The relationship of the papilloma virus to a carcinoma derived from the virus-induced rabbit papilloma has been the subject of numerous studies. Early work indicated that the carcinoma (V2) did not contain papilloma virus demonstrable by the usual infectivity tests. Nevertheless, there was reason to believe that papilloma antigen was present in the V2 carcinoma, since the host rabbits developed specific papilloma complement-fixing antibodies during the course of intramuscular passage of the carcinoma (7). However, some time between the fifth and eighth years of continuous passage, a change occurred in the antigenic make-up of the V2 carcinoma so that host rabbits no longer developed papilloma antibodies. Despite the apparent loss of papilloma antigen, the V2 carcinoma has retained all its original characteristics (6). Recent studies of seven other papilloma-derived carcinomas have produced significant new findings (9). When these tumors were established in baby domestic rabbits, it was discovered that the carcinomas elicited little or no papilloma antibody; but small amounts of papilloma virus were demonstrated in extracts of the carcinomas, although the rabbits bearing the tumors did develop specific papilloma complement-fixing antibodies in their sera.

It has long been recognized that it was difficult, or often impossible, to demonstrate papilloma virus in the papillomas of domestic rabbits, although large amounts of virus usually could be isolated from cottontail rabbit papillomas (10). Therefore, it seemed probable that the papilloma virus, if present in V2 carcinomas, would be difficult to demonstrate when the tumor was growing in the domestic rabbit. Therefore, from time to time, unsuccessful attempts have been made to establish the V2 tumor in cottontail rabbits with the hope that the papilloma virus could thereby be isolated.

In the present studies of the V2 carcinoma, it was found that the tumor could be successfully transplanted to the anterior chamber of the domestic rabbit eye. By using V2 carcinoma tissue derived from tumor growing in the domestic rabbit eye, it was also possible to transfer V2 carcinoma into a series of cottontail rabbits in the form of iris tumors. This paper describes the characteristics of the V2 eye tumor and the results of serologic and virus tests on the eye tissues and fluids.

MATERIALS AND METHODS

The V2 carcinoma was obtained from Dr. John G. Kidd. This tumor has been continuously passed since 1928 by the intramuscular route in domestic rabbits. It has been maintained in this laboratory since 1948 by intramuscular inoculation of tumor cells at approximately monthly intervals. Adult New Zealand white male rabbits, purchased from a local dealer, were used in experiments with domestic rabbits. Cottontail rabbits were purchased from a Kansas trapper.

Intraocular inoculation.—V2 tumor tissue was passed through a monel metal sieve (40 mesh) with 0.9 per cent saline and was centrifuged 5 minutes at 800 r.p.m. The sediment was diluted with an equal amount of saline containing penicillin and streptomycin in a final concentration of 800 units and 200 

µg/ml, respectively. The suspension was then inoculated in 0.08-ml amounts into the anterior eye chamber with a 22-gauge needle by a technic previously described (8).

Harvest of eye tumors and eye fluids.—The rabbits were killed by intravenous injection of air. The eyes were aseptically removed. Adherent blood and tissue were washed or cut away. The eye was then opened in a mortar. The iris and tumor were cut away; the lens was discarded. Aqueous and vitreous humors from both eyes of a given rabbit were pooled, separated from the tissue fragments. After clarification by centrifugation at 2,000 r.p.m. for 20 minutes, fluids were stored (−20°C.) undiluted or diluted tenfold in 0.9 per cent saline containing 200 units of penicillin and 200 

µg/ml. Ten or 20 per cent extracts of V2 iris tumors were made in 0.9 per cent saline. After light centrifugation, the materials were frozen at −50°C. until used in infectivity tests or until further prepared for complement fixation tests.

Sections for microscopic examination were made from tumor and adjacent tissue or the whole eye.

Complement fixation.—The procedure of Casals and Palacios was employed (1). Papilloma and V2 carcinoma antigens were prepared as previously described (8, 5). All antigens were centrifuged at 2,000 r.p.m. for 50 minutes before use. Appropriate positive and negative control antigens and sera were included in all tests.

Tests for papilloma virus.—Eye fluids and extracts of eye
tumors were inoculated on the hyperplastic skin of domestic rabbits according to a previously described technic (2).

EXPERIMENTAL

V1 tumors in the anterior eye chamber of the domestic rabbit.—V1 carcinoma was obtained from a 3-week-old intramuscular growth in a New Zealand white rabbit. Necrotic material was removed, and healthy tumor tissue was passed through a sieve and injected into the anterior eye chambers of New Zealand white rabbits as described in "Methods."

Immediately after inoculation, the visible tumor fragments settled to the iris angle at the inferior border of the anterior eye chamber. A diffuse conjunctivitis developed within 24 hours and persisted for 3 days. By the fourth day, the injected tumor fragments were no longer visible. Evidence of tumor growth, as indicated by generalized iris edema and linear red and gray lines radiating to the pupil, was first noted the fifth day. From the seventh to fifteenth day, the entire iris rapidly became enlarged and thickened by increasing tumor infiltration. In the 4 days before harvest, progressive edema and bulging of the cornea were observed, and the color of the tumor appeared to change from gray to white. The eyes were harvested 15 days after inoculation, when one of the corneas burst. Gross examination of the eyes after removal of the cornea revealed that the abnormalities were confined to the iris, ciliary body, and the cornea (Fig. 1). Microscopic examination of whole eye sections revealed that the tumor cells observed in the iris and ciliary body appeared to be identical with the type of cell seen in V1 intramuscular growths (Fig. 4).

A suspension of the V1 tumor cells from the eyes of the first rabbit passage was prepared and injected into the anterior chamber of both eyes of two normal domestic rabbits. Tumor growth was similar to that of the first passage, and the eyes were harvested after 11 days. Thereafter, consecutive passages were made at 9—12-day intervals until a total of seven passages was made. Tumor growth was uniformly successful in both eyes of all rabbits. The size and extent of the tumor was similar in all eyes. Later passages did not grow faster than the initial passages. Histologic examination of eyes from later passages revealed that occasionally V1 cells infiltrated eye structures adjacent to the ciliary body. Detailed studies to determine the minimal amount of tissue required to initiate growth were not carried out. However, suspensions containing 1 part of cells and 32 parts of diluent caused the development of tumors which were only slightly smaller than the usual ones. V1 carcinoma from intramuscular growths was used to initiate three additional series of iris tumors. The original findings were duplicated in each series.

V1 tumor in the anterior eye chamber of the cottontail rabbit.—The next experiments were designed to determine whether the V1 carcinoma would grow in the anterior chamber of the cottontail rabbit. It was found that the tumor grew in the iris of both eyes of two cottontail rabbits inoculated with an aliquot of the same V1 intramuscular tumor suspension that was employed to initiate the first eye passage in domestic rabbits. Growth of the tumor followed the same general pattern already described in detail for the domestic rabbit eye tumors. When the eyes were harvested 15 days after inoculation, it was noted that white tumor masses occupied the entire iris. Histologic examination revealed no significant differences from the microscopic appearance of the V1 tumor in the domestic rabbit eye.

Further passages of the iris tumor were attempted. A suspension of the first passage eye tumors was inoculated into both eyes of two more cottontail rabbits. At harvest 12 days later, localized iris tumors 5 X 10 ml. in size were noted. A third passage with the use of the small tumors of the second passage produced only minute growths in the iris, which were insufficient for passage.

An attempt was made to produce transplantable tumors by inoculating V1 tumor tissue obtained from the domestic rabbit eye. Third passage V1 eye carcinoma (domestic rabbit), harvested 12 days after inoculation, was prepared and inoculated in both eyes of two cottontail rabbits as described in "Methods."

In the first passage, white tumors with sharp borders developed and occupied one-half to two-thirds of the iris surface. Thereafter, six successful passages were carried out at 11- to 19-day intervals, with V1 tumor from the preceding passage. Certain differences from the growth of V1 eye tumor in the domestic rabbit were apparent. Although the tumors were confined to the iris, sharply localized single or multiple tumors were noted instead of complete infiltration of the iris. Only 75 per cent of the inoculated eyes developed tumors. The histologic character of the V1 cells showed no definite differences from those of other V1 tumors in the anterior eye chamber.

A year after the first experiments with cottontail rabbits, fresh domestic rabbit V1 iris tumor cells were used to initiate further cottontail eye tumors. The resulting growths differed from those of the first series in that they were smaller and not always confined to the iris. Ap-
proximately one-third of the tumors extended across the pupil from the adjacent border of the iris.

**Serologic and virus studies.**—Before tests were made of eye fluids and tumors and of the sera from rabbits with V2 eye tumors, complement fixation tests were performed with sera from domestic rabbits bearing V2 intramuscular tumors. Tests of the sera from rabbits of fourteen consecutive passages revealed no complement-fixing antibodies for the papilloma virus. This confirmed the findings of Smith, Kidd, and Rous (11).

Eye fluids and eye tumor extracts from sixteen domestic rabbits representing seven eye passages were tested for evidence of papilloma complement-fixing antigen without success. In twelve of these rabbits, infectivity tests also failed to demonstrate papilloma virus in the eye fluids or tumors. Although papilloma complement-fixing antibodies could not be detected in any of sixteen domestic rabbit sera, four eye fluids were also tested for papilloma complement-fixing antibodies. These later tests were also negative. Therefore, there was no evidence of local antibody formation that might have masked papilloma virus.

Tests next were made with materials from cottontail rabbits bearing V2 eye tumors despite the lack of evidence of papilloma antigen in domestic rabbit V2 intramuscular and eye tumors. Again, eye fluids and eye tumors were tested for papilloma complement-fixing antigen. These tests were negative in four cottontail rabbits. No papilloma virus was demonstrated in infectivity tests employing whole eye extracts from ten additional cottontail rabbits bearing V2 eye tumors. Finally, in the sera of the latter ten cottontails, no papilloma complement-fixing antibodies could be detected.

Previous studies (8) have shown that the blood of rabbits carrying the V2 carcinoma contains antibodies which will fix complement in the presence of saline extracts of various normal and neoplastic rabbit tissues. The next experiments were performed to determine whether a distinctive antigen could be demonstrated in extracts of the V2 eye tumors or in the fluids from these eyes. Extracts of eye tumors were prepared, and fluids were obtained from twenty eyes of domestic rabbits and ten eyes of cottontail rabbits with V2 eye tumors.

Repeated tests failed to reveal a specific complement-fixing antigen in the eye fluids from rabbits with V2 eye tumors. These results are in keeping with previous findings that the complement-fixing V2 antigen is associated with a sedimentable particle contained in V2 carcinoma. The results of tests with saline extracts of the eye tumors were similar to those previously obtained with extracts of the intramuscular growths (8).

**DISCUSSION**

The striking features of the growth of V2 carcinoma in the anterior eye chamber of the domestic rabbit were the apparent disappearance of the tumor cell inoculum before tumor growth was noted in the iris and the diffuse multicentric origin and rapid growth of tumor over the entire iris surface. With the possible exception of the Kato sarcoma (4), this sequence of events has not been previously described. Usually, tumors have been implanted in the iris angle of the eye in the form of a single piece of tumor tissue. After a variable period of time, the tumor tissues have grown and have become attached to the iris or other eye structures. It is likely that the diffuse type of iris tumor growth seen with the V2 tumor is attributable to the physical character of the inoculum rather than to any special character of the V2 carcinoma. In the act of inoculation, minute tumor fragments may diffusely seed the iris. The tumor inoculum that disappeared from its settling place was probably completely absorbed and had nothing to do with the growth of V2 carcinoma in the iris. It seems certain that no soluble tumor substance invades the iris, because inoculations of cell-free fluids from eyes with the V2 tumor failed to produce eye tumors. Furthermore, all previous attempts to pass V2 carcinoma with cell-free extracts of intramuscular growths have been unsuccessful.

One of the primary objectives of this study was to attempt to demonstrate papilloma virus or antigen in the V2 carcinoma by growing the tumor in the cottontail rabbit. Although it was possible to grow and to transfer serially the V2 carcinoma in the cottontail anterior eye chamber, attempts to demonstrate papilloma virus or antigen in cottontail V2 tissue were unsuccessful. As indicated, however, there is evidence that the papilloma antigen had been lost from the V2 carcinoma before its passage to the cottontail eye. Successful transfer of the more recently established papilloma-derived carcinomas to the cottontail eye might make it possible to demonstrate larger amounts of papilloma virus in the tumors (9).

**SUMMARY**

1. V2 carcinoma was successfully grown and carried through seven consecutive passages in the anterior eye chamber of the domestic rabbit. Rapid confluent growth in the iris and ciliary body necessitated the harvest 9–12 days after inoculation to prevent rupture of the cornea.

2. V2 carcinoma from the eyes of domestic rab-
bits was utilized to initiate six consecutive passages of $V_2$ tumor in the anterior eye chamber of the cottontail rabbit. Rapid growth occurred as single or multiple isolated tumors on the iris and ciliary body in 75 per cent of the inoculated eyes.

3. Confirmatory evidence was obtained that the $V_2$ carcinoma no longer carries a masked papilloma antigen. No papilloma virus nor any papilloma complement-fixing antigen was detected in the eye fluids or the eye tissues of domestic or cottontail rabbits with $V_2$ eye tumors. Complement-fixing antibodies against the papilloma virus were not demonstrable in the sera of rabbits with $V_2$ eye or $V_1$ intramuscular tumors.

REFERENCES


V₂ Carcinoma in the Rabbit Eye

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