

A STUDY OF QUANTITATIVE CHANGES OF THE SHOPE RABBIT PAPILLOMA VIRUS AT THE SITE OF INOCULATION IN THE SKIN OF THE COTTONTAIL AND DOMESTIC RABBIT

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During the course of certain experiments concerning the mechanism of the "masking" of the papilloma virus in the domestic rabbit, it was thought of importance to determine from the moment of inoculation to the appearance of papillomas the quantitative changes of virus inoculated into the skin of the domestic and cottontail rabbit. It seemed to be of particular interest to know: (a) The rate at which the inoculated virus disappears or is inactivated at the site of inoculation in the skin of the domestic rabbit. (b) Whether the active virus persists at the site of inoculation during the whole incubation period in the cottontail rabbit or disappears for a time only to reappear in the developing papillomas. In the latter case, does the rate of inactivation in the skin of the cottontail rabbit differ from that in the skin of the domestic rabbit?

Several experiments have been published in which a temporary disappearance or decrease in the amount of the inoculated virus has been demonstrated at the site of inoculation; rabies (Remlinger, 1), vaccinia (Noguchi, 2; Widelock, 3), St. Louis encephalitis (Webster and Clow, 4), yellow fever (Whitman, 5). In other instances such a negative phase has not been observed, but the virus starts to multiply after a stationary lag, as has been claimed for the poliomyelitis virus in the monkey brain by Fairbrother and Hurst (6).

EXPERIMENTAL

10 per cent extracts of papillomas from cottontail rabbits were prepared by grinding with sterile sand and physiological saline solution, and subsequently centrifuging the extract for 15 to 20 minutes at about 2700 R.P.M. 5 drops of 1:1000 dilutions of these extracts gave rise to several (10 to 20) papillomas. At several sites on the closely clipped skin of 3 domestic and 2 cottontail rabbits, 0.1 cc. of the 10 per cent extract was injected intracutaneously. The borders of the resulting wheals were marked. Then the epithelium over the wheal was lightly punctured 15 times with the same needle used for

injection with a view to increasing the chances of a "take." After varying intervals the skin areas comprising the site of the wheal were excised, weighed, and made up to 10 per cent extracts in the way described for the papilloma extracts. The excised skin pieces weighed between 50 and 150 mg. Provided that none of the virus injected with the 0.1 cc. of the 10 per cent virus extract had been transported from the site of inoculation, and that the whole inoculation area had been excised, the final dilutions of the inoculated papilloma virus should correspond roughly to dilutions varying from 1:50 to 1:150 or to an average of 1:100. When excisions were made very soon after the inoculation, it was not possible, however, to be certain that a part of the injected fluid did not ooze from the excised piece, thus making the final dilution somewhat higher.

5 drops of these skin extracts were inoculated into 3×5 cm. areas of the scarified skin of domestic rabbits in the manner described by Shope (7).

A papilloma extract diluted 1:100 in saline was inoculated as a control. As an additional control 0.1 cc. of the virus suspension used in the experiments was ground with an excised piece of normal domestic or cottontail rabbit skin and physiological saline to make a 1:100 virus dilution in a 10 per cent skin extract. The supernate obtained after centrifugation was used. While some of these control suspensions gave a papilloma growth identical with that produced by a direct dilution at 1:100 of rabbit papillomas in saline, others showed a reduction of the number of resulting papillomas from "semi-confluent" growth to "many discrete" (see below) corresponding approximately to an additional tenfold reduction of the virus.

The intracutaneous route of inoculation is not the best one since it gives but irregular takes (Shope, 7). An attempt to use the usual method of rubbing the virus into the scarified skin which takes in practically 100 per cent was, however, for the purpose of these experiments unsatisfactory.

An area approximately 5×17 cm. of the abdomen of a domestic rabbit was inoculated broadcast with 20 drops of a 10 per cent papilloma extract. Thus each 1.2×1.2 cm. received about 0.1 cc. of the virus extract. After 15 minutes, 18 hours, 2, 4, 7, 11, 15, and 21 days, respectively, skin pieces (on the 21st day already displaying developing papillomas) of that size were excised and made up to 10 per cent extracts, which, as to the amount of virus originally inoculated into them, roughly corresponded to a 1:100 to 1:150 dilution of the papilloma material used. 5 drops of these extracts were inoculated in the usual way into normal domestic rabbits. Not even the extract of the skin piece excised 15 minutes after inoculation gave rise to any papillomas. A control dilution of 1:200 in physiological saline solution of the papilloma extract used in this experiment gave a semiconfluent growth.

Friedewald (8) reported the recovery of small amounts of virus in a few instances from papillomas of domestic rabbits 21 days after inoculation. He considers the possibility of the virus having persisted since the inoculation but thinks this unlikely and is inclined to assume a real production of new virus. An experimental confirmation or rejection of this assumption by repeated titrations of the virus content of the inoculated skin during these 21 days should be of great interest. A gradual decrease should indi-

cate a mere persistence of the virus, whereas a complete disappearance and a subsequent reappearance should give evidence of an actual multiplication.

The results of the experiments in which the intracutaneous route was employed are shown graphically in Figs. 1 and 2 where the amount of active virus, as roughly estimated by the numbers of papillomas developed,¹ is plotted along the axis of ordinates and the time interval from the inoculation to the excision on the abscissa. For comparison, the serial tenfold

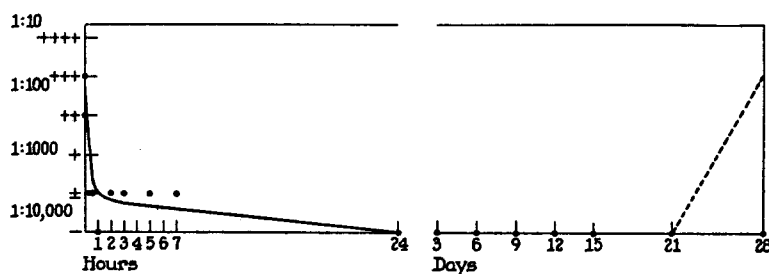


FIG. 1. Results of titrations of papilloma virus in the skin of cottontail rabbits at various intervals after intracutaneous inoculation.

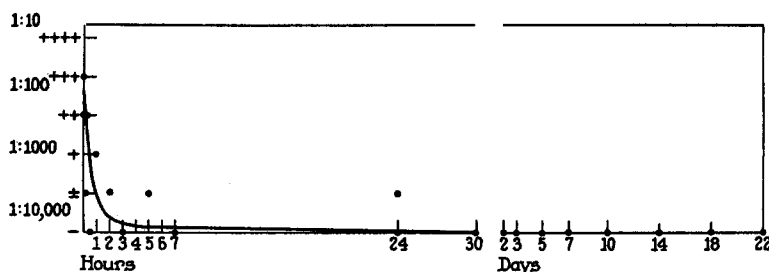


FIG. 2. Results of titrations of papilloma virus in the skin of domestic rabbits at various intervals after intracutaneous inoculation.

dilutions of the papilloma virus used in these experiments are inserted along the ordinate according to the extent of papilloma growth produced by them.

It will be seen that the amount of active virus at the site of inoculation decreased and disappeared within the first few hours in both the domestic and the cottontail rabbit. It should be mentioned in this connection that

¹ The scale of Kidd and Rous (*J. Exp. Med.*, 1940, **70**, 831) has been used, except that their \pm and \pm groups have been fused to one.

++++	confluent growth of papillomas
+++	semiconfluent " " "
++	many discrete " "
+	5 to 10 " "
\pm	1 to 4 " "

the wheal at the site of inoculation persisted, at least to some extent, and the skin area comprising it, when excised, still was more or less succulent during the first 5 to 7 hours, *i.e.*, when virus was recoverable.

DISCUSSION

Though the experiments recorded above are rather preliminary in character, and though a very arbitrary quantitative scale has been used in giving the "titers," there seems to be sufficient evidence to indicate that active virus in demonstrable amounts is not present at the site of inoculation for more than approximately 24 hours after the intracutaneous injection and reappears, as a rule, only with the appearance of papillomas, in the cottontail rabbit, and not at all in the domestic rabbit. The fact that most of the virus disappears within the first hour (in the cottontail rabbit even within the first 15 minutes) seemed to indicate that the virus is not transported away but is inactivated *in loco*. The question of whether it is completely destroyed or only reduced to a pathogenically inactive, but antigenically still active state, remains to be answered by serological methods.

It is possible that the rate of disappearance of the virus may alter to some extent when a more extensive study is made, particularly if a stronger virus suspension is used enabling more adequate titrations with increasing dilutions of the extracts. Another question of interest which will require further experimental work, is the exact time when active virus reappears in the cottontail rabbit; whether it appears with the formation of macroscopical papillomas, the development of the first histological changes, or prior to either of them.

SUMMARY

By excising after varying intervals the site of inoculation of the rabbit skin injected intracutaneously with the Shope papilloma virus and titrating its virus content, it has been shown that demonstrable active virus disappears from the skin within approximately 24 hours. The disappearance of most of the virus within such a short time as 15 to 60 minutes makes a transportation of virus from the site of inoculation as a responsible factor unlikely. The rate of disappearance seems to be the same both in the domestic and in the cottontail rabbit.

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