divisions show abnormalities similar to those found in tumor cells of man and of the common laboratory animals. The cytological behavior of these chemically induced tumor tissues in the mouse is identical with that of other known spontaneous tumors of this animal. Cellular abnormalities of tumor tissue are interpreted as a stage in tumor development and not necessarily as associated with tumor degeneration.

Filtrates of P. tumefaciens injected into mice with well developed tumors produce hemorrhages and tumor necrosis, but regression of the tumors was not observed.

**Literature Cited**