The Transplantation of Tumors to the Brains of Heterologous Species*

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The transfer of heterologous tumor tissue to the brain was reported by Shirai in 1921 (5) and confirmed by Murphy and Sturm in 1923 (4). Despite early recognition, however, the ability of the brain to support heterologous growth has not been utilized in experimental work, nor has its application to the general problems of transplantation been explored.

One phase of a study undertaken in this laboratory to determine the factors allowing heterologous growth in the anterior chamber of the eye has been a systematic investigation of other bodily regions, including the brain, with respect to transplantation reactions. The Brown-Pearce rabbit tumor has been successfully transplanted to the subcutaneous space and testicle of the mouse (1), and several mouse tumors have been grown intramuscularly in hamsters and rats; but, in general, tumors will not survive transfer to such regions in alien hosts. In contrast, the brain has proved an excellent nidus for heterologous tissues, rivaling the anterior chamber as a growth site, and in one case providing a medium for growth after consistent failure of eye transfer.

Although in individual cases special conditions inherent in the region many act to produce differences in the incidence and rate of growth, the basic factors determining the success or failure of brain transfer appear to be identical with those concerned in anterior chamber transplantation. Adult, embryonic, and cancer tissues grow on homologous transfer, while benign tumors and precancerous tissues fail to survive, and heterologous transfer is successful only in the case of embryonic tissue and cancer. The object of the present paper is to report the heterologous growth of tumors with particular reference to those of human origin. Experiments concerned with embryonic tissues will be described at a later date.

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MATERIALS AND METHODS

The simple procedure devised for transfer to the brain allows rapid operation and is rarely associated with infection or mortality. After nembutal anesthesia and preparation of the skin by shaving and washing, a small incision is made through the scalp, exposing the upper anterior portion of the right parietal bone. A burr hole is drilled in the bone at a point equidistant between the sagittal and parietal sutures and approximately 0.5 cm. from the bregma. Metal drilling bits, as obtained in ordinary hardware stores, are adequate for this procedure. The bore of the bit should be slightly larger than that of the inoculating trocar, and it is essential that the cutting edge be sharp. Piercing of the inner table of the diploe imparts a recognizable sensation to the drilling hand, and, with short practice, the manipulation can be stopped before the underlying brain is injured. The use of a drill is not necessary when mice are employed, and a satisfactory opening can be obtained by the manual rotation of a pointed knife blade.

The tissue is introduced through the burr hole into the brain by means of a trocar. The trocar utilized in mouse transfers is an altered 20-gauge hypodermic needle, but for larger animals an anterior chamber trocar measuring 1½ mm. in diameter is employed. The locus at which the tissue is deposited apparently does not influence the incidence of takes. Care should be exercised, however, that the transplanted fragment remains in the brain substance when the trocar is withdrawn.

The operation is terminated by approximating the edges of the skin wound and sealing them with a drop of collodion.

The species employed included rabbits, guinea pigs, rats, and mice. All the mice were of the DBA strain.

RESULTS

Homologous transfer.—The transplantation of the various stock tumors carried in the laboratory was readily effected in the brains of unrelated animals of the same species. The mouse tumors included MT-8 (3); C1300, a neuroblastoma origi-
nating at the Jackson Laboratory in Bar Harbor; and MT-66, a glioblastoma obtained from Dr. Harry Zimmerman at the Montefiore Hospital in New York. The single rabbit tumor used was the Brown-Pearce carcinoma. The tumor MT-8 originated in a CBA mouse, C1300 in an A strain animal, and MT-66 in a C3H mouse. All these tumors are maintained in our colony by serial intramuscular transfer in DBA mice, and brain transfers were made in this strain. The Brown-Pearce tumor is carried by serial anterior chamber transfer in rabbits, and in homologous brain experiments the breed of the recipient was always different from that of the donor.

In all cases, takes were obtained in 100 per cent of the animals, and the tumors grew rapidly to cause death in from 1 to 4 weeks. The Brown-Pearce was the most rapidly fatal tumor, and animals rarely survived more than 9 days. MT-66 grew more slowly, and its bearers occasionally lived for as long as 4 weeks.

Histologically, the noteworthy features of growth in the brain were the abundant vascular supply and the absence of necrosis. In some cases, particularly in growths of MT-8, the vascular content of the tumor was so great as to suggest a hemangioblastoma. Supporting connective tissue stroma was extremely scanty, and, aside from thin-walled blood vessels, the tumors were almost completely parenchymatous. The size attained before death was related directly to location. Spread to the midbrain was associated with short survival and small tumors, while cerebral growths often involved the greater part of a hemisphere before death occurred.

Heterologous transfer between laboratory animals.

—The stock tumors listed above, together with the Rous chicken sarcoma, were tested for hetero-transferability to the brain, and growth was obtained in each case (Figs. 1-6).

Experiments involving the mouse tumors MT-8, MT-66, and C1300 were limited to guinea pigs. The frequency of growth was extremely high, with many transfers resulting in 100 per cent of takes, and it seems probable that the rare failures were related to errors in technic. The tumors grew rapidly and led to death in from 4 to 6 weeks. At death, the growths usually occupied the major portion of a hemisphere and were characterized by a solid medullary structure without necrosis. Occasionally, secondary growths within the brain were found resulting from rupture of the transplant into a ventricle with “seeding.” Serial transfer from brain to brain was readily effected.

The Brown-Pearce tumor was successfully transferred from the rabbit’s eye to the brains of rats, mice, and guinea pigs and has been carried by serial passage in the latter two species. The incidence of takes was uniformly high, regardless of species, and the few failures were related to infection.

Growth was rapid in the mouse and rat, and adult animals rarely survived for more than 12 days. Longer survival periods, occasionally extending to 20 days, were noted when newborn mice were used. In such cases, the intracranial pressure incident to the expanding tumor was apparently relieved by the stretching of the membranous calvarium, and large growths replacing the greater part of a hemisphere were not uncommon. In adult animals, the tumors at death averaged 0.4 cm. in diameter. On section the tumors were solid, medullary, and generally free of necrosis. Growth was invasive as well as expansive, but seeding within the brain was not observed. The tumor cells were identical with those found in the rabbit, and their presence in the alien host invoked no inflammatory reaction.

The ready transplantability of the Brown-Pearce carcinoma to the guinea pig’s brain is in sharp contrast to the behavior of the tumor in other bodily regions of this species. Despite numerous attempts in many sites, including the anterior chamber of the eye, transplantation has not been effected. Although tumor cells may persist for several weeks when transferred to the eye in contact with transplants of embryonic guinea pig lung, or when the animal is given large quantities of Vitamin C, proliferation is minimal and growth can only be detected by microscopic examination. Transfer to the brain, on the contrary, is successful in 100 per cent of cases and results in large, progressively growing tumors.

While transfer is almost invariably successful, the rate of growth in the guinea pig’s brain is remarkably slow, and animals survive for long periods of time. A mass of sufficient size to kill is attained in other species in 1 or 2 weeks, but in the guinea pig, indicative neurological signs rarely appear before the tenth week. If the animals are killed previous to this time, gross sections of the brain may fail to show the rather typical, demarcated mass resulting from the transplantation of other tumors, and the instance may be dismissed as a failure of transfer. However, after histological staining, a surprisingly large area of involvement becomes apparent. An explanation of this paradox is found on microscopic study and concerns a highly characteristic mode of growth (Fig. 7).

Early in the course of the transplant, the tumor cells invade Virchow-Robin spaces and with con-
tinued proliferation extend in many directions from the site of inoculation. There is little evidence of expansive growth, and the intervening brain substance is not altered. The tumor may extend in this manner to involve an area of 0.5 cm. in diameter without producing sufficient distortion to render it grossly visible. Eventually, the spaces become filled with tumor cells, and with further growth and expansion the cords of tumor coalesce, replacing brain parenchyma, and a solid mass is produced. This mass is easily recognized upon sectioning of the brain and at the time of death may occupy the major portion of a cerebral hemisphere.

Death from brain damage or increased intracranial pressure usually occurs near the end of the third month after transfer. On the other hand, the cells may persist with little growth for much longer periods, and small foci of healthy tumor only slightly larger than the transplanted fragment have been found in animals killed during the fifth month. Areas of necrosis or of glial proliferation, such as might result from regression of a tumor, have not been observed.

The tumor has been transferred from brain to brain for six guinea pig generations without alteration in the growth rate or other transplantation characteristics. Attempts have been made to transfer from the brain to the eye at each passage but have been uniformly unsuccessful.

The Rous chicken sarcoma was transferred to the brains of rabbits, guinea pigs, and mice, with an incidence of takes exceeding 85 per cent. The characteristics of the transplanted tumor were similar in all species. Growth was rapid but short-lived and invariably terminated in regression within 2 weeks of transfer. The brains of animals killed on the seventh or eighth day contained solid masses of living tumor, sometimes measuring as much as 0.5 cm. in diameter, and serial transfer undertaken at this time was always successful. The tumor was carried by consecutive passage from brain to brain for six generations in all three species, and there is no reason to believe that it could not have been maintained indefinitely by this means.

On section, the tumor was characteristically mucoid in texture and easily distinguishable from brain substance. Histologically, it was identical in cellular content and architecture to growths obtained in chickens. There was no evidence of inflammatory reaction in adjacent brain tissue, and the tumor was abundantly supplied with thin-walled blood vessels (Fig. 8).

**Heterologous transfer of human cancer.**—In past experiments human cancer has been grown in various regions of the guinea pig’s body, but such growth was obtained only after the tumor had been carried for one or more generations in the eye. Direct passage from the human patient to extra-ocular sites was never achieved, although many bodily locations were repeatedly tested. In the present experiments, the two human cancers carried for stock purposes in guinea pigs’ eyes proved readily transplantable to the brain, but a finding of greater significance, sharply differentiating the brain from other bodily regions, was the fact that cancer tissue derived immediately from the human patient survived and grew.

The two human cancers maintained in the laboratory to provide stock experimental material were a glioblastoma multiforme and a carcinoma of the colon. The glioblastoma was first transplanted to the guinea pig’s eye in January, 1948, by Dr. Edward Krementz,1 and has been carried to date by serial anterior chamber transfer. In the present study, the tumor was successfully transferred to the brains of guinea pigs, mice, rats, and rabbits and has been passed for three consecutive generations in the two former species. The intestinal carcinoma has been maintained in guinea pigs’ eyes since May, 1950, and fragments of eye growths proved readily transplantable to the brain.

Transplants of the glioblastoma are of particular interest inasmuch as they represent a human tumor growing in its natural site in an experimental animal and thus offer a unique opportunity for investigation. Transfer of the tumor is almost invariably successful, and if careful technic is employed, takes occur in all the animals used. The behavior of the transplants has been studied in guinea pigs and mice, and several significant variations have been observed. The course and termination of the tumor will be followed in rats and rabbits, but up to the present all such animals have been killed at various periods after transfer to determine the presence or absence of growth.

The presence of growth in the brains of guinea pigs and mice is not associated with neurological signs until shortly before death, and animals bearing tumors 0.5 cm. in diameter may appear in perfect health. As a rule, the guinea pigs die between the 90th and 100th day after transfer, but occasionally animals have survived to the 150th day. At death the tumors are large, free of hemorrhage and necrosis, and are well demarcated from the surrounding brain substance (Fig. 9). The survival periods of mice are more irregular in extent. Several animals have died early in the second month, with tumors occupying the greater part of a

1 Jane Coffin Childs Fellow.
hemisphere, while others with tumors of comparable size have survived for as long as 120 days. The majority die between the 70th and 90th days, and the average survival period, based on 56 fatal cases, has been 83 days. At autopsy the tumors are commensurate with those found in guinea pigs but are poorly demarcated. No distinct boundary can be made out, and the tumor tissue blends gradually with the surrounding brain substance.

Histologically, the growths in the guinea pig show all the characteristics of the parent tumor (Figs. 11, 12). Mitotic figures are numerous, the cells are pleomorphic, and giant forms are common. Palisading is often a pronounced feature, and capillary endothelial hyperplasia is constant. The brain tissue about the periphery of the growth is compressed, and although it contains scattered infiltrating cells, the transition from tumor to brain tissue is quite abrupt (Fig. 13). In the mouse brain the tumor is much more sarcomatous in appearance. There are abundant mitotic figures, but the cells show little variation in size or shape. Palisading and endothelial proliferation are not common. The relationship between tumor and brain tissue is in sharp contrast with that found in the guinea pig. No boundary exists, and tumor tissue extends in irregular tongues from a central solid mass into the adjacent brain (Fig. 14). The tumor projections may extend for several millimeters with a gradual decrease in their cellular content, and the intervening brain substance appears normal. Occasionally, an area of intact brain tissue is observed completely inclosed by such “cross country” growth without evidence of degenerative cellular changes, and isolated patches of tumor are rarely found without traceable connection with the main tumor mass.

The carcinoma of the colon grows slowly and produces large amounts of mucus in the anterior chamber of the eye. Brain transplants behave in a similar manner. At 100 days, the bulk of the brain tumor consists of mucus, and most of its cells are of the “signet ring” variety (Fig. 15). Animals have been held for as long as 200 days without the development of neurological signs, and no fatalities attributable to the growth have occurred. Old transplants consist almost entirely of mucus, and tumor cells are only found after careful and prolonged search.

The transfer of cancer directly from the human patient to the brain of the guinea pig or mouse has been accomplished in ten instances. Three of the tumors were ovarian in origin, three were glioblastomas, two were derived from the intestine, one was a malignant melanoma, and one was a mammary carcinoma. With the exception of the glioblastomas, all the growths were known to have metastasized at the time of transfer, or metastases have since become evident. Concurrent transfer to the anterior chamber of the eye was successful in each instance. A further series of twelve tumors failed to grow in the brain and also failed to grow in the eye. These included five brain tumors, a hypernephroid carcinoma of the kidney, a seminoma of the testicle, a fibrosarcoma of the chest wall, and four mammary carcinomas. The patients bearing the seven bodily tumors were free of metastasis at the time of operation and are without clinical evidence of tumor at the present time.

The ovarian cancers consisted of two papillary serous cystadenocarcinomas and one pseudo-mucinous cystadenocarcinoma. All the tumors were transferred to guinea pig brains, and mice were employed as additional hosts for one of the papillary carcinomas. In the latter case, the mouse brain proved as good a transplantation site as the guinea pig’s brain, and at autopsy 40 per cent of the animals bore tumors. Approximately the same incidence of takes obtained in the transfer of the other tumors of the group and the behavior of the transplants was similar. Growth was rapid in all instances. Several mice killed as early as the sixth day showed microscopically visible tumors, and in one guinea pig the growth was of sufficient size by the sixteenth day to allow serial transfer. Histologically, the transplants in both guinea pigs and mice were identical with the tissue used for transfer (Figs. 16, 17).

The three glioblastomas showed the usual variations in histological structure and incidence of mitotic figures common to tumors of this type. It is of interest, although no significance can be based on data derived from only three tumors, that the growth rate of the transplants was directly proportional to the mitotic index of the primary tumor. One of the tumors containing many mature astrocytes and rare mitotic figures gave rise to neurological signs in 101 days; one with an intermediate number of mitoses required 66 days, while the tumor with the highest mitotic index resulted in death in 41 days. It is also suggestive that the slowest growing transplant was derived from the only surviving patient of the group.

The histological appearance of the transplants was similar to that of the stock glioblastoma and showed the same variation associated with growth in different species—a circumscribed, pleomorphic tumor in guinea pigs and a diffuse, sarcomatous tumor in mice.

Attempts to transplant five additional brain
tumors failed. The tumors of this group consisted of two astrocytomas, two ependymomas, and one so-called hemangioblastoma. It should be emphasized that these tumors also failed to grow when transferred to the guinea pig's eye.

The two intestinal cancers successfully transplanted were a poorly organized adenocarcinoma of the sigmoid and a colloid carcinoma of the rectum. The carcinoma of the sigmoid grew rapidly to produce neurological signs in approximately 90 days, but animals killed as early as 19 days bore tumors of sufficient size to allow large scale serial transfer. The growths in the brain were invasive, and there was no evidence of inflammatory reaction. Histologically, they resembled the parent tumor, but in some areas a higher degree of organization was evident with the production of a well defined glandular pattern (Figs. 10, 20, and 21). The colloid carcinoma of the rectum produced small growths in the brain which did not give rise to neurological signs. Guinea pigs and mice killed at varying periods from the 20th to the 80th day all showed tumors of comparable size and structure. These rarely exceeded 0.5 cm. in diameter and consisted for the most part of mucoid material with widely scattered, apparently inactive cancer cells. In the great majority of cases there was no reaction in the adjacent brain, but occasional transplants were found surrounded by phagocytosing microglia.

The breast cancer was medullary in structure, and its cells were clear, resembling those found in a hypernephroma. Takes were obtained in both mice and guinea pigs, and second generation transfers were successfully effected in both species. The mice were killed at various periods before the development of signs, and it was of interest that growths of sufficient size to be visible on gross section were found as early as the twelfth day. Neurological signs became evident in guinea pigs about the 60th day, and the brains of animals killed at this time contained large tumors histologically indistinguishable from the primary growth (Figs. 10, 20, and 21). Control transplants in the anterior chamber of the eye grew very slowly and had no more than doubled in size by the time brain transplants had attained a diameter of 0.5 cm.

The malignant melanoma was derived from the skin of the back of a 2-year-old child and, contrary to the usual behavior of this type of tumor in pre-pubertal age groups, had metastasized widely. Takes were obtained in the majority of mice and guinea pigs used. The transplants grew slowly in both species, and deaths did not occur until after the 90th day. The tumors found at death, however, were the largest noted in the present series and, in several cases, had ruptured into a ventricle; and secondary growth was found in other parts of the brain (Fig. 22). Histologically, the tumors were amelanotic and resembled the parent growth in all details. As in the case of the previously described mammary cancer, brain growth was more rapid than anterior chamber growth.

DISCUSSION

The major disadvantage of the brain as a site for heterologous tumor transplantation is the inability of the investigator to see or to palpate the growing transplant. Neurological signs suggestive of intercranial growth do not become apparent until shortly before death, and there is nothing in the animal's behavior to indicate the presence or absence of growth. This handicap can be overcome to a certain extent by brain biopsy. The drill hole in the calvarium remains open for a long period of time, and if the biopsy needle is directed along the path taken by the trocar at transfer, specimens can be obtained with relative ease. However, this becomes a laborious procedure when many animals are involved, and in investigations concerned with quantitative measures the anterior chamber is the superior transplantation site.

On the other hand, some features of brain growth render it preferable for certain types of experimentation. The transplants grow to larger size, they are always medullary with a minimum of connective tissue stroma, and, representing the tumor in almost pure culture, offer a unique material for chemical or immunological study. From a more particular point of view, the ability to grow human brain tumors in the brains of laboratory animals offers an opportunity for the investigation of a highly fatal and poorly understood group of human cancers under conditions closely approaching their natural state.

The fact that human cancer will survive and grow in the brain substance of mice is of significance from both biological and clinical standpoints. In previous experiments, it had been found that the mouse eye was far inferior to the guinea pig's eye as a nidus for the growth of human cancer, but in contrast it offered a much better medium for the growth of rabbit cancers (2). Other observations in line with this finding have suggested that a grouping of species with reference to the ability to synthesize vitamin C coincides with susceptibility or resistance to heterologous transfer. Transfer between species with the same type of C metabolism (man and guinea pig) is comparatively easy, while transfer between species with different types (man and mouse) is
difficult. The point to be emphasized in the present connection is that while these considerations pertain to the eye and other bodily regions, they do not apply to the brain. A further indication that the conditions prevailing in the brain differ from those in the rest of the body is given by the behavior of the Brown-Pearce rabbit cancer in guinea pigs, for this tumor fails to survive transfer to the eye or other bodily region yet grows readily in the brain. Whether or not the different status of the brain concerns Vitamin C metabolism or some other factor is the subject of continued study. In any case, it would appear, on a basis of transplantation reactions, that the brain substances of different animal species bear a closer relationship to each other than do other bodily tissues.

The rapidity with which human cancer grows in the mouse brain, together with the comparative low cost of mice, are factors of importance in the clinical use of heterotransplantability as a prognostic test. It has been found that with the exception of brain neoplasms, only metastasizable tumors are heterotransplantable, and this fact is utilized to determine the status of human tumors with respect to metastasizability at the time of their removal. Guinea pigs are relatively expensive as regards both cost and maintenance, and in the case of certain tumors a month or more is required to determine the results of transfer. It is suggested that the mouse brain may be used in place of the guinea pig’s eye with advantage from the standpoint of both time and economy.

REFERENCES
Fig. 9 (left).—Cut surface of brain of guinea pig bearing growth of a human glioblastoma multiforme. The animal was killed 94 days after transfer.

Fig. 10 (right).—Cut surface of brain of guinea pig bearing growth of a human mammary carcinoma. The animal was killed 60 days after transfer.
FIG. 11.—Section of transplant of human glioblastoma in guinea pig’s brain. X200.

FIG. 12.—Section of transplant of human glioblastoma in guinea pig’s brain showing the characteristic capillary endothelial hyperplasia. X200.

FIG. 13.—Human glioblastoma growing in guinea pig’s brain. Section taken at edge of tumor to show the relatively abrupt boundary between tumor and adjacent brain. Compare with following figure. X200.

FIG. 14.—Human glioblastoma growing in mouse brain. There is no distinct boundary between tumor and brain substance. The tumor is infiltrating white substance between rows of ganglion cells for a considerable distance beyond main tumor mass. X200.

FIG. 15.—Section of guinea pig’s brain bearing a transplant of a carcinoma of a human colon. Note signet ring cells and abundance of mucus. X260.

FIG. 16.—Section of guinea pig’s brain bearing a transplant of a human ovarian papillary serous cystadenocarcinoma. The animal was killed 10 days after transfer. X200.

FIG. 17.—Section of mouse brain bearing a transplant of a serous cystadenocarcinoma derived from the ovary of a different human patient. The mouse was killed 6 days after transfer. X100.

FIG. 18.—Cross section of guinea pig brain bearing a transplant of a carcinoma of a human sigmoid colon. The animal was killed 57 days after transfer. X5.
Fig. 19.—Higher power view of tumor shown in Fig. 21. X200.

Fig. 20.—Section of guinea pig brain bearing a transplant of a human mammary carcinoma. This is a magnification of the tumor shown in Fig. 10. X190.

Fig. 21.—Section of mouse brain bearing transplant of human mammary carcinoma. The mouse was killed 8 days after transfer. X100.

Fig. 22.—Section of a guinea pig’s brain bearing a transplant of a human malignant melanoma. Section shows growth of a secondary tumor in ventricular wall as a result of “seeding” from the main mass. The animal was killed 90 days after transfer. X35.
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