Infectiousness of the Virus in Shope Papillomas Induced or Grown in the Variant Rabbit Strain and in a Resistant Species

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In the great majority of cases, the pathogenicity of the Shope papilloma virus is lost after transfer from cottontail to domestic rabbits. There is adequate evidence that the virus is present in the papillomas arising in domestic rabbits, for such animals develop neutralizing and complement-fixing antibodies. Further, the intraperitoneal inoculation of extracts of noninfectious papillomas results in the production of antibody and in resistance to reinfection (4, 5). It has been concluded, on this basis, that the virus exists in an altered or "masked" form, but the nature or mechanism of the masking process is not understood. Evidence has been obtained to suggest that antibody is concerned in the phenomenon (3), but the significance of its action is questionable in view of the high titer found in cottontail rabbits bearing infectious papillomas.

The experiments to be presented were carried out to determine whether the masking effect was dependent on the body fluids of the domestic rabbit or on the constitution of the epithelial cells harboring the virus. To this end, papillomas derived from cottontail and domestic rabbit skin were tested for the presence of infectious virus after induction in or transplantation to the variant strain.

MATERIALS AND METHODS

Transposition of the papilloma tissue was effected in two ways. In one group of experiments, papillomas were induced in the skin of cottontail and domestic rabbits and subsequently transplanted to the variant strain (cottontail papilloma to domestic rabbits and domestic papilloma to cottontail rabbits). In a second experiment, normal adult domestic and cottontail skin, removed by biopsy, was infected with the virus in vitro and transferred to the variant strain. In a further series, undertaken to determine the effect of residence in a naturally resistant host on the infectiousness of the virus, both of the previous experiments were repeated with the hamster as the recipient host.

The technics employed have been described in detail (1, 2), but general procedures may be repeated here. Both papillomas and normal skin were washed in several changes of physiological saline after removal and treated for 1/2 hour in a mixture of penicillin and streptomycin. The normal skin was scarified in vitro and bathed for an additional half-hour in virus fluid. Living portions of the papilloma tissue and epidermal layers of the normal skin were selected for transplantation. Fragments measuring approximately 1/2 mm. in diameter were used, and transfer was effected by means of a trocar. The brain was employed as a transplantation site in the transfer of tissue to the rabbit, but the subcutaneous space was used in all experiments involving the hamster.

The rabbits and hamsters bearing transplants were killed at varying periods after transfer. The transplants were dissected free of normal tissue, ground to a paste, and suspended in saline. After standing, the supernatant fluid was withdrawn and tested for the presence of infectious virus by inoculation in the skin of domestic rabbits.

In brief, two types of experiments were carried out. One involved the use of papilloma tissue arising in intact rabbits. Three steps were employed: (a) induction of papillomas in domestic or cottontail rabbits with stock glycerolated cottontail virus, (b) transplantation of living fragments of the induced papillomas to domestic rabbits, cottontail rabbits, or hamsters, and (c) assay of the resulting growth for the presence of infectious virus by preparing a saline extract and rubbing this on the scarified skin of domestic rabbits. In the second type of experiment the starting ma-
terial consisted of fragments of skin obtained by biopsy from normal adult domestic or cottontail rabbits and the procedure involved also consisted of three steps: (a) infection of the skin fragments in vitro with virus obtained from glycerolated cottontail papillomas, (b) transplantation of the infected skin fragments to the variant rabbit strain and to hamsters, and (c) assay of the resulting papilloma for infectious virus in domestic rabbits.

RESULTS

The successful homologous transplantation of papilloma tissue between domestic rabbits as well as the heterologous transfer of such tissue to the subcutaneous space of the hamster has been described (1, 2). Comparable results were obtained when the cottontail rabbit was used as donor or recipient. The transfer of adult skin is also generally successful when the brain of the same or variant strain is used as a transplantation site; and, if the skin fragment is infiltrated with the Shope virus, a characteristic papilloma develops. The use of the brain as a transplantation site is apparently a significant factor in such transfers, and relatively few takes occur in the subcutaneous space. In contrast, the subcutaneous space of the hamster has proved as suitable a nidus for growth as the brain of this species, and, although the incidence of takes is considerably less than following homologous transfer, a small percentage of the infected transplants grow and eventuate as papillomas.

Several precautions apply to both the homologous and heterologous transfer of adult skin. The skin of the adult rabbit consists of a thin sheet of epithelial cells covering a thick dermis, and whole thickness sections may appear to consist almost entirely of the latter. It should be emphasized, however, that the upper portion of this layer contains the hair follicles and that the cells comprising these structures constitute the site of predilection of the Shope virus. Accordingly, the preferential fragment for transfer consists of epidermis and subjacent dermis, and thin shavings obtained by horizontal sectioning are usually inadequate. Satisfactory fragments may be obtained by excising the upper half of the cut surface or by pinching the epidermal surface with sharp-pointed forceps and removing the raised area with a razor blade or sharp curved scissors. In either case, the proportion of epithelial cells in the fragment is relatively small and the possibility that they may escape infection can be partially counteracted by further infiltration of the fragment with virus-containing fluid utilizing a cutting surgical needle.

The transplantation of infected adult rabbit skin to the brain of other rabbits results in takes in approximately 70 per cent of cases and half of the successful transplants show typical papillomatous transformation at the end of a month (Figs. 1, 2). In many other successful transplants, the epidermis is thickened and hyperkeratotic, and, although characteristic papillary formations are absent, it seems probable that the changes represent reaction to the virus.

The growths found at the end of a month are rarely as large as those obtained with infected embryonic skin and usually represent no more than a three- to fourfold increase in size of the transplanted tissue. Occasional transplants show no apparent increase in size and approximate the fragments used for transfer but contain typical papillomatous lesions on microscopic examination. Growth of sufficient size to kill the hosts are present in only a few instances at the end of a month but are the rule in animals held beyond the 50th day.

The transfer of infected adult rabbit skin to the subcutaneous space of hamsters results in takes in about 25 per cent of cases, but three-quarters of these show characteristic papillomatous changes (Figs. 3 and 4). The transplants are usually palpable in 10 days and may increase to nodules 1 cm. in diameter by the end of a month. Like brain transplants, they consist of a central core of keratin surrounded by thick walls made up of epithelium in papillomatous arrangement.

A point of considerable interest concerns the transplants of infected adult rabbit skin showing no histological evidence of papillomatous reaction. The transfer of normal adult rabbit skin to the subcutaneous space of hamsters is associated with an inflammatory reaction, and the transplants do not survive. Infected transplants of adult rabbit skin fail to excite a foreign body reaction but become vascularized and persist for long periods of time. Further, such behavior does not appear to depend on the occurrence of papillomatous transformation, and frequently the transplants persist as histologically intact normal skin (Figs. 5 and 6).

Infectiousness of the virus in transplanted papillomas.—The infectiousness of the virus contained in cottontail papillomas grown in domestic rabbits, domestic papillomas grown in cottontail rabbits, and both types of papillomas grown in hamsters was tested by applying saline suspensions of ground tissue to the scarified skin of domestic rabbits. The results obtained are shown in Table 1 and summarized in Table 2.

The infectiousness of the virus remained unchanged after transfer of the papilloma to the
brains of normal animals of the strain of origin—that is, cottontail papillomas grown in the brains of cottontail rabbits were infectious, while domestic papillomas grown in the brains of domestic rabbits failed to induce skin lesions. Further, the infectiousness of the virus was not altered by residence and growth of the papilloma in the variant strain or in an alien species. Cottontail papillomas retained their infectiousness despite transfer to the domestic rabbit although, characteristically, the property is lost following inoculation of the cell-free virus in this strain. Further, there was no indication of a modification of the virus after growth of the papilloma in the hamster, an animal species distinguished by possession of natural resistance to the infection. On the other hand, the domestic papilloma did not acquire infectiousness following transfer to the cottontail rabbit or the hamster.

The skin lesions induced with cottontail papillomas after growth in domestic rabbits and hamsters were similar to those obtained with cottontail papillomas grown in the brains of cottontail rabbits. The lesions in the skin of the test animals were rarely confluent and generally appeared as isolated growths spaced irregularly throughout the scarified area. Occasionally their appearance was delayed for as long as 5 weeks, whereas emulsions obtained directly from the primary tumor in the cottontails' skin produced growths in 3–4 weeks. Further, papillomas did not develop in all the test animals, and in the two cases, Tests 1 and 6 (Table 1), no lesions occurred in three of the eleven rabbits employed. Identical results, however, were obtained with cottontail papillomas grown in the brains of cottontail rabbits, and, inasmuch as comparable amounts of material were used for scarification, it seems probable that the factors determining variations from the infectious behavior of primary cottontail papillomas were of a quantitative nature and not related to the site of transplantation or the constitution of the new host.

Attempts to induce papillomas serially from the lesions of test animals used for assay were unsuccessful. Although the growths in domestic rabbit brains resulting from the transplantation of cottontail papillomas induced papillomas in the skin of other domestic rabbits, the induced papillomas themselves were not infectious. Similarly, papillomas induced in domestic rabbits by suspensions of hamster-grown cottontail tissue could not be passed serially to other domestic rabbits. Attempts to induce lesions in the skin of adult hamsters with such material were also unsuccessful.

The failure of the natural resistance of the hamster to influence the infectiousness of the cottontail papilloma was duplicated in experiments involving the rat, mouse, and guinea pig (Fig. 7). Cottontail papillomas grown in the brains of these species retain their ability to infect the skin of domestic rabbits and transplants of domestic papillomas remain noninfectious.

Infectiousness of the virus in transplanted skin.—It was concluded from the preceding experiments

**TABLE 1**

<table>
<thead>
<tr>
<th>Test no.</th>
<th>Host of induced papilloma</th>
<th>DURATION (days)</th>
<th>Host of transplanted papilloma</th>
<th>DURATION (days)</th>
<th>Infectivity test No. with papillomas</th>
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<tr>
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<td>7</td>
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<td>&quot;</td>
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<tr>
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<td>&quot;</td>
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</tr>
</tbody>
</table>

**TABLE 2**

**INFECTIOUSNESS OF THE VIRUS AFTER TRANSFER OF THE PAPILLOMA TO THE VARIANT STRAIN AND RESISTANT SPECIES (SUMMARY)**

<table>
<thead>
<tr>
<th>Host of induced papilloma</th>
<th>Host of transplanted papilloma</th>
<th>Infectiousness of virus for domestic rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottontail rabbit</td>
<td>Domestic rabbit</td>
<td>+</td>
</tr>
<tr>
<td>Domestic rabbit</td>
<td>Cottontail rabbit</td>
<td>-</td>
</tr>
<tr>
<td>Cottontail rabbit</td>
<td>Hamster</td>
<td>+</td>
</tr>
<tr>
<td>Domestic rabbit</td>
<td>Hamster</td>
<td>-</td>
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</tbody>
</table>

That residence of the Shope virus in cottontail papilloma cells, irrespective of the cells' environment—domestic, rabbit, or hamster—protected the virus from "masking." Susceptibility to "masking," however, was not altered by the experimental procedures, and the virus underwent such a process upon infection of domestic rabbit cells. It seemed desirable to determine whether the protection afforded by the papilloma cell was provided by normal cells. Accordingly, the transplantation experiments were repeated with nor-
normal adult domestic and cottontail skin infected in vitro rather than tissue from mature lesions. The resulting papillomas from the recipient host were then tested for the presence of infectious virus in the scarified skin of domestic rabbits.

The data obtained are shown in Table 3 and summarized in Table 4. The results were identical with those found in the preceding experiments. Infectious virus was recovered from papillomas induced in cottontail skin after transfer to domestic rabbits and hamsters, whereas the papillomas arising in domestic skin in cottontail rabbits or hamsters were noninfectious.

**DISCUSSION**

Although the actual presence of the Shope virus in the papillomas of domestic rabbits can be readily demonstrated by immunological techniques (4), the infectiousness of the virus is generally lost in a single passage. There have been experiments to suggest that antibodies, evoked in response to the infection, are concerned in this alteration (3). However, the antibody found in high titer in papilloma-bearing cottontails does not appear to be different, and the virus remains infectious in this strain.

The present experiments were undertaken in an attempt to supply additional data pertinent to the problem. The experiments were based on the transplantability of papillomas and of adult skin and the consequent capacity to transfer these tissues from the environment of their natural host to that of an animal of the variant strain or even of different species. Accordingly, papillomas induced in the skin of cottontail rabbits could be removed and transplanted to domestic animals and, in such a situation, be subjected to any influence present in the body fluids of this strain that might operate to alter the infectiousness of the virus. Further, the activity of factors existing in the environment of naturally resistant species could be similarly determined.

The results showed that the body fluids of the new host were without influence on the infectiousness of the virus contained in the transplanted papilloma. Cottontail papillomas remained infectious after growing in domestic rabbits and hamsters, and the pathogenicity of domestic papillomas was not restored by growth in cottontails or in the alien species.

The transplantation procedures were repeated with freshly infected adult skin used in place of tumor tissue, and identical results were obtained. Infected cottontail skin transplanted to domestic rabbits or hamsters developed papillomas containing infectious virus, while the papillomas derived from domestic skin growing in cottontails and hamsters proved to be noninfectious. It should be noted that, in the latter experiments, the only elements in the complex representative of the domestic rabbit. The animal was killed 30 days after transfer. ×30.

**FIG. 1.—Transplant of adult domestic rabbit skin infected in vitro with the Shope papilloma virus growing in the brain of a cottontail rabbit. The animal was killed 23 days after transfer. X57.**

**FIG. 2.—Transplant of adult cottontail rabbit skin infected in vitro with the Shope papilloma virus growing in the brain of a domestic rabbit. The animal was killed 30 days after transfer. X30.**

**FIG. 3.—Hamster bearing subcutaneous transplant of adult domestic rabbit skin infected in vitro with the Shope papilloma virus. The skin over the transplant has been removed. Photograph taken 25 days after transfer.**
tumors (7). The relatively slight difference between these two series for mice with AK4 leukemia early massive blood-borne invasion of organs. For transition from peritoneal exudate was overshadowed by organ invasion from various sites (charts) was opposite tendenciespto spread extensively into and nonleukemic tumors with regard to their two primary growth and to be implanted into connective organs by blood route at the expense of the primary growth and--indirectly and directly--in the death of the host. This factor is least significant for leukemic cells is an important factor in the amount of tumor cells on the 6th day) was higher for leukemias (AK4, P1534, C1498) than for nonleukemic tumors, inversely to the results on malignant tumors (S-37, lymphoma) tumors. An inverse proportional growth and to be separated from the primary growth, to be carried to organs was the essential lethal factor in leukemias. However shorter survival of animals with large tumor growths in the scalp as suggested that metastatic infiltrative growth of all leukemic strains the difference in the amount of cell implantation into abdominal organs from peritoneal exudate. It appears from the results on "malignant cells is based on their interdependence, while the ability to grow as free cells in body fluids, to be separated from the tissue, and to infiltrate into" tissues growth 'from benign and malignant growths (6) that both tendencies vary only quantitatively from subcutaneous growth. This was attributed to their reduced adhesiveness (1). Their high tendency to early spread in the blood (AK4); however, shorter survival of patients with AK4 leukemia than for nonleukemic tumors, inversely to their probability to early massive blood-borne invasion of organs. For leukemias reported above and those on other tumors (7) as well as their shorter survival (estimated from total cell number and percentage distribution of Blood-borne Tumor Metastases: A Preliminary Investigation. J. Clin. Path., 4:139-36, 1951.)
FIG. 4.—Section of transplant shown in Figure 3. ×30.

FIG. 5.—Transplant of adult domestic rabbit skin infected in vitro with the Shope virus growing in the brain of a domestic rabbit. The animal was killed 38 days after transfer. Note absence of papillomatous changes and preservation of normal architecture. ×57.

FIG. 6.—Transplant of adult domestic rabbit skin infected in vitro with the Shope virus growing in the subcutaneous space of a hamster. The animal was killed 88 days after transfer. There is no evidence of papilloma and the heterologous skin appears in good condition. ×125.

FIG. 7.—Transplant of adult domestic rabbit skin infected in vitro with the Shope virus growing in the brain of a guinea pig. The animal was killed 28 days after transfer. ×175.
tumors (7). The relatively slight difference between these two series for mice with AK4 leukemia is stated, indicating that early massive blood-borne invasion of organs from peritoneal exudate was overshadowed by the transition between various strains of leukemias. While compared to those bearing scantier local growths, shorter survival suggests that metastatic infiltrative growth of cells in the flank indicates the role of primary growth as a factor in leukemias. However, shorter survival of animals with large tumor growths in the scalp as leukemic cells into organs was the essential lethal factor. Eventually, being the direct lethal factor.

In the organs, it was observed that invasion from various sites (charts) was paralleled by a difference in survival (Table e), suggesting that for this strain the role of implantation from peritoneal exudate into abdominal organs was the essential lethal factor. For this strain, the role of implantation from peritoneal exudate was overshadowed by the transition between various strains of leukemias. The purpose of this paper was to study the primary growth of leukemic cells.

**SUMMARY**

- The primary growth in the peritoneal exudate paralleled by a difference in survival (Table e).
- The role of implantation from peritoneal exudate into abdominal organs was the essential lethal factor.
- The primary growth in the peritoneal exudate indicated the role of primary growth as a factor in leukemias.

**REFERENCES**

8. GOLDIE, H.; WEST, H. D.; JEFFRIES, B. R.; and CLARK, M. C.; and HAHN, P. F., Growth Characteristics of Leukemias AK4, P1534, and C1498; malignant lymphoma, S-37, S-180; carcinomas in C57-6 and in C3H mice) and that the tendency of tumor cells to invade blood-borne infiltrative growth in organs is a specific characteristic of malignant cells due to their interdependence, while the ability to grow as free cells in body fluids, to be separated from the tissue, and to infiltrate into organs by blood route at the expense of the primary growth and to be implanted into connective tissue either at the site of inoculation or from the opposite tendencies to spread extensively into organs was the essential lethal factor.
mestic rabbit were the epidermal cells; these received a cottontail blood supply and were bathed in cottontail fluids. However, masking of the virus occurred precisely as though the cells were part of the skin of an intact domestic rabbit. Conversely, the milieu of a cottontail skin transplanted was that of the domestic rabbit, and its humoral relationships to the new host were not essentially different from those of the domestic rabbit's own skin; still, the infectiousness of the virus contained in its cells remained unchanged.

It is apparent that in these experiments the body fluids of the domestic rabbit were not concerned in the masking process. In the case of domestic skin transplants, masking occurred in the absence of domestic fluids, and in the case of cottontail transplants masking did not occur despite the intimate domestic relationships. It might be suggested that in the first instance alteration of the virus was effected during the course of infection, for this represents the only period when contact between virus and domestic body fluids was possible. However, the skin fragments were bathed in antibiotic mixtures and in several changes of saline, and it seems probable that the body fluids present were either washed away or greatly diluted prior to addition of the virus. The depletion of body fluids in tissue fragments by such procedures is demonstrated by the fact that skin removed from immune rabbits and so treated is susceptible to infection with the virus.¹

A further point warrants consideration in this connection. The Shope virus occupies an intracellular position in infected skin, and the cell membrane may be impermeable to factors in the body fluids concerned with masking. In such case, the protected virus would remain unchanged throughout its intracellular existence irrespective of the cell's environment—whether cottontail, domestic, or hamster in nature. Consequently, masking could only occur when the extracellular virus and body fluids were in contact. Other investigations have been concerned with the possibility that alteration of the virus took place at such periods, but the results are controversial (3, 6).² As has been noted, the present techniques minimized the opportunity for reaction between virus and extravasated fluid.

It would appear, therefore, that these experiments support the view that the body fluids are not concerned in the masking process and that alteration of the virus leading to loss of infectiousness is a function of factors resident in the epidermal cells of the domestic rabbit.

It is of interest that the virus survives, unaltered, in the environment of a host possessing natural resistance to the infection. Such circumstances suggest that the natural resistance of the hamster to infection with the Shope virus relates to a factor associated with its epidermal cells rather than with its humoral constitution.

The successful transfer of infected adult rabbit skin to the subcutaneous space of the hamster requires some comment. Despite numerous attempts, the heterologous transplantation of normal adult tissues has not been effected. Normal adult rabbit skin transferred subcutaneously to the hamster excites a foreign body reaction, and takes do not occur. It would seem, therefore, that infection of the skin with the Shope virus was a significant factor in its survival. Further, growth and survival of the transplant are not necessarily associated with papillomatous transformation, for, in many instances, such a reaction does not occur, and microscopic examination at the end of 6 weeks shows a normal, healthy, unchanged epidermis. An interpretation of this effect is not possible from present data, and pertinent studies are being continued.

**SUMMARY**

Shope papillomas derived from cottontail and domestic rabbit skin but induced or grown in the variant strain or in a resistant species were tested for the presence of infectious virus. It was found that papillomas induced in the intact animal or in isolated fragments of skin were unaltered in infectiousness following growth in the new environment. It was concluded that the body fluids of the domestic rabbit were not concerned in the masking process and that alteration of the virus leading to loss of infectiousness was a function of factors resident in the epidermal cells of this strain.

**REFERENCES**

2. **—. Transplantation of the Shope Papilloma and Rous Sarcoma during Early Developmental Stages. Ibid., pp. 726.
4. **—. The Detection of a “Masked” Virus (the Shope Papilloma Virus) by Means of Immunization. Results of Immunization with Mixtures Containing Virus and Antibody. Ibid., 74:321, 1941.

¹ H. S. N. Greene, unpublished experiments.
² J. T. Syvertton, unpublished experiments.
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