Methylcholanthrene Papillomas and the Virus Problem

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Papillomas of proved virus origin are no rarity in the animal kingdom (1, 5, 6, 7), but none has been so minutely investigated as the Shope papilloma of the rabbit (8). Though this tumor yields virus in generous amounts when it is grown in western cottontail rabbits, the etiological agent can no longer be obtained (4) from the carcinomas into which the warts in these hosts are occasionally transformed (4, 9).

Does an analogous situation obtain with induced papillomas of the mouse and the carcinomas that arise from them? The answer to this question will be found in the following paragraphs.

MATERIALS AND METHODS

To obtain the necessary papillomas, young adult RIII male and female mice of a brother-to-sister bred strain were painted thrice weekly with a 0.6 per cent solution of methylcholanthrene in benzol.

It is not easy to decide on the exact day upon which a papilloma first appears, but those employed were judged to be about 7, 11, 18, 42, 51, 82, and 114 days old.

Only such warts were used as showed no sign of thickening at the base, an indication of the malignant transformation that is conceded to be trustworthy and that had to be relied upon in any case, since there was not sufficient material available for microscopic examination.

As papillomas were required they were excised, after they and the surrounding skin had been gently cleansed with a cotton pledget just barely moistened with benzol to remove any adherent carcinogen, then with cotton similarly wetted with alcohol to get rid of the benzol, and immediately mopped dry with sterile cotton. It is not thought that either of the cleansing fluids thus used could have destroyed any virus present.

It was necessary to kill three or four mice each time in order to get sufficient material. After the warts had been removed the 0.3 to 0.6 gm. of tissue so obtained was thoroughly ground with sand in physiological saline solution and the resulting extracts were centrifuged for 15 minutes at about 2,000 r. p. m. They were then either watery-clear or faintly opalescent, and sometimes lightly blood-tinged. Had any of them elicited tumors, adequate filtration of subsequent extracts would of course have been required.

Thus prepared, the extracts were inoculated in the usual way, by rubbing them with a glass rod into shaven skin that had been lightly scarified with a needle. For this purpose young adult male and female mice of the strain that furnished the papillomas were employed, with resort in most cases to procedures that it was hoped might enhance the susceptibility of the organism or the efficacy of the extracts. The details appear below.

Experiment 1.—A 5 per cent papilloma extract was rubbed into the scarified but otherwise normal skin of 6 male and 6 female mice.

Experiment 2.—Similar, except that a 10 per cent extract was used.

Experiment 3.—Here the skin was made hyperplastic by painting it with equal parts of turpentine and acetone, the method so successfully employed by Friedewald (2), who increased the effective titer of the Shope papilloma virus from 10 to 100 times in this way. Twelve male and 12 female mice were thus treated, but the skin of this RIII strain seems to be less delicate than that of the rabbits described by Friedewald, for 9 applications had to be made, at intervals of 2 days, before the skin reached the thickened and inflamed condition that he achieved with 4 or 5 paintings at similar intervals. Into this hyperplastic skin a 10 per cent papilloma extract was rubbed after the customary scarification.

Experiment 4.—It has been said that some viruses gain efficacy as a result of glycerol storage in the cold. Such an occurrence must be rare if, indeed, it takes place at all, for it is clearly stated of most viruses that they lose strength with the passage of time. Nevertheless, and as a forlorn hope, some papillomas were stored in the refrigerator in equal parts of glycerol and physiological saline solution for 48 days. At the end of this period the glycerol was washed away in 3 changes of saline solution, and the usual 10 per cent extract was inoculated into 12 male and 12 female mice whose skins had been previously irritated with the turpentine-acetone mixture.
Experiment 5.—An effort was made to diminish the resistance of the animals by exposing their entire bodies to as large a dose of x-rays as a mouse can safely stand. Six males and 6 females received 300 r (184 kv. peak, 2 mm. Cu+ 1 mm. Al filter), and a similar dose 6 days later. Twenty-four hours after the second raying the white cell count in the females had fallen to 4,500, in the males to 2,750, and on the following day all were inoculated in the customary manner with a 10 per cent papilloma extract.

Experiment 6.—The inoculated area was covered immediately with paraffined gauze, which was held in place by adhesive plaster until healing had occurred, about 5 days later. According to Friedewald (3), necrosis and scabbing are almost entirely obviated by this procedure, many susceptible cells are provided for the virus far earlier than they otherwise would be, and the inoculum is conserved instead of being largely lost amidst necrotic tissue and scab. The experiment comprised 12 male and 12 female mice inoculated with a 10 per cent extract of papilloma.

Experiment 7.—Similar to Experiment 6 except that the skin was made hyperplastic before the inoculation, for Friedewald (3) estimates that the combination of inflamed skin and a paraffined gauze pad increased the efficacy of the Shope papilloma virus from 100 to 10,000 times. Eleven male and 12 female mice were employed.

DISCUSSION

Though the incubation period of the Shope rabbit papilloma falls well within 2 weeks, the mice of these experiments were kept under observation for many months. Yet among 131 animals not the slightest indication of a papilloma has appeared. The scarifications have healed smoothly in a week or so and the skin has regained its normal appearance in every instance.

Nevertheless it would be unsafe to conclude that methylcholanthrene papillomas do not contain a virus. All that can be said is that none was disclosed under the conditions governing these experiments.

It may be of significance that viruses are recoverable from certain forms of leukemia in fowls and from many of their tumors, but not from mouse tumors or mouse leukemia. It is not beyond the bounds of possibility that the mouse holds on more tenaciously to some of its viruses or that some of them are more labile than certain avian viruses.

SUMMARY

Of 131 mice inoculated with extracts of methylcholanthrene papillomas, not a single one developed a tumor.

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