The Heterologous Transplantation of Mouse and Rat Tumors*

Harry S. N. Greene, M.D., and Edwin D. Murphy, M.D.**

(From the Departments of Pathology and Surgery, Yale University School of Medicine, New Haven 11, Connecticut)

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Several years ago it was shown that human and rabbit cancers can be readily transferred to animals of alien species by utilizing the anterior chamber of the eye as a transplantation site (2-4). The study of heterotransplantability has since been extended in various directions, and one phase of the investigation has been an examination of cancers of other species with respect to this property. Cancers from a variety of species have been successfully transplanted to guinea pigs and rabbits, but tumors of mice and rats formed the bulk of available material and have been studied from several viewpoints in relation to their ability to survive and grow in foreign hosts. The present paper will report the results of heterologous transfer of a number of propagable growths of rats and mice used in cancer research, while later papers will be concerned with a study of the developmental course of spontaneous and experimental mouse tumors with special reference to the evolution of autonomy as expressed by heterotransplantability.

The extensive literature dealing with the heterologous transplantation of mouse and rat tumors was reviewed in 1933 (8). Successful transfer between species was undoubtedly effected in several instances. Thus Murphy transferred the Jensen rat sarcoma to the developing chick embryo and was able to carry the tumor serially in this host (9); transfer back to the rat resulted in takes, but all attempts to transplant the tumor to adult chickens failed. Shirai reported briefly the transplantation of a rat sarcoma to the brains of adult mice, but apparently serial transfer was not attempted (11). Putnoky transplanted Ehrlich’s mouse carcinoma subcutaneously into adult rats and has maintained the tumor by serial transfer since 1929 (10). A large inoculum of 300 to 500 mgm. of tumor is used, and growth is rapid. Regression begins by the tenth day, and transfer must be made at this time. Histologically the appearance of the tumor is identical with that in the mouse host.

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MATERIALS AND METHODS

The mouse tumors used in the present experiments consisted of an epidermoid lung carcinoma, sarcoma 180, an ovarian embryoma, an experimentally induced hepatoma, and 5 different mammary carcinomas. The rat tumors were R39 and 2426.

The anterior chamber of the eye was used as a transplantation site and the technic employed has been described in detail (5). Testicular and intramuscular transfers were performed in several instances, and both tissue fragments and cellular emulsions were employed.

Guinea pigs, rabbits, rats, hens, and ducks served as foreign hosts. Animals of both sexes and of different age groups were used, with no significant variation in the results obtained. The animals were not subjected to special treatment before or after transfer but were maintained under ordinary conditions of cage life.

Before heterologous transfer, all the mouse tumors were tested to determine their transplantability in various mouse breeds. The breeds used were the C3H, C57 black, dba, and the A and Bagg albinos.

RESULTS

The results of transfer of the different mouse and rat tumors to the anterior chambers of the eyes of guinea pigs are summarized in Table I. The tumors varied in growth rate and in behavior in the foreign hosts, and will be described separately.

BRONCHOGENIC CARCINOMA

This tumor, which arose spontaneously in a bronchus of a CBA mouse and was characterized histologically by prickle cells, has been carried by serial subcutaneous transfer in this strain by Dr. Gardner. It grows equally well in C3H mice, and has been maintained in this line in our laboratory. Takes occur regularly

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1 These tumors were made available through the courtesy of Dr. William H. Woglom, Department of Cancer Research, Columbia University; Dr. William U. Gardner, Department of Anatomy, Yale University School of Medicine, and Dr. Austin M. Brues, Massachusetts General Hospital, Boston, Massachusetts.
in 100 per cent of the animals used, and the transplants grow rapidly. It is invasive and extends through the abdominal muscles and lymphatics to involve the peritoneum in 3 to 4 weeks. Metastasis to the lungs and other viscera is common.

On test, takes were obtained by subcutaneous transfer in dba's, A's, and Bagg albinos, but not in C57 blacks. There were relatively few takes in the first generation transfers to the new strains, but with serial passage the transplantability increased and after several generations equaled that obtained in the parent line. No significant difference in the morphology or behavior of the tumor was noted in the new strains.

growth is progressive; the cornea ruptures, and the tumor protrudes externally as a fungating mass.

The factors concerned in regression have been studied in a series of reinoculation experiments in which living tumor was transferred to the anterior chamber of the opposite side after regression of the first transplant. If the transplant regresses before filling the chamber, the animal is invariably found resistant to further transfer. A similar situation occasionally obtains when regression follows the complete filling of the chamber, but in the majority of such cases transfer to the second eye results in takes. It is suggested, therefore, that in the former instances

Transfer to the anterior chamber of guinea pig eyes.—The tumor has now been carried by serial anterior chamber passage in guinea pigs for more than a year. Growth occurred in approximately 66 per cent of the animals in the entire series, but an examination of succeeding generations shows a gradual increase in the incidence of takes, to reach 100 per cent in the last 4 passages.

Growth and vascularization of the transplants are invariably evident by the tenth day following transfer, and occasionally the tumor increases to fill one-half of the chamber in this period. In rare instances regression follows a short period of growth, but in the great majority of cases the transplant enlarges to fill the chamber by the 20th day. Thereafter partial regression followed by renewed growth or complete and permanent regression may occur. In other animals

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* Tumors 355, BR, ST, and 2426 did not take in 33, 30, 30, and 25 guinea pigs respectively.
be a general rule that growth in the guinea pig is associated with a slightly higher degree of differentiation and organization than is observed in the primary host. Guinea pig transfer is thus of considerable value in the classification of anaplastic, poorly organized tumors, and is frequently used in our laboratory as an aid to the microscopic diagnosis of human tumors.

The tumor cells invade the guinea pig’s iris early in the course of growth, and may extend deeply into its substance before an increase in the circumference of the transplant is noted. This is apparently not associated with invasion of vascular walls, for hemorrhage is much less common than in the case of rat sarcoma 39, which also is characterized by early iris invasion.

Growth in the bulk of the transplant is accompanied by an extension of tumor cells over the surface of the iris in the manner of a lining membrane. Invasion occurs in many areas but does not extend into its retinal portion. Subsequent growth is purely expansive, and the bulging posterior surface of the iris is invariably covered by stretched but intact retina.

After rupture of the cornea the tumor extends under the bulbar conjunctiva and invades the eyelid. Infection invariably follows and necessitates sacrifice of the animal.

Transfer to guinea pig testicles.—Testicular transfer of the tumor is readily accomplished, either with tissue obtained directly from the mouse or after growth in the guinea pig’s eye. The growth rate of the transplants varies widely, and occasionally exceeds that observed in the anterior chamber. Usually the testicular parenchyma is completely replaced by the 40th day, but in some instances the growth persists as a pean-sized nodule for many months.

Histologically the testicular and anterior-chamber transplants are identical in cellular character and structure (Fig. 3). The tumors are sometimes surrounded by a connective tissue capsule containing numerous eosinophils, and growth appears to be expansive in nature. In other animals, however, the tumor has no discrete boundary, but extends in strands and columns throughout the parenchyma, invading and destroying the tubules (Fig. 4).

Transfer to the anterior chamber of rabbit eyes.—Surprisingly, transfer of the tumor to the eyes of hens and ducks gives rise to nearly 100 per cent of the cases, and identical results are obtained regardless of the derivation of the tissue. Vascularization of the fragments is apparent within 2 weeks of transfer, but subsequent growth is extremely slow and the transplants may not increase by more than 3 diameters in the following 4 months.

The transplants have been studied histologically at different periods of growth. Between the first and second weeks the great majority of tumor cells die and a large portion of the transplant is converted into a necrotic, amorphous mass. However, islands of cells in scattered areas survive and grow (Fig. 9). The surviving cells are large, contain abnormal nuclear forms, and mitosis is common. At a later period the graft is made up entirely of living cells with supporting stroma and blood vessels. The cells are smaller than usual, largely because of a diminution in the amount of cytoplasm, and are closely packed (Fig. 10).

Tumor 180

Mouse tumor 180, propagated for many years in the Department of Cancer Research of Columbia Uni-
FIG. 1-6
grown in many different strains of mice, and in our laboratory proved readily transplantable to dba's, C57's, A's, and Bagg albinos. For stock purposes the tumor is carried in C3H mice, where it grows regularly in 100 per cent of the animals. In this strain it grows rapidly and is invasive. The abdominal wall is invaded and peritoneal extension occurs in from 3 to 4 weeks after subcutaneous transfer. The growths frequently ulcerate, with consequent infection, and death usually occurs before metastasis. However, metastasis eventually takes place in animals that survive infection or peritoneal extension.

Transfer to the anterior chamber of guinea pig eyes.—Transfer of the tumor to the eyes of guinea pigs gives rise to growth in 75 per cent of the animals used but, as in the case of the lung carcinoma, the frequency of takes increases with continued passage.

The transplants undergo considerable increase in size before vascularization becomes evident, and subsequent growth is so rapid that the chamber may be completely filled within 8 days of transfer. Thereafter, regression invariably occurs. Regression may be rapid and result in the death of the transplant in 24 hours but is sometimes delayed so that foci of living, transplantable cells persist for 3 or 4 weeks. Neither corneal rupture nor renewed growth has been observed.

Histologically the transplants are indistinguishable from the original mouse tumor (Figs. 11 and 12). Sections of early grafts are devoid of blood vessels, suggesting that primary growth may occur in the manner of a tissue culture, the tumor cells imbibing nutriment directly from the surrounding aqueous humor. At a later stage, however, the growths are abundantly supplied with thin-walled blood vessels. Invasion of the iris is a constant feature, but does not extend beyond the pars iridica retinae.

Transfer to guinea pig testicles.—Testicular takes occur after transfer of tumor derived from the mouse or the guinea pigs' anterior chamber, but in the great majority of cases the transplants regress within 10 days. In 3 of 25 cases the growths persisted and increased to the size of marbles in a period of 30 days. One of these animals was killed for microscopic study, while the remaining 2 were held to determine the eventual fate of the tumor. In both instances the tumors decreased in size and no trace remained on palpation of the testicles on the 50th day.

Histologically the essential tumor cells in early transplants are identical in structure and arrangement with those found in the eye, but in contrast to the situation in the anterior chamber the foci of growth are surrounded and compressed by capsules of dense fibrous connective tissue. A definite capsule was not observed in the 1 animal with persistent growth. Here tumor cells, many of which were in mitosis, were diffusely intermingled with young fibroblasts and strands of older connective tissue. The mass composed of these elements was not circumscribed but of irregular outline, with projections extending into adjacent testicular parenchyma, engulfing and destroying tubules in a manner comparable to that observed in the case of the previous tumor. Remnants of tubules were also present in the center of the tumor, suggesting that in this instance growth had been infiltrative rather than purely expansive as in the earlier transplants.

Intramuscular transfer in guinea pigs.—Growth has followed transfer of the tumor to the thigh muscles in 8 of 15 animals. The transplants increase in size with extreme rapidity and by the tenth day the thigh is converted into a diffuse incapacitating mass extending from hip to knee joint. However, regression occurs with the same rapidity and no clinical evidence of tumor remains on the 25th day.

Histologically the appearance of the transplants is comparable with that of persistent testicular growths. In scattered areas the tumor cells are aggregated in masses, but elsewhere are diffusely interspersed with young fibroblasts and muscular elements (Fig. 13).

Transfer to the anterior chamber of rabbit eyes.—Transfer to rabbit eyes has been successful in only about 10 per cent of cases (3 of 24). The clinical course of the tumor is comparable with that observed in guinea pigs, and its histological appearance is identical (Fig. 14).

Transfer to the rabbit's testicle.—Transfer to the...
testicle with tissue derived from both rabbit and guinea pig eyes has been uniformly unsuccessful, no takes having been obtained in more than 30 attempts.

Transfer to the anterior chamber of rat eyes.—Anterior-chamber transfer results in 100 per cent of takes in rats, but, as in the case of the lung carcinoma, the transplants are invariably infected and undergo a similar course (Fig. 15).

Ovarian Embryoma

This tumor arose in the ovary of a C3H mouse and has been carried serially in this strain by Jackson and Brues. Takes occur on subcutaneous transfer in approximately 75 per cent of animals but the growth rate varies widely. The tumor is composed of a mixture of embryonal and mature tissues, which persist on transfer with variation of the predominant cell type in different animals (7).

The tumor has been carried in C3H mice in this laboratory and has also been successfully transplanted to the A albino strain. Its histological characteristics in our C3H mice are identical with those described by Jackson and Brues, but in A albinos the growth consists of sheets of embryonal cells without architectural organization (Figs. 16 and 17). Growth of the tumor is largely expansive, with minimal muscular invasion. Lymphatic extension has not been observed, but metastasis to the lungs and other viscera occurs in the terminal stages.

Transfer to the anterior chamber of guinea pig eyes.—Experiments involving the heterologous transplantation of this tumor have been limited to the guinea pig’s eye, and the tumor has not been carried serially in this species. First generation transfers from 4 separate mouse tumors have given rise to growth in 15 of the 21 animals used, or approximately 70 per cent.

Growth of the transplants is generally much more rapid than in the natural host, and in many instances the anterior chambers of guinea pigs’ eyes are completely filled with tumor by the 11th day, at least a week before growth can be detected in control mice. On the other hand, growth may be greatly retarded; in one particular instance the transplant in an animal released to the breeding population as negative remained dormant for 3 months and then grew slowly to fill the chamber. In such cases the transplant may reach a large size and result in bulging of the cornea with destruction of the internal structures of the eye. Usually, however, after regression of the tumor the iris is little damaged and shows only a minor degree of scarring. Regression has been the eventual termination of the tumor in all animals held for continued observation.

In contrast to the parent tumors in C3H mice, the guinea pig transplants are composed of a single cell type. This is an embryonal cell suggestive of early embryonic epithelium, and appears to be identical with the elements producing the tumor in A strain mice. In the guinea pig, however, the cells are arranged in a definite architectural pattern closely imitating glandular acini (Fig. 18).

Hepatoma

This tumor is a liver carcinoma produced by α-amidoazotoluene in the Department of Medicine at Columbia University and carried serially by subcutaneous transfer in Bagg albino mice. In our laboratory it has also been transferred successfully to dba’s, where it grows in approximately 50 per cent of the animals inoculated. Its morphology is identical in both mouse strains (Fig. 19). Growth is predominantly expansive, but muscular invasion occurs in late stages. Neither lymphatic extension nor metastasis has been observed.

Transfer to the anterior chamber of guinea pig eyes.—Study of this tumor in alien species has also been limited to the guinea pig’s eye. Early transfers to this site gave rise to few takes, but the incidence of growth increased to 100 per cent in the fourth and fifth serial generations.

Vascularization of the transplants becomes evident within a week of transfer, but subsequent increase in size is slow and the chamber is rarely filled in less than 2 months. In occasional cases growth is further delayed, and may persist for as long as 150 days before regressive changes become noticeable. Early growth is almost entirely expansive in character and invasion of the iris...
is never so pronounced as in the case of other tumors. After filling of the chamber regression is the rule, but renewed growth may occur in scattered foci at any time during the process. Histologically the transplants are identical with the mouse tumors in appearance (Fig. 20).

MAMMARY CARCINOMA RC

This growth has been carried serially by subcutaneous transfer in dba mice at the Biochemical Institute of The University of Texas and in the Department of Cancer Research of Columbia University. In our laboratory it has also been transferred successfully to the C57 and A strains. The tumor grows expansively to produce a large subcutaneous mass with little muscular invasion. Invasion of the overlying skin, however, is a relatively constant feature, leading to ulceration with subsequent infection, and in the majority of cases death occurs without metastasis. Histologically the tumor consists of anaplastic epithelial cells arranged in solid rounded masses separated from each other by varying amounts of connective tissue stroma (Fig. 21).

Transfer to the anterior chamber of guinea pig eyes.—The tumor grows readily in the guinea pig’s eye and 100 per cent of takes was obtained in the fourth serial generation. Growth is rapid and the chamber is almost invariably filled with tumor by the eighth day after transfer. Regression generally follows, as is usual with rapidly growing tumors, and is often succeeded by a period of renewed growth. In some cases the cornea ruptures at the site of the incision through which the graft was introduced, and the protruding tumor may survive for a number of weeks before infection and necrosis supervene.

The usual histological picture closely duplicates that found in the mouse (Fig. 22). Occasionally, however, there is an overgrowth of guinea pig connective tissue and the essential cancer cells are scattered singly or in small groups throughout the loose stroma, actually occupying only a relatively small part of the tumor mass.

Transfer to the anterior chamber of rat eyes.—Takes occur regularly in the anterior chambers of rats’ eyes, but in our experience transfer to this species is always associated with infection of the transplants and the final picture is one of acute infection and abscess formation. Areas of growing tumor are found in animals killed during the first 2 weeks following transfer, and show the same morphological features observed in mice (Fig. 23).

YALE TUMOR NUMBER 1

This tumor is also a mammary carcinoma. It arose in an A strain mouse and has been under transfer at Yale for many years. It has been successfully transplanted also to mice of the C3H strain in our laboratory. The dominant mode of growth is expansive, but the tumor also invades and metastasizes. Histologically it is a well differentiated adenocarcinoma (Fig. 24).

Transfer to the anterior chamber of guinea pig eyes. Anterior-chamber transfer results in takes in approximately 50 per cent of the guinea pigs used, and despite its comparatively well organized structure the tumor grows with surprising rapidity. Vascularization and increase in size can usually be detected by the fourth day, and the chamber may be completely filled by the tenth. The growing tumor, like other well differentiated growths of glandular type, is characterized clinically by a cherry red color, which apparently reflects an abundant and complex blood supply. Regression and necrosis are easily detected by the appearance of white opacities, and their occurrence in scattered focal arrangement is a distinctive feature of the tumor during the third or fourth week of growth. These areas gradually coalesce and eventually involve all but a minute portion of the transplant. The opaque material slowly disappears over a period of weeks and the persistent pinkish tissue undergoes renewed growth.

Histologically the anterior chamber transplants are identical with the subcutaneous growths in mice (Fig. 25).

MAMMARY TUMOR 755

This arose in the mamma of a C57 black mouse and has been maintained in this strain by serial subcutaneous transfer. Transplants grow slowly to produce a

Fig. 13.—Transplant of sarcoma 180 growing in guinea pig’s muscle. Mag. X 275.
Fig. 14.—Transplant of sarcoma 180 growing in anterior chamber of rabbit’s eye. Mag. X 340.
Fig. 15.—Transplant of sarcoma 180 growing in anterior chamber of rat’s eye. Mag. X 275.
Fig. 16.—Transplant of embryoma growing in subcutaneous tissues of C3H mouse. The photograph represents the least organized area found in the growth, but even here a definite glandular pattern can be recognized. Mag. X 275.
Fig. 17.—Transplant of embryoma growing in subcutaneous tissues of an A mouse. Note complete disorganization. Mag. X 275.
Fig. 18.—Transplant of embryoma growing in anterior chamber of guinea pig’s eye. Appearance is that of a well differentiated adenocarcinoma. Mag. X 275.
bulky tumor, but growth appears to be entirely expansive and neither invasion nor metastasis has been observed. Histologically there is only a slight suggestion of glandular pattern, and the essential cells are arranged in solid masses separated from one another by connective tissue septa.

In attempts to transfer the tumor to other lines of mice more than 50 animals of each strain, including the C3H, dba, A, and Bagg albino have been used, but in no instance has any suggestion of growth been noted. In like manner all attempts to transfer to guinea pigs and rabbits have been unsuccessful.

Mammary Tumor BR

This tumor arose in the mamma of a C3H mouse in our laboratory. A portion of the growth was removed at biopsy 2½ weeks after discovery, and has since been carried by serial subcutaneous transfer in the same strain. All attempts at transfer to other mouse strains, including C57, dba, A, and Bagg albino have been unsuccessful. In C3H mice growth is entirely expansive, and neither lymphatic extension nor metastasis has been observed. Histologically the tumor is a well differentiated adenocarcinoma.

Attempts have been made to transfer this tumor from 3 different mice bearing it to the anterior chambers of guinea pigs' eyes. Ten pigs were used in each transfer, and all were held under observation for a period of 2 months. No indication of growth was observed in any instance.

Mammary Tumor ST

This tumor arose in the mamma of a C3H mouse and has been carried by serial transfer in Dr. Gardner's laboratory. It is a fairly well differentiated adenocarcinoma and neither invasion nor metastasis has been observed. All attempts to transfer the tumor to other mouse strains have been unsuccessful, and in no instance has growth been obtained in the anterior chamber of the guinea pig's eye.

Rat Sarcoma 39

This tumor originated as a fibroadenoma in the mamma of an old female market rat in the Department of Cancer Research in Columbia University, but its subsequent development was that of a polymorphous cell sarcoma, and it has been propagated there as such for many years. Takes occur in from 50 to 100 per cent of the Wistar strain of rats used, and approximately 30 per cent of the resulting tumors regress. The tumor has been successfully transferred to several varieties of rats in our laboratory, but in all these the eventual fate of the growth was regression. The mode of growth is predominantly expansive but invasion does occur.

Transfer to the anterior chamber of guinea pig eyes.—Transfer to guinea pig eyes is successful in approximately 50 per cent of cases. Growth is extremely rapid and apparently proceeds for a time in the manner of a tissue culture, for the chamber may be one-third filled with tumor before the appearance of a vascular supply. This is particularly evident when small particles become detached from the main fragment during passage through the chamber and give rise to multiple isolated foci of growth. Here the growth of individual cell clumps can be easily followed with the aid of a magnifying lens, and enlargement in all directions to a rounded translucent mass preceeds penetration by blood vessels. After vascularization the growth rate is further increased, and the chamber may be completely filled by the sixth day. The appearance of large hemorrhagic areas in the substance of the transplant, and of free blood in the chamber, is a constant feature of growth after vascularization and obscures the sequence of later events. Eventually, however, after a lapse of several weeks regression occurs. It may be interrupted by a period of renewed growth, but ultimately becomes complete. The eye is extensively damaged and after healing presents the picture of phthisis bulbi.

Histological examination of the transplants after vascularization shows extensive invasion of the iris, with growth along the choroid and consequent dislocation of the retina. Tumor cells are found both in large mass formations and as single isolated units throughout the eye. Morphologically the appearance and arrangement of the cells suggest a carcinomatous rather than a sarcomatous character and resemble the growing edge rather than the main bulk of the rat tumor (Figs. 26 and 27).

Transfer to the anterior chamber of rabbit eyes.—

Transplants of the tumor grow in 40 per cent of

DESCRIPTION OF FIGS. 19 TO 24

Fig. 19.—Transplant of hepatoma growing in subcutaneous tissues of Bagg albino mouse. Mag. X 275.

Fig. 20.—Transplant of hepatoma growing in anterior chamber of a guinea pig's eye. Mag. X 275.

Fig. 21.—Transplant of mammary carcinoma RC growing in subcutaneous tissues of dba mouse. Mag. X 275.

Fig. 22.—Transplant of mammary carcinoma RC growing in anterior chamber of guinea pig's eye. Mag. X 275.

Fig. 23.—Transplant of mammary carcinoma RC growing in anterior chamber of rat's eye. Mag. X 275.

Fig. 24.—Transplant of Yale Tumor No. 1 growing in subcutaneous tissues of C3H mouse. Mag. X 275.
rabbits, and their behavior is comparable to that described in guinea pigs. Histologically the resemblance to carcinoma is even more pronounced (Fig. 28).

**Rat Mammary Tumor 2426**

This tumor arose in the mamma of a female rat and the characteristics of the growth in the primary host and in experimental animals have been described (1). It is a highly differentiated growth and, according to Eisen, does not metastasize. In our laboratory neither invasion nor metastasis has been observed.

Attempts to transplant the tumor subcutaneously into rats of several different strains have been unsuccessful, and in no instance has growth been obtained in the guinea pig.

**RETURN TO THE MOUSE AFTER GUINEA PIG PASSAGE**

After growth for a generation or more in the anterior chamber of the guinea pig’s eye, the tumors have been transferred back to the subcutaneous tissues of mice of the strain in which they originated, and without exception the speed of growth, power to invade, and metastatic rate are increased. Thus when the bronchogenic carcinoma is returned to the abdominal subcutaneous tissues of C3H mice, takes are apparent within 2 days. Expansive growth is reduced to a minimum, and in contrast to the local swelling characteristic of ordinary subcutaneous transplants the tumor appears as a diffuse, plaque-like thickening of the greater part of the abdominal musculature, resembling in many respects the growth of a lymphosarcoma transplanted to the same region. Invasion of the peritoneal cavity occurs with great rapidity, and the animals frequently die with lung metastases in less than 2 weeks after transfer.

**DISCUSSION**

The ability of some human and rabbit tumors to survive and to grow in animals of alien species has been demonstrated, and it is apparent from the experiments described in the present paper that this attribute also distinguishes certain tumors of the mouse and rat. The property of heterotransplantability is not shared by all tumors of these species but appears, on the other hand, to be a common characteristic of a special group, and thus offers a means of classification based on biological behavior. The significance of such a classification is the subject of present investigation, but the evidence already at hand is highly suggestive and warrants consideration.

In all the species studied the ability to survive heterologous transfer is restricted to a group of tumors characterized by distinctive properties with respect to manner and range of growth in the parent species. In the rabbit the heterotransplantable tumors, in contrast to those not transferable in this manner, are distinguished by the capacity to invade and metastasize and to grow in unrelated animals of the same species. Similarly, in man, only those tumors that invade and metastasize are capable of growth in alien species, but whether or not they possess the power to grow in unrelated individuals of the same species is obviously a question not amenable to experimentation. An examination of the neoplasms utilized in the present investigation reveals a situation comparable with that observed in the rabbit—that is, the tumors that invade and metastasize are transplantable to unrelated strains and to alien species, whereas those that show neither invasion nor metastasis in the parent stock fail to grow when transferred to unrelated strains or to foreign species.

Thus the ability to invade and to metastasize characterizes the heterotransplantable tumors of all the species studied. This same ability is apparently an essential property of neoplasms that are capable of survival and growth in unrelated strains of the parent species. Tumors that invade and metastasize can be transferred both to unrelated animals of the same species and to animals of entirely unrelated species, and there is no evidence to indicate that the two types of transfer reflect different biological potencies. On the contrary, it is suggested that heterotransplantability does not imply the assumption of new and different properties on the part of the tumor, but that the attributes allowing true homologous transfer also allow heterologous transfer.

It is essential in the present connection to emphasize the biological distinction between successful transfer to animals of the same strain and successful transfer to animals of an unrelated strain. All mouse tumors appear to be transplantable in animals of the strain in which they originate. But this does not constitute homologous transfer, for as a result of long inbreeding the donor and the recipient in such an experiment bear a genetic relationship somewhat comparable to that of the fore and hind quarters of the same individual. Successful transfer of this type, therefore, does
not attain the same biological significance as homologous transfer in species such as the rabbit, where inbreeding has not been practiced. In effect, such transfer in the mouse is comparable to autologous transfer in the rabbit, and just as all mouse tumors grow in related animals so, also, all rabbit tumors grow on autologous transfer. Homologous transfer in the rabbit is possible only after progressive development of the tumor, and in terms of biological behavior marks the distinction between dependency and autonomy or, in clinical terms, between benignancy and cancer. It seems probable that a similar situation obtains in the mouse, and that with respect to autonomy successful transfer within a strain is of no greater significance than autologous transfer in the rabbit.

It is suggested that at this stage of development, when only limited transfer is possible, the tumors are dependent for continued survival and growth on factors peculiar to the primary host and closely related individuals. These factors are not supplied by unrelated animals, and such animals will not support growth of the tumors on transfer. At a later stage autonomy is attained, the tumors become independent of the factors concerned in this development, and gain the ability to survive and grow in their absence. The independence thus achieved eliminates genetic and species barriers and allows growth in unrelated animals and in alien species.

The tumors employed in the present study may be classified with these points in mind. On such a basis the dependent growths are the mammary tumors 755, BR, ST, and 2426, while the autonomous growths are the bronchogenic carcinoma, 180, Yale 1, RC, the embryoma, the hepatoma, and R39.

It has been found by means of successive biopsies and transplantation experiments, performed throughout the developmental course of cancer in rabbits, that the tumors undergo phases of dependency and autonomy and that the attainment of autonomy is only evidenced by invasion and metastasis (6). Comparable experiments utilizing spontaneous mouse tumors are in progress, and will be reported in detail in a later paper. However, for present purposes it may be noted that the developmental course of tumors in this species is also characterized by dependent and autonomous phases. During early or dependent stages the growths are transplantable only into animals of the same strain, but after continued development with invasion and metastasis they become transplantable in unrelated strains and in alien species. On this basis it seems probable that the dependent tumors in the series under study were transferred from the primary host before development had been completed. On the other hand, the tumors found to be autonomous may have been transferred at a later period in their developmental course, after the attainment of independence or, conceivably, during a dependent phase with later inadvertent transfer to animals supplying factors necessary for their continued development to cancer.

In any case, it is clear that the mouse and rat tumors studied are divisible into two classes on a basis of fundamental differences in biological behavior, and that tumors of the different groups can not be used as comparable materials in cancer research.

SUMMARY

A number of mouse and rat tumors including a bronchogenic carcinoma, sarcoma 180, an experimentally induced hepatoma, 2 mammary carcinomas, and sarcoma 39 were successfully transplanted to animals of alien species. All the heterotransplantable tumors, in contrast to a group not transferable in this manner, possessed the ability to invade and metastasize in the parent strain and to survive and grow in unrelated strains. On this basis it is concluded: first, that in mice, as well as in man and in the rabbit, invasion marks the attainment of autonomy; and second, that from the point of view of autonomy true homologous transfer and heterologous transfer possess the same significance.

REFERENCES

The Heterologous Transplantation of Mouse and Rat Tumors

Harry S. N. Greene and Edwin D. Murphy

*Cancer Res* 1945;5:269-282.

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