Transplantation of the Shope Papilloma and the Rous Sarcoma during Early Developmental Stages*

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Serial transplantation experiments undertaken at various stages in the development of spontaneous animal tumors indicated that the ability to grow on transfer to normal unrelated animals was a developmental acquisition and not a property of the tumors from their inception (3). Transplantability of this order appeared to be coincidental to the attainment of the ability to metastasize and extended to heterologous as well as homologous species. Heterologous transplantation experiments involving human tumors disclosed the occurrence of comparable developmental phases and suggested the existence of a similar relationship between transplantability and metastasizability (4). However, in the course of this study a striking exception in respect to behavior was found to characterize the transplantation reactions of four human tumors. These were the meningiomas, the Schwannomas, the mixed salivary gland tumors, and the bladder papillomas. Such tumors were transplantable from the time of clinical discovery, and the ability to grow in normal animals bore no relationship to metastasizability. Among animal tumors, the Shope rabbit papilloma and the Rous chicken sarcoma have also been found to be anomalous in this respect, and both tissues possess the ability to grow in homologous and heterologous hosts at periods of development long antedating metastasis (1, 5).

In past experiments the homologous and heterologous transplantation of the two animal tumors was successfully effected with tissue obtained at various times during their early course, and no attempt was made to determine whether or not the property of transplantability was present from their initiation. Investigation of this question was expedient, for the absence of a nontransplantable phase would constitute a significant variation from the biological history of other tumors studied in this laboratory and would apply directly to a conception of cancer autonomy as a developmental state. Data relative to the human tumors was not applicable to the problem, and human material adequate for a pertinent study was obviously unobtainable. Accordingly, an investigation of the animal tumors with respect to the inception of transplantability was instituted, and the results obtained are described in the present report.

MATERIALS AND METHODS

The tumors utilized were induced by inoculation of domestic rabbits and chickens with cell-free filtrates of the Shope papilloma and the Rous sarcoma. The region of inoculation was biopsied and tissue obtained for transfer at intervals prior to and immediately following the appearance of lesions.

The tissue obtained from rabbits inoculated with the Shope papilloma virus was transplanted to other unrelated domestic rabbits and to guinea pigs, hamsters, and mice. Chicken tissue was transferred to rabbits, guinea pigs, and mice, but homologous hosts were not used, for the connective tissue present in the chicken's brain reacts to the Rous virus with the production of sarcomas, and a distinction from successful transplants cannot be made with certainty. The brain was used as a transplantation site in all cases. The technic employed has been described in detail (2).

RESULTS

Shope papilloma.—The filtrate was derived from glycercinated cottontail papilloma tissue known to produce visible changes in the scarified skin of domestic rabbits in from 18 to 20 days, and in order to study early lesions transplantation experiments were begun on the 14th day. Further biopsy specimens were transplanted to normal animals on the 16th and 21st days, and the series was

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controlled by the transfer of normal scarified skin obtained prior to treatment with the virus.

The transplantation of normal, uninfected skin resulted in takes in rabbit brains, but the tissue failed to survive transfer to the brains of heterologous species. At the end of a month the homologous transplants had increased in size and were well vascularized. Both dermis and epidermis were histologically intact. Mitoses were common in the lower layers of the epidermis and in hair follicles, and formed hairs were occasionally present. Epithelial-lined cysts filled with keratin were found in many transplants, but changes comparable to those characteristic of the Shope papilloma were absent (Figs. 1, 2).

Biopsy specimens obtained on the 14th day after treatment showed microscopic, scattered areas of thickened epidermis with early hyperkeratotic changes in the nature found in typical papillomas, and transplantation of the tissue was successfully effected in both homologous and heterologous species. Characteristic tumors were found in the brains of six of the thirteen rabbits used. The tumor masses were comparable in size to those resulting from the transfer of fully developed papillomas and frequently occupied the greater part of a cerebral hemisphere. Histologically, they were composed of papillomatous epitheloid epithelium and keratin (Fig. 3). The transplants in four of the remaining seven rabbits consisted of normal adult skin without papillomatous changes, and it seems probable, in these cases, that infected cells were not included in the fragments used for transfer.

The brains of guinea pigs and mice were used as sites for heterologous transplantation. No takes occurred in the twelve guinea pigs used, but twelve of the twenty mice bore growths (Fig. 4). These were larger in relation to brain size than the corresponding tumors in rabbits and in a number of instances resulted in death of the mouse in less than a month after transfer.

On the 16th day after scarification, a slight papular eruption was apparent on examination with a hand lens, and histological examination showed, in minor degree, the epithelial proliferation characteristic of the fully developed papilloma. Transfer was successful in the six rabbits used and in eight of the ten mice, but again there were no takes in guinea pigs.

Minute papules were visible to the naked eye on the 21st day, and individual lesions were dissected free of adjacent skin for transplantation. Transfer was made to the brain of rabbits and to the brain and subcutaneous space of guinea pigs, mice, and hamsters. No takes occurred in the subcutaneous space of guinea pigs, but large growths were present in the brain at the end of a month and in both sites in the other species (Figs. 5–9).

Rous sarcoma.—A cell-free filtrate of the Rous sarcoma was inoculated in the breast muscle of young chickens which were killed at 8, 10, and 12 days to provide material for transfer. Microscopic examination of tissues obtained on the 8th and 10th days showed foci of growth, but the tumor was not sufficiently demarcated to allow a clean separation from adjacent muscle before the 12th day. Accordingly, the material used for transfer on the 8th and 10th days consisted largely of random fragments of muscle from the inoculation site, while that employed on the 12th day was composed solely of tumor.

The brains of rabbits, guinea pigs, and mice were used as sites for transplantation, and the animals were killed 10 days after transfer. Microscopic examination of the transplants prior to the 12th day showed degenerating muscle surrounded by a zone of inflammatory reaction, and there was no evidence of tumor growth. However, the transplants of the 12th day were made up of characteristic sarcoma tissue with little or no evidence of foreign body reaction and had increased by 2–8 diameters in size. Takes occurred in all the six rabbits used, in eight of the twelve guinea pigs, and in eleven of the fifteen mice (Figs. 10–16).

**DISCUSSION**

It is apparent from these results that the Shope papilloma and the Rous sarcoma possess the ability to survive and to grow on homologous and heterologous transfer from their earliest stages of development. This property is not an attribute of other tumors at corresponding periods but, on the contrary, is a late development coincident with the attainment of metastasizability and antecedent to a fatal termination. Such associations are not found in the cases of the Shope and Rous tumors. Transplantability is present from inception, yet metastasizability is only acquired after an extended developmental course.

An investigation of transplantation reactions in the pre-metastasizable phase of the development of ordinary tumors suggests that the failure of growth in normal animals relates to a dependence on factors peculiar to the tumor-bearing animal (3). Such factors are not supplied by normal animals but are shared by the primary host and other animals bearing spontaneous tumors of the same nature. They are present from the earliest stages of tumor development and appear to be constitutional in nature. The early Shope and Rous tumors are of special interest from this point of view, for
their transplantability suggests either that they are autonomous from inception or that the factor on which their continued existence depends is not constitutional in nature but is transmitted to the new host along with the tumor cell.

Both the Shope papilloma and the Rous sarcoma are virus-induced tumors, and, during the greater part of their course, their continued growth appears to be conditioned on the continued presence of the virus. The virus is intracellular in location and necessarily accompanies the tumor cell in its transfer to a new host. It is suggested that the intracellular virus is analogous in its operation to the constitutional factors concerned in the dependence of other tumors and that the transplantability and growth of the Shope papilloma and the Rous sarcoma in normal animals is conditioned on the concomitant transfer of their dependent factors. The attainment of autonomy, or independence of conditioning factors, is a final stage in the development of other tumors, and there is evidence of a parallelism in the course of the Shope papilloma; for in the final stage of epidermoid carcinoma, its independence of conditioning factor is manifest by growth and transplantability in the absence of detectable virus (6–8).

The latter point is of some interest, as it may apply to investigations concerned with the possible virus etiology of other tumors. If the virus operates as a conditioning factor in such instances and the attainment of independence of the factor is a sequence in the natural development of the tumor, it is possible that, as in the case of the Shope papilloma, the virus may disappear with the assumption of autonomy. Accordingly, tumors in early or dependent phases of development would constitute a more logical material for virus search than the fully evolved, autonomous cancer.

A conception of the virus as a conditioning factor analogous in its action to the constitutional factors concerned in the dependence of other tumors and located in the cell rather than in the host implies that transfer of an infected cell would necessarily be accompanied by transfer of the factor on which its continued growth depends. Thus, the special environmental conditions requisite for the growth of other tumors transplanted during dependent phases of development are replaced by intracellular components in the case of virus tumors, and in this respect the survival and growth of transplants are independent of the constitutional status of the host. Accordingly, a question of a virus etiology is associated with tumors capable of growth in normal unrelated hosts during the dependent or pre-metastasizable phase of development and applies to the four human tumors characterized by such behavior—the meningioma, the Schwannoma, the mixed salivary gland tumor, and the bladder papilloma.

SUMMARY

Unlike other tumors studied, the Shope papilloma and the Rous sarcoma are heterotransplantable from their inception. The significance of this finding is discussed.

REFERENCES

3. ———. A Conception of Tumor Autonomy Based on Transplantation Studies: A Review. Ibid., 11:916-918.
Fig. 5.—Transplant of early papilloma, growing in the brain of a DBA mouse. The fragment used for transfer was obtained by biopsy from the skin of a rabbit 21 days after application of the Shope virus. The mouse bearing the transplant was killed 24 days after transfer. ×175.

Fig. 6.—Transplant of early papilloma growing in the brain of a guinea pig. The fragment used for transfer was obtained by biopsy from the skin of a rabbit 21 days after application of the Shope virus. The guinea pig was killed 31 days after transfer. ×175.

Fig. 7.—Transplant of early papilloma growing in the brain of a hamster. The transplanted fragment was obtained from the skin of a rabbit 21 days after treatment with the Shope virus and the hamster killed 32 days after transfer. ×175.

Fig. 8.—Transplant of early papilloma growing in the subcutaneous space of a DBA mouse. The transplanted fragment was obtained from the skin of a rabbit 21 days after treatment with the Shope virus, and the mouse was killed 33 days after transfer. ×175.
FIG. 9.—Transplant of early papilloma growing in the subcutaneous space of a hamster. The transplanted fragment was obtained from the skin of a rabbit 21 days after application of the virus and the hamster killed 58 days after transfer. ×40.

FIG. 10.—Transplant of Rous chicken sarcoma in the brain of a rabbit. The tissue was obtained from a chicken 12 days after injection of the virus, and the rabbit was killed 10 days after transfer. Note area of chicken muscle included in transplanted tissue. The muscle is dead and necrotic in contrast to actively growing tumor. ×85.

FIG. 11.—Transplant of Rous chicken sarcoma in rabbit’s brain. The tumor was obtained from a chicken 12 days after injection of the virus, and the rabbit was killed 10 days after transfer. ×920.

FIG. 12.—Transplant of Rous chicken sarcoma in a guinea pig’s brain. The tumor was obtained from a chicken 10 days after injection of the virus, and the guinea pig was killed 10 days after transfer. ×85.
Fig. 13.—Transplant of Rous chicken sarcoma in a guinea pig’s brain. The tumor was obtained from a chicken 10 days after injection of the virus, and the guinea pig was killed 10 days after transfer. \( \times 320 \).

Fig. 14.—Transplant of Rous chicken sarcoma in the brain of a DBA mouse. The tumor was obtained from a chicken 10 days after injection of the virus, and the mouse was killed 10 days after transfer. \( \times 230 \).

Fig. 15.—Transplant of Rous chicken sarcoma in the brain of a DBA mouse. The tumor was obtained from a chicken 10 days after injection of the virus, and the mouse was killed 10 days after transfer. \( \times 320 \).

Fig. 16.—Transplant of Rous chicken sarcoma in the brain of a DBA mouse. The tumor was obtained from a chicken 10 days after injection of the virus, and the mouse was killed 10 days after transfer. \( \times 320 \).
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