The Virus-induced Papilloma-to-Carcinoma Sequence

IV. Carcinomas in Domestic Rabbits Infected while in Utero*

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The growth pattern of the epithelial tumor induced by the rabbit papilloma virus (Shope) (11) is characterized by a sequence of changes that is well defined and predictable (4). Three successive growth phases occur: proliferative, stationary, and involutionary. The involutionary phase can terminate either in regression and disappearance, or in cancer. Since earlier published studies of the virus-induced papilloma and of the cancer that may follow (1—11, 13, 15) left open the comparative incidence of malignancy in the two host species, it was of particular interest to learn (15, 16) that cancer occurred without interference with the natural sequence of alternative changes in about 85 per cent (32 out of 187) of the cottontail rabbits and in about 75 per cent (22 out of 30) of the domestic rabbits that had been under observation for 6 months or longer. Moreover, it became apparent from the results of this same study (15), and from the other two communications in this series (16, 17), that papilloma virus can operate as a remarkably efficacious carcinogenic agent, for both domestic and cottontail rabbits. This observation made it desirable to select for experimental study certain factors that may operate to condition the papilloma-to-carcinoma sequence or to influence differences in species and host reactivity. Among these factors is the age of the host. Since age is recognized commonly as such an exceedingly important determinant for conditioning the extent and type of response to infectious, immunogenic, and carcinogenic agents, the present experimental study was carried out to learn what influence the immaturity of the host might exert upon the papilloma-to-carcinoma sequence. For this purpose fetal rabbits, while still in utero, were inoculated intracutaneously at multiple sites with papilloma virus. These fetal rabbits were kept under observation after birth so as to record for each animal (a) the time of appearance of papillomatous lesions, (b) any differences from the sequence of changes which had been observed for 187 mature rabbits (15), and, particularly, (c) the time interval for the development of cancer. The findings of this study are contained in the present paper.

MATERIALS AND METHODS

Virus.—The papilloma virus (Shope) was obtained from papillomas that had been incurred under natural conditions in Kansas by cottontail rabbits (genus Sylvilagus). The papillomatous tissue was kept in 50 per cent neutral glycerol at 4°C. until it was made ready for transfer either to fetal or to control adult rabbits. The tissue was thoroughly triturated with alundum in 0.85 per cent NaCl solution to yield a 10 per cent suspension. This suspension was centrifuged in an angle-head at 2,000 r.p.m. for 30 minutes and the supernatant fluid withdrawn for inoculation.

Rabbits.—Fetal rabbits, provided by purebred American Dutch does, were employed as the test animal. Since the period of gestation for domestic rabbits (genus Oryctolagus) is 30 days, the does were bred and subjected 20 days thereafter to a laparotomy when their fetuses were from 18 to 20 days of age.

Method of inoculation.—The mother rabbit was placed on its back with its legs extended and fastened by ties to an operating table; the abdominal region was shaved, cleansed with soap and water, and zephiran chloride was applied. Deep anesthesia was induced and maintained by utilizing in combination sodium pentothal intravenously and ether by inhalation. The bicornuate uterus
with its contained fetuses was exposed quickly by making a 2-inch left rectus incision extending from the level of the umbilicus to the symphysis pubis. Since it was impractical to employ the cross-hatch scarification technic for transfer of papilloma virus for the inoculation of embryos in utero, another method was devised. The approach consisted of grasping between the thumb and forefinger each uterine horn with its contained fetuses, so as to make known the bodily configuration of each fetus. A 26-gauge needle attached to a 1-ml. syringe containing 0.5 ml. of virus suspension made it possible to pierce the uterine wall and to make multiple insertions into the underlying fetal skin. It can be assumed that the virus was thereby brought regularly into contact with injured epithelial cells. Each embryo was inoculated in this manner at multiple sites for a total of approximately 0.5 ml. of virus suspension.

The female rabbits usually gave birth to litters within 10 days of injection. However, some do abort, and other rabbits practiced cannibalism to destroy their entire litter soon after birth.

**RESULTS**

The offspring that were inoculated while in utero were kept for observation of the papillomatous-tocarcinoma sequence. The successive changes were found to conform to the growth pattern in domesticated rabbits (15).

None of the young at birth exhibited any signs of skin injury or papillomatous growth. However, in from 7 to 10 days after birth or from 17 to 24 days after inoculation, discrete papillar growths appeared. Many of these papillomas merged to form aggregative masses. None of the animals was devoid of growths. The final number of papillomatous lesions on single animals ranged from three to seventeen. These papillomas proliferated for 8 months. Lateral extension subcutaneously of the papillomatous growths was observed in two of the animals. The proliferative phase was followed by the stationary, or quiescent, stage. Alternative changes began to appear about the fifth month to result during the succeeding 8 months in carcinomatous degeneration in lesions of five of the hosts. It is of interest that none of the papillomas was observed to regress completely, although a number showed marked diminution in size. The six rabbits that survived for 6 months or longer provided tissues at biopsy for histopathological study. Five of the six rabbits within 7 months after injection yielded tumors that were proved microscopically to be epidermoid carcinomas, and the sixth rabbit was observed to have lesions undergoing degenerative changes during its eighth month of infection. However, these lesions were not established microscopically as cancerous when the rabbit died from intercurrent injection. The rabbits are described below in the order of their survival.

**Rabbit no. 1.**—Rabbit no. 1 presented on examination 14 days following its birth three papillomatous growths which measured 0.3 cm. in diameter and 0.3 cm. in height. By the sixth week, eight papillomas, ranging in size from 1 × 1 × 1 cm. to 1.8 × 2 × 2 cm., were noted. Three of these papillomas coalesced during the next 30 days to form a large papillomatous mass measuring 7 × 5 × 5 cm. It was during the fifth month that this large growth underwent a change in appearance: necrosis occurred, the base of the growth appeared fleshy, and extension into the subcutaneous tissues was noted. Similar changes were observed in another papilloma. These two growths, when removed at autopsy and when subjected to microscopic examination, were proved to be epidermoid carcinomas (Figs. 1–5).

**Rabbit no. 2.**—Evidence of early papillomatous growths was noted on Rabbit no. 2, 15 days after birth, when each of three lesions measured approximately 0.2 × 0.2 × 0.2 cm. On examination 2 weeks later, six papillomas were present, ranging in size from 0.2 to 1 cm. in diameter and from 0.2 to 2 cm. in height. Five weeks following birth, eight papillomatous growths were recorded. These papillomas grew progressively to range in size by the fifth month from 3 × 3 × 4 cm. to 4.5 × 4.5 × 5 cm. During the succeeding 60 days, the tumors underwent necrosis and for one an extension peripherally was noted. One of the tumorous growths was observed, during the seventh month, to have undergone a radical transformation in appearance. This lesion was sectioned at biopsy and found on microscopic examination to be an epidermoid carcinoma (Figs. 6–9).

**Rabbit no. 3.**—Three newly appeared papillomas were present on examination of Rabbit no. 3, 2 weeks after birth. No additional papillomas were noted during the 7 months that this rabbit was under observation. By the end of the sixth month, the papillomas had increased in size to 5 × 3 × 2.5 cm., and one of the three growths had undergone necrosis, revealing a fleshy base and evidence of extension peripherally into the subcutaneous tissues. This tumor was examined at biopsy when the rabbit was 7 months old to yield evidence on microscopic examination of malignancy.

**Rabbit no. 4.**—Three papillomas were noted on examination 14 days after birth, and four were recorded when the rabbit was 5 weeks of age.
These papillomas increased in size to reach \(3 \times 2.5 \times 3.5\) cm. It was during the sixth month that one of the tumors on the back of the rabbit began to show degenerative changes. The surface growth sloughed, the base became fleshy, and evidence was noted of extension into the underlying tissues. The central part of the papillomatous tumor underwent necrosis. The degenerative lesions were removed at biopsy during the seventh month and shown by microscopic examination to be carcinomatous.

Rabbit no. 5.—Rabbit no. 5 had four papillomas 21 days following birth. During the next 60 days these four papillomas grew to reach a size approximating \(2.5 \times 2 \times 4\) cm. It was during the fifth and sixth months that evidence of extension and necrosis was recorded. A single lesion underwent further changes. It was removed at biopsy 30 days later and examined microscopically. A histologic diagnosis of epidermoid carcinoma was made.

Rabbit no. 6.—The papillomas on Rabbit no. 6 were limited to two growths that were observed first 37 days after birth of the rabbit. These tumors measured in diameter 0.4 cm. and 0.1 cm., respectively. Four months later the greatest size was reached when both tumors were \(2.5 \times 3.0 \times 2.5\) cm. No change in size or appearance occurred in the next 60 days. However, the tumors became dry during the seventh and eighth months and underwent a reduction in size to \(1.5 \times 1.0 \times 1.0\) cm. and \(2.2 \times 1.3 \times 1.0\) cm., respectively. The rabbit died from pasteurellosis in the eighth month following infection. The lesions on microscopic examination were benign hyperplastic, keratinized epithelial growths without evidence of malignant degeneration.

DISCUSSION

The observations recorded in the present paper make known the sequence of changes and the ultimate fate for experimentally induced papillomas that were induced by the transuterine injection of papilloma virus into the skin and other tissues of fetal rabbits (genus Oryctolagus). Epidermoid carcinomas resulted at one or more of the sites which had been occupied by virus-induced papillomas in five of the six rabbits that survived for from 5 to 7 months. The sixth rabbit in the group died of infection 8 months after inoculation. The two papillomatous lesions on this rabbit had arisen after a long incubation period and had grown slowly to reach the regressive phase when examined microscopically.

The findings summarized above make it apparent that the susceptibility of domestic laboratory rabbits is not unduly influenced by the immaturity of the host. Thus, the results in this series of animals show that the immature rabbit is fully receptive to infection with papilloma virus and, particularly, to the development of the successive changes that may result ultimately in an epidermoid carcinoma. The evidence in support of this conclusion is the observation that five of the six animals in this study harbored lesions that underwent carcinomatous degeneration, whereas in similar studies (15) employing fully mature adult rabbits of a similar purebred line only eight of fourteen which had been kept under observation for from 6 to 12 months provided lesions that had undergone malignancy.

This study did not produce evidence for any significant alteration in the host cell-papilloma virus relationship. Thus, the injection of large amounts of papilloma virus into skin of fetal rabbits did not result, as anticipated, in widespread dissemination, or in evidence for the infection of cells other than of skin. The possible bearing upon these findings of earlier studies by Rous and Beard (1, 8, 9) is worthy of comment, since the results of these investigators may be interpreted (9) as evidence that embryonic epithelium is less susceptible and responsive than adult skin. However, the failure of papilloma virus in embryonic tissues which had been kept in contact with papilloma virus in vitro for from 30 to 120 minutes and implanted subcutaneously to show evidence for proliferation is not comparable to studies carried out in vivo. Other evidence (9) to suggest for immature epithelial cells a limited receptivity and response was the observation that the inoculation of domestic rabbits within a few days after birth produced less vigorous growth than resulted from the same inoculum in adult animals. However, this difference in age reactivity was not apparent when adult and newborn rabbits were tested for reactivity by employing for injection by cross-hatch scarification serial fivefold dilutions of a single inoculum.1

1 J. T. Syverton, unpublished observations.
natural host, the cottontail rabbit, from which virus is readily recoverable.

SUMMARY

Neoplasms developed in rabbits which were infected in utero as readily as in mature animals. Thus, fetal domestic rabbits (genus Oryctolagus) were found fully susceptible to infection by papilloma virus, as manifested after birth by the papilloma-to-carcinoma sequence. The immaturity of the host was without any remarkable alternative effect in this sequence. Since the observation that cancer developed in five of six animals within 7 months of birth makes it apparent that the time required in immature rabbits for the papilloma-to-carcinoma sequence to eventuate in cancer is probably shorter than in adult rabbits, it is more than suggestive that the provocative carcinogenic effect of papilloma virus results from the initial host cell-virus relationship and that it is not significantly conditioned by the age of the host.

REFERENCES


The microscopic sections were stained with hematoxylin and eosin. The photographs were made by Mr. Henry Morris.

FIG. 1.—Tumor 1 of Rabbit 1, to show juncture where cancer arises from adjoining gnawed-off base of a benign papilloma. The underlying tissues are infiltrated and replaced by cancerous tissue. X10.

FIG. 2.—The carcinoma of Figure 1 a few millimeters below the surface photographed to show total replacement by cancerous tissue. X125.

FIG. 3.—Another portion of the cancer of Figure 1. A squamous-cell carcinoma is seen. X185.

FIG. 4.—Tumor 2 of Rabbit 1, to show the gnawed base of a virus papilloma (upper left), a squamous-cell carcinoma (lower left), and beyond the artifact a malignant papilloma (right half). X10.

FIG. 5.—A section showing the tissues underlying the malignant papilloma of Figure 4. An epidermoid carcinoma is seen. X32.

FIG. 6.—Tumor 1 of Rabbit 2. The overlying virus papilloma shows evidence of regression. The underlying tissues are largely replaced by a squamous-cell carcinoma. X10.

Figs. 7, 8, and 9.—Figure 7 (X90) shows better the squamous-cell carcinomas immediately underlying the residuum of the virus papilloma; Figure 8 (X125) shows at a higher magnification the adjoining cancer to the right; Figure 9 (X90) the cancer to the left.
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