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THE INDUCTION OF THE ROUS SARCOMA IN HETEROLOGOUS TRANSPLANTS OF VIRUS-INFECTED ADULT CHICKEN TISSUE**

The capacity of the Rous chicken tumor to survive heterologous transplantation has been demonstrated in both early and late stages of development.[1,4,6] Such investigations were concerned with the transformed cell while the question at issue in the present study involves the cell during the process of transformation.

It has been observed that adult rabbit skin infected either *in vivo* or *in vitro* with the Shope papilloma virus attained the ability to survive transplantation to the subcutaneous space of the hamster.[5] In contrast to the destructive foreign body reaction characteristic of the heterologous transfer of normal adult rabbit skin, the infected epithelial cells remained viable in the hamster for a sufficient time to develop a typical papillomatous transformation and occasionally survived as histologically intact epidermis without papilloma formation. It appeared, accordingly, that incident to infection and prior to neoplastic transformation, the cells of the adult rabbit's epidermis acquired the property of heterologous transplantability. The experiments described in the present paper were instituted to determine whether an analogous acquisition was associated with the process of Rous sarcoma transformation in chicken cells.

MATERIALS AND METHODS

A filtrate of fresh Rous tumor was injected into the breast muscle of a group of adult Leghorn chickens, and the animals were killed at intervals from 1 to 24 hours. The injected area was removed by sterile technique and minute fragments were dissected for transfer.

The recipient animals were adult mice of DBA strain and received no conditioning treatment before or after transfer. Transfer was accomplished by means of a No. 18 trocar inserted into the substance of the brain through a small burr hole in the

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right parietal bone. Six mice were used in each experiment, and all were killed for histological brain examination on the eighth day after transfer.

RESULTS

The results are shown in Table 1. A total of 48 mice received fragments of infected muscle and, after eight days' residence in the mouse brain, 39, or 80 per cent, of the transplants contained proliferations of Rous transformed cells. The frequency of the successful transfer of infected cells did not vary significantly throughout the experiment, and the incidence at 1 and 24 hours was identical.

Histological sections of muscle derived from the infected area in the chickens at the time of transplantation were not recognizably different

TABLE 1. THE INCIDENCE OF NEOPLASTIC TRANSFORMATION IN FRAGMENTS OF ADULT CHICKEN MUSCLE INFECTED *in vivo* WITH THE ROUS SARCOMA VIRUS AND TRANSPLANTED TO THE BRAINS OF MICE

Hours between infection and transfer	1	2	3	4	5	17	19	24
Number of mice bearing transplants	6	6	6	6	6	6	6	6
Number of transplants with neoplastic transformation	5	5	4	4	5	6	5	5

from sections of normal control muscle, and there were no changes indicative of virus infection (Fig. 1). However, the appearance of transplants from the mouse brain was identical with that of the Rous sarcoma produced in chickens (Figs. 3, 4, 5, 6, 8). In many instances, no remnants of muscle were found, but in others, fragments presented as swollen, homogeneous fibers without nuclei or cross striations. A lymphocytic and polymorphonuclear infiltrate surrounded the growing mass but permeation through the proliferating fibroblastic zone to centrally placed muscle fibers was much less pronounced and contrasted with the dense concentration characteristically found about transplants of normal uninfected muscle (Figs. 2, 7).

In several cases, fragments of the transplants were transferred to the brains of other mice, and the tumor was successfully carried for a number of serial generations. The inflammatory reaction found in the primary transplants did not occur in subsequent generations. In other cases, the fragments were transplanted back to chickens with the production of tumors of typical appearance and behavior.

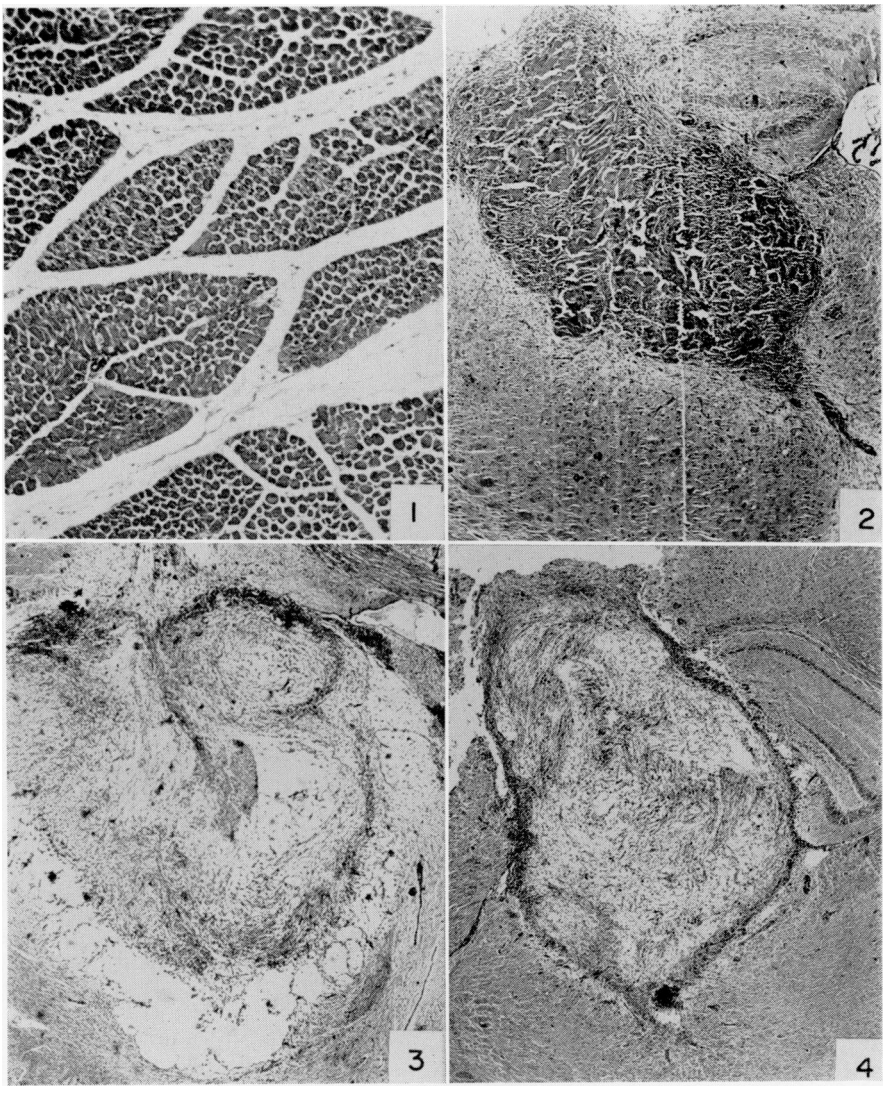

FIG. 1. Chicken muscle 24 hours after infection with the Rous sarcoma virus. The histological appearance of muscle and connective tissue is identical with that found in control sections of normal chickens. Mag. X 150.

FIG. 2. Section of mouse brain containing a transplant of normal chicken muscle eight days after transfer. The muscle is necrotic and is surrounded and permeated by a dense infiltrate of lymphocytes and polymorphonuclear leukocytes. Mag. X 40.

FIG. 3. Section of mouse brain containing a transplant of muscle removed from a chicken three hours after infection with the Rous virus. The mouse was killed eight days after transfer. There is a small remnant of necrotic muscle near the center of the transplant, but its bulk is made up of transformed sarcomatous fibroblasts. Note the decreased inflammatory reaction as compared with Figure 2. Mag. X 35.

FIG. 4. Section of mouse brain containing a transplant of muscle removed from a chicken five hours after infection with the Rous virus. The mouse was also killed on the eighth day, but the muscle tissue has disappeared and the transplant consists entirely of Rous sarcoma cells. Mag. X 35.

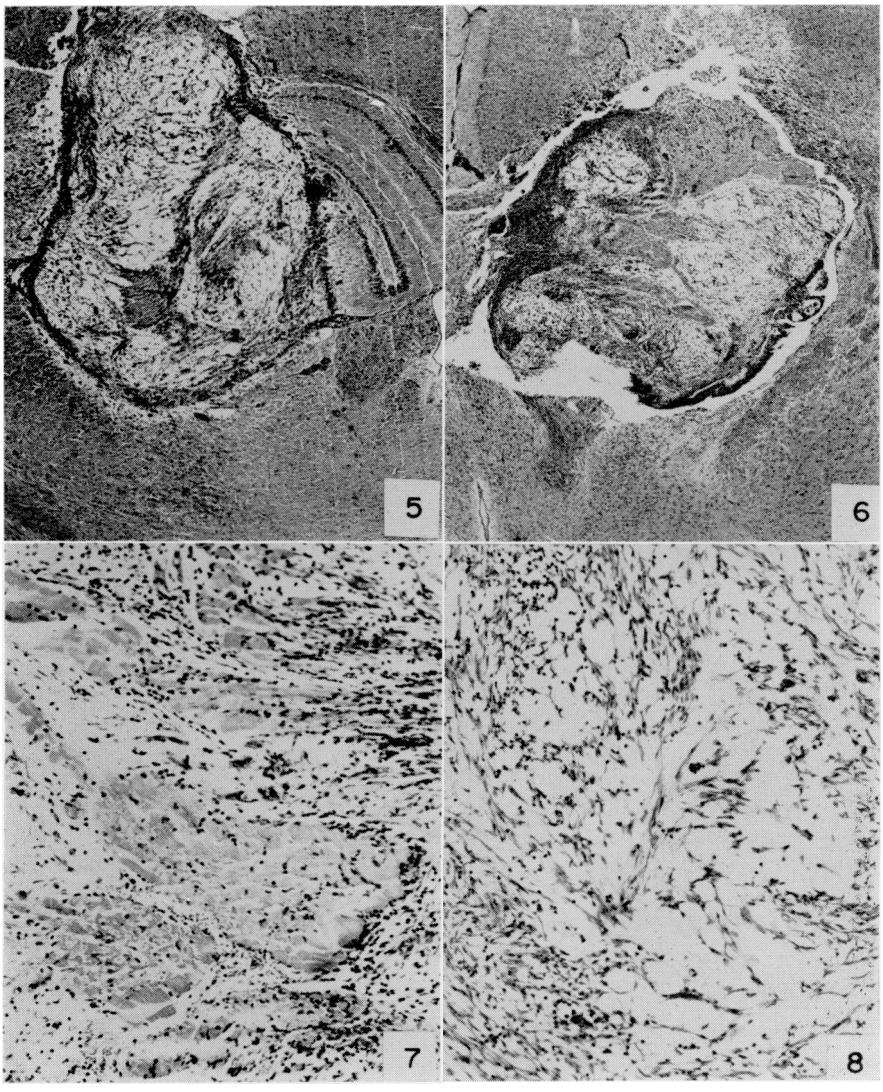

FIG. 5. Section of mouse brain containing a transplant of muscle from a chicken 17 hours after infection with the Rous virus. Mag. X 35.

FIG. 6. Section of mouse brain containing a transplant of muscle from a chicken 24 hours after infection with the Rous virus. Mag. X 35.

FIG. 7. Section of degenerated muscle from the center of a transplant in a mouse brain. The fibers are homogeneous, lack cross striations and nuclei are absent. Rous cells are present between muscle bundles and the lymphocytic reaction is less intense than that found encircling uninfected muscle transplants. Mag. X 150.

FIG. 8. Section from periphery of same transplant (Figure 7) showing typical Rous transformed fibroblasts. Mag. X 150.

DISCUSSION

In previous experiments, it was found that fragments of mesodermal tissue from ten-day-old chick embryos infected with the Rous virus *in vitro* and transplanted to the brains of normal mice afforded a nidus for the development of the Rous sarcoma.[1] Similar results have been obtained by others using the brain of conditioned rats as a transplantation site,[7] and it is clear that infected embryonic cells retain the capacity to undergo neoplastic transformation during residence in an alien species. However, the results of experiments utilizing embryonic tissue do not constitute evidence that virus infection influenced the successful transplantation of the tissue, since normal embryonic chick tissues without virus infection are also transplantable to the mouse brain.[1]

Adult chicken tissues, on the other hand, like adult tissues of all species will not survive heterologous transfer, but excite an inflammatory foreign body reaction accompanied by necrosis of all components of the graft including fibroblasts as well as muscle. In the present experiments, the histological appearance of the virus infected muscle used for transfer was not different from that of normal adult muscle, and there was no alteration in the number or morphology of fibroblasts to suggest that neoplastic transformation had occurred at the time of transplantation. However, heterologous transplants of such tissue contained proliferating masses of transformed fibroblasts in addition to degenerated muscle, demonstrating that their parent cells, unlike normal fibroblasts, possessed the capacity to survive transfer to an alien species. It would appear, therefore, that the virus-infected fibroblast in the process of transformation shares this property wtih the fully transformed neoplastic cell.

The significance of this observation lies in the fact that the transplantation reactions of adult chicken cells are modified by infection with the Rous virus and that this modification precedes neoplastic transformation. The relationship between these events is of interest from several points of view. It has been postulated that the early stages of chemical carcinogenesis are associated with the loss of specific cellular antigens[8] and a comparable occurrence in viral carcinogenesis would supply a rational explanation for the acquired capacity of the transforming chicken fibroblasts to survive heterologous transfer. On the other hand, the acquisition of heterologous transplantability following the infection of rabbit epidermal cells with the Shope virus is occasionally associated with survival of the cells without neoplastic transformation, suggesting that the two phenomena may represent independent effects of viral infection rather than causally related events.

SUMMARY

Normal adult chicken tissue transplanted heterologously to the mouse brain induces an inflammatory foreign body reaction accompanied by destruction of all elements of the graft. In contrast, adult chicken tissue infected *in vivo* with the Rous sarcoma virus and transplanted during the first 24 hours after infection gives rise to the growth of typical Rous sarcoma cells in the brain of the alien species. Inasmuch as the infected fibroblasts are transplanted prior to neoplastic transformation, it is concluded that the property of heterologous transplantability is attained by infected cells during this process.

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