

## THE RECOVERABILITY OF VIRUS FROM PAPILOMAS PRODUCED THEREWITH IN DOMESTIC RABBITS

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(Received for publication, February 28, 1944)

The papilloma virus is readily recoverable in large amount from most cottontail rabbit papillomas but it cannot ordinarily be obtained from the papillomas of domestic rabbits that result from inoculation with it (1). Differential high speed centrifugation of extracts of cottontail papillomas usually yields a considerable pellet of the virus, whereas none has been demonstrable in extracts from domestic rabbits when similarly treated (2). Nevertheless the virus is present in some growths of the latter sort, as shown by the not infrequent recovery of small amounts of it (3), by the regular appearance of a specific antiviral antibody in the blood of animals carrying the growths (4), and by the antigenicity of extracts of the latter,—which elicit the antibody on injection into normal rabbits (5). Because of these facts the conception has arisen that when the virus cannot be recovered it is masked in some peculiar way. This possibility has large interest, since the carcinomas which derive from papillomas due to the virus fail to yield it though they contain the virus or some nearly related antigen, as indicated by the serological findings in rabbits to which the growths have been successfully transplanted (6).

The present paper shows that active virus can be demonstrated in extracts of domestic rabbit papillomas yielding none to ordinary methods of test, if such extracts are inoculated into epidermis so prepared previously as to provide especially favorable conditions for infection.

Shope reported tests with 58 extracts of domestic rabbit papillomas (1, 3), and of these 13 yielded virus. Attempts to carry the latter in domestic rabbits beyond the second serial passage were made in 7 instances, and of these 4 were successful, but the virus thus passed was always slow in producing papillomas and the growths were small and discrete. Those procured from rabbits over 2 years old yielded virus more frequently than those from rabbits 2 to 4 months old, but in general the breed of the rabbit and period of growth of the papillomas seemed to be without influence on the result.

On the assumption that virus is absent from extracts of domestic rabbit papillomas which prove innocuous on test, the hypothesis has recently been put forward that it is degraded in the tissue as fast as formed and that the antigenicity of the material from domestic rabbits is due to some product of this degradation (7).

### *Methods*

The most successful of ordinary methods of inoculation is to scarify large patches of skin and rub into them an extract of papillomatous tissue prepared

by grinding in sand and suspending in saline. But under such circumstances nearly all of the epithelium is scraped away from the inoculated patches, as successive biopsies have shown, and the virus has immediate access only to epidermal cells where the mouths of the hair follicles have been cut across, though after some days, if persisting at the site of inoculation, it may come into contact with the regenerating sheet of epidermis as this extends laterally from the follicles (8). But when the epidermis has been rendered hyperplastic by irritants, not only does it form a thick, actively proliferating layer, of which some survives scarification, but it regenerates with great rapidity. It has been found that under these favorable conditions the virus will cause papillomas in dilutions 10 to 100 times as great as those which are effective under ordinary circumstances.

Areas of the skin of the rabbits on which the extracts were to be tested were shaved and prepared by painting them with a 0.3 per cent solution of methylcholanthrene in benzene or with a mixture of turpentine and acetone in equal parts. Adult domestic rabbits of the gray-brown (agouti) breed were used throughout and the paintings were done with a soft brush four to seven times at 2 to 7 day intervals (8). Saline extracts of the papillomas were rubbed into the prepared skin areas following light scarification with sandpaper. The character of the papillomas arising was recorded every few days according to a standard scale: + + + + = confluent papillomas; + + + = semiconfluent papillomas; + + = many discrete growths; + = 5 to 15 papillomas; ± = 2, 3, or 4 papillomas; ± = 1 papilloma; 0 = negative.

In some of the experiments it was important to know the amount of antiviral antibody in the circulating blood. The complement fixation test (9) was utilized for this purpose, with dilutions of serum tested in mixture with 2 units of complement and an optimal dose of antigen. This last consisted of a centrifugalized extract of cottontail rabbit papillomas, which contained a known amount of virus.

#### EXPERIMENTAL

It was necessary first of all to determine whether any of the virus which might be procured from domestic rabbit papillomas represented a survival of the original inoculum.

*Experiment 1.*—A 10 per cent virus extract (W.R. 1-28) was tattooed into twelve spots each about 2 to 4 mm. across on the abdomens of four normal domestic rabbits. Excess virus was immediately removed by blotting with a gauze sponge. One hour later all the sites of inoculation were washed with liquid soap and thoroughly rinsed with tap water,—a procedure used in most of the later tests. From time to time,—after 1 hour, 1 day, 2 days, 4 days, 8 days, and 12 days,—the skin at two of the inoculation sites was excised, as well as a small area round about them, and frozen immediately at  $-67^{\circ}\text{C}$ . At first there was scabbing at these sites, but by the 12th day, when the last specimens were taken, the areas were completely healed and minute papillomas were appearing on them in three of the four rabbits. Two days after the last piece of skin had been obtained, the materials were ground separately with sand and

suspended in 2 cc. of saline. The whole crude suspensions were then rubbed into scarified skin areas of six domestic rabbits which had been prepared with seven applications of 0.3 per cent methylcholanthrene in benzene at 2 day intervals prior to inoculation.

Table I shows the results of the experiment. It will be seen that all of the suspensions contained virus 1 hour after inoculation. A somewhat smaller amount of virus was recovered 1 day and 2 days after inoculation, but no virus could be demonstrated on the 4th, 8th, or 12th day. The experiment shows clearly that a small amount of detectable virus remains on the inoculated area for a short time after inoculation and is then lost. Most of this virus is prob-

TABLE I  
*The Infectivity of Extracts of the Skin of Domestic Rabbits Procured at Various Times after Inoculation*

Time to excision of virus-inoculated skin	Yield of virus from the excised skin of four rabbits											
	D.R. 2-73			D.R. 2-74			D.R. 2-75			D.R. 2-76		
	a	b	c	a	b	c	d	e	f	d	e	f
1 hr. ....	0	±	±	++±	+	++	±	++±	±	0	±	±
1 day. ....	±	0	±	±	0	+	0	+	±	0	0	0
2 days. ....	0	0	0	±	±	0	0	±	±	0	±	0
4 days. ....	0	0	0	0	0	0	0	0	0	0	0	0
8 days. ....	0	0	0	0	0	0	0	0	0	0	0	0
12 days. ....	0	0	0	0	0	0	0	0	0	0	0	0

The skin of two tattooed spots was removed from each animal at the times indicated and the specimens saved frozen until all had been assembled. They were then ground separately and suspended in 2 cc. of saline and the suspensions rubbed into the prepared skin of test rabbits a-f. The readings were those of the 42nd day. (See Experiment 1.)

ably incorporated in the scab that forms following scarification, and this scab comes away soon after epithelial regeneration has been completed (8).

The results of the experiment make plain that any virus procured from domestic rabbit papillomas which had proliferated for some time could be regarded as formed in the growths and not as residual from the inoculation. Experiments reported by Packalén (10) yielded similar results. He could not demonstrate the presence of virus at the site of inoculation in domestic rabbits even within a few minutes after intradermal injection of it; but he used the ordinary method of skin test.

#### *Comparative Infectivity Tests with Normal and Hyperplastic Epidermis*

Tests were next done to compare the infectivity of extracts of domestic rabbit papillomas on inoculation into normal and hyperplastic epidermis. The following experiment is typical of many tests of the sort.

*Experiment 2.*—The papillomas of five gray-brown domestic rabbits were utilized, which had resulted from inoculation in two different ways. Those of three of the animals (3-93, 3-94, 3-96) had been produced by tattooing a highly infectious 10 per cent saline extract of the glycerolated papillomas of cottontail W.R. 1-28 into ten spots about 4 mm. across on the skin of the abdomen. The growths were scrubbed with liquid soap and rinsed well with tap water 42 days after the inoculation, and they were then excised. They were washed again in physiological saline, diced after connective tissue and keratinized or blood-tinged material had been trimmed away, and placed in a mixture of equal parts of glycerol and Locke's solution at about 4°C. The papillomas of two other domestic rabbits (2-31, 2-32) had been produced by rubbing

TABLE II  
*Pathogenicity Tests with Extracts of Domestic Rabbit Papillomas Inoculated into (a) Normal Skin and (b) Skin Treated with Methylcholanthrene*

Source of papillomas			Growths resulting from inoculation of papilloma extracts into											
Cottontail papilloma extract inoculated	Duration of growths	Rabbit No. D.R.	Normal skin			Methylcholanthrene-treated skin								
			20th day		30th day		42nd day		20th day		30th day		42nd day	
			a	b	a	b	a	b	a	b	a	b	a	b
W.R. 1-28	42	3-96	0	0	0	0	0	0	±	±	±	±	+	+
		3-94	0	0	0	0	0	0	±	0	±	0	±	0
		3-93	0	0	0	0	0	0	±	0	±	±	±	±
W.R. 1-72	30	2-32	0	0	0	0	0	0	±	0	±	±	±	±
		2-31	0	±	0	±	0	±	+	+++	+	+++	+	+++

a, b = test rabbits.

another 10 per cent virus extract (cottontail W.R. 1-72) into scarified skin areas. These were harvested in like manner 30 days after inoculation.

For the test 10 per cent saline extracts of the papillomas of each animal were prepared by rinsing weighed portions of the glycerolated tissue in salt solution, grinding thoroughly with sand, suspending in 10 volumes of 0.9 per cent NaCl, and spinning at about 2,000 R.P.M. for 5 minutes. The supernatant fluids thus procured were then rubbed into scarified skin areas of three gray-brown domestic rabbits. Some of these areas had been rendered hyperplastic prior to inoculation by six applications of 0.3 per cent methylcholanthrene in benzene at weekly intervals, while other comparable areas on the same rabbit were left untreated.

The results of the experiment are shown in Table II. One of the test rabbits died, and hence the results with only two are available. Four out of five of the papilloma extracts failed to produce any growths when rubbed into areas of normal skin scarified in the usual way, whereas inoculation into the prepared skin revealed virus in all of the extracts. The fifth extract (2-31) elicited a

few papillomas on the normal skin of one of the rabbits, but it produced many more growths on the hyperplastic epidermis. Yet the amount of virus demonstrated was not large, considering that 10 per cent extracts had been inoculated.

TABLE III  
*Recoverability of Virus from Papillomas of Domestic Rabbits*

Source and character of material			Pathogenicity of suspensions of the papillomas†									
Rabbit No.	Duration of growths days	Serum antibody titer of host*	Character of growths	20th day ●			30th day			42nd day		
				a	b	c	a	b	c	a	b	c
1-90	30	1:2	Large, confluent	+	±	0	+±	++	+	+±	++	+
1-95	"	1:2	" "	±	0	0	+±	±	±	+±	±	±
2-17	"	1:16	" "	0	0	0	0	0	0	0	0	0
2-00	"	1:2	Large, semiconfluent	±	0	0	±	±	0	±	±	0
1-98	"	1:8	" "	0	0	0	±	±	0	±	±	0
2-03	"	1:8	" "	+	0	0	+±	+±	+	+±	+±	+
1-99	"	1:4	Small, discrete	±	0	±	±	±	+	±	±	+
2-08	"	1:2	" "	0	+	++	±	++	+++	±	++	+++
2-09	"	1:2	" "	±	0	±	±	+±	+	±	+±	+
3-72	42	1:16	Large, confluent	0	0	0	0	0	±	0	0	±
3-73	"	1:32	" "	+	±	±	+±	+++	+±	+±	+++	+±
3-74	"	1:16	" "	0	0	0	0	±	+	0	±	+
				d	e	f	d	e	f	d	e	f
2-19	61	1:8	Discrete, tattoo	0	±	0	±	±	0	±	±	0
2-20	"	1:2	" "	±	++	0	++	+++	+	++	+++	+±
2-21	"	1:2	" "	+±	++	++	+++	+++	+++	+++	+++	+++

All of the papillomas had been produced with a single cottontail virus extract (W.R. 1-28).

\* Complement fixation tests.

† 0.3 per cent methylcholanthrene in benzene applied to skin areas five times at 2 day intervals prior to inoculation of papilloma extracts.

a-f = test rabbits.

For the next test, extracts of papillomas of varying size and duration from fifteen domestic rabbits receiving the same inoculum were tested on hyperplastic epidermis. Comparative observations were made to find whether the amount of antibody circulating in the blood of the animals influenced perceptibly the demonstration of virus in the growths.

*Experiment 3.*—A 10 per cent extract of the glycerolated papillomas of cottontail W.R. 1-28 was inoculated into fifteen gray-brown domestic rabbits. Large confluent papillomas were produced in nine of the rabbits by rubbing the virus into skin expanses scarified with sandpaper, whereas small discrete growths were elicited in six of the rabbits by inoculation of the virus into four small spots with a tattoo machine.

No preparation of the skin had been done. The papillomas from nine of the animals were removed on the 30th day after inoculation, from three on the 42nd day, and from three more on the 61st day. All were harvested as described in Experiment 2 and kept in glycerol-Locke's solution in the ice box. Blood was procured from each rabbit at the time of removal of the papillomas and the serum was tested for antiviral antibody by means of the complement fixation test. For inoculation 10 per cent saline extracts of the glycerolated papillomas were prepared as usual and rubbed into scarified skin areas already rendered hyperplastic by the application of methylcholanthrene in benzene.

Table III shows that active virus was demonstrable in extracts of the papillomas from fourteen of the fifteen rabbits. It will be noted that in general the discrete papillomas yielded somewhat more virus than the confluent papilloma masses, although the difference is not striking save in the case of D.R. 2-21. Scrutiny of the table fails to reveal any close correlation between the serum antibody titer of the host and the yield of virus from the papillomas, though it is clear that the growths from rabbits with most serum antibody generally yielded little virus (D.R. 2-17, 1-98, 2-03, 3-72, 3-74, 2-19). D.R. 3-73 provides a noteworthy exception in that its serum had the highest titer of antibody (1:32), yet the papillomas yielded as much virus as those with little or no demonstrable serum antibody. As bearing upon the differences observed in the case of animals with approximately the same antibody titer, it may be recalled that this antibody escapes to a differing extent into individual papillomas and that its presence there greatly affects the yield of virus on extraction (11). Relatively little of it gets out into tattoo papillomas.

#### *Influence of the Virus Strain on the Infectivity of the Extracts*

Domestic rabbit papillomas produced by a number of different cottontail virus materials were now tested for comparative yield of virus. The materials are designated by the serial number of the cottontail rabbit providing the growths and the virus specimens they yielded will be referred to as virus *strains*. They consisted of the naturally occurring papillomas of cottontail rabbits, which had been plucked, washed free of blood in saline, and preserved in separate bottles in 50 per cent glycerol-Locke's solution at about 4°C.

*Experiment 4.*—10 per cent saline extracts of the glycerolated cottontail rabbit papillomas from twelve different cottontail sources were each tattooed into two areas about 4 mm. across on the skin of four normal domestic rabbits. Three of the virus materials (A, B, and C) consisted of the pooled papillomas from nine or more cottontail rabbits, whereas the others came from individual rabbits. The growths resulting from the twelve inocula in two of the domestic rabbits (D.R. 13-97 and 14-04) were harvested on the 54th day, by the same procedures as in Experiment 2, and from two more of the animals (D.R. 3-88 and 14-00) on the 74th day. A representative section of each growth was taken for microscopic study and the remainder frozen. The papil-

lomas on each rabbit, produced by the different virus strains, were remarkably alike in the gross as well as on microscopic examination—discrete hassocks, 1 to 2 cm. across, with fleshy bases and dry, keratinized tops. The serum of each host (taken just before removal of the growths) was tested for antibody by means of the complement fixation test. 20 per cent saline extracts of the papillomas were then prepared as usual and rubbed into scarified areas on each of four or five normal domestic rabbits which had been prepared by four applications to the skin of turpentine and acetone at 2 day intervals.

TABLE IV

*Influence of the Virus Strain and of the Inoculated Animal on the Demonstration of Virus in Extracts of Domestic Rabbit Papillomas*

Virus strain producing papillomas W.R.		Pathogenicity tests*															
		Domestic rabbits providing papillomas															
		14-04				14-00					13-97					3-88	
		Test rabbits a b c d				Test rabbits e f g h i					Test rabbits j k l m n					Test rabbits o p q r s	
1-27	+	+±	+±	+	+++	+++±	+++	+++±	+++	+	++	+	+	++	0 ±	0 0 ±	
1-52	+++	+++	++	+±	±	+±	+±±	++		0	0	0	0	0	0	0 0 ±	
A	+++	+++	++	+++	±	±	+	+	±	0	0	±	+	±	0 ±	0 0 0	
B	++	+++	++	++	±	±	++	++		±	+	±	±	±	0	0 0 0 0	
2-00	±	+±±	+±	+±	0	0	±	0	0	0	±	±	±	±	0	0 0 0 0	
53	±	+	±	0	0	0	0	±		0	0	0	0	0	0	0 0 0 0	
C	±	±	0	0	0	0	±	±		0	0	0	0	0	0	0 0 0 0	
TX <sub>1</sub>	0	+	0	0						0	0	0	0	0	0	0 0 0 0	
TX <sub>2</sub>	0	0	0	0	0	±	0	0		0	0	0	0	0	0	0 0 0 0	
3-31	±	0	0	0	0	0	±	0		0	0	0	0	0	0	0 0 0 0	
1-68	0	±	0	0						0	0	0	0	0	0	0 0 0 0	
2-95	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0 0 0 0	

\* 20 per cent saline extracts of papillomas rubbed into scarified skin areas prepared with a mixture of turpentine and acetone applied four times at 2 day intervals.

In this experiment each cottontail papilloma extract was inoculated into four or five domestic rabbits in order to minimize the factor of individual susceptibility of the test animal; and it will be seen that the results were so consistent (Table IV) that it can be dismissed from consideration. A first finding to be noted is that the yield of active virus from the papillomas produced by the twelve virus strains varied with the domestic rabbit from which they came, eleven of the twelve inocula from D.R. 14-04 giving rise to growths whereas only three from D.R. 3-88 did so and these to exceedingly few, the other two hosts occupying an intermediate position. Yet D.R. 11-04 and D.R. 3-88 were wholly comparable as concerns age, species and weight, and character of the papillomas tested. It will further be seen that some virus strains (1-27, 1-52, A, B, and 2-00) proved “recoverable” much more frequently than others

and that this held true from host to host. Only the virus strains which were most readily recoverable gave rise in D.R. 3-88 to growths that yielded demonstrable virus on extraction. Circulating antibody can scarcely have been responsible for this last finding since antibody was not demonstrable by the complement fixation test in the blood of D.R. 3-88 (nor in that of 13-97), whereas the sera of D.R. 14-04 and of D.R. 14-00 fixed complement in a dilution of 1:2.

Many subsequent tests gave similar results. A total of 365 domestic rabbit papillomas, produced by 21 different virus strains, have now been tested in various experiments. About one-fourth of the strains proved to be readily demonstrable in extracts of most of the papillomas produced with them in domestic rabbits when tested in the way just described, whereas the remainder were recoverable only from hosts in which the conditions were favorable for reasons unknown, as in Experiment 4. By utilizing papillomas from these favorable hosts virus has been recovered from every one of the 21 strains tested. Further analysis of the group showed that virus could be demonstrated in extracts of from 86 to 90 per cent of the growths produced by the more readily recoverable virus strains (strain 1-27 and strain A, for example—see Table IV), whereas only 34 per cent of the growths produced by the poorest virus strain (2-95) yielded virus.

*Comparative Yield of Virus from Domestic and Cottontail Rabbit Papillomas*

*Infectivity Tests with Growths Produced by Different Virus Strains.*—The preceding experiments have shown that domestic rabbit papillomas yield virus in varying amount, this largely depending upon the virus strain used to produce the growths and the host in which they grow. Do these same factors influence the recoverability of virus from the papillomas of cottontail rabbits? In an experiment to test the point cottontail and domestic rabbit papillomas produced by a readily recoverable and a poorly recoverable strain of virus respectively were tested.

*Experiment 5.*—10 per cent saline extracts of the glycerolated papillomas of W.R. 1-52, one of the most readily demonstrable strains of virus, and W.R. 2-95, the poorest in this respect (see Table IV), were each tattooed into six spots on the skin of six normal cottontails and four normal domestic rabbits. The resulting papillomas were carefully traced on sheets of cellophane with a wax pencil on the 80th day after the inoculation. The domestic rabbit growths were 1 to 2 cm. across at the base and 0.4 to 0.8 cm. high, whereas the cottontail growths were up to 1.5 cm. high. No difference was evident in the size or character of the papillomas produced by the two virus strains in the individual host though as usual they had enlarged more rapidly in some rabbits than in others and in some were fleshy, in others dry. The papillomas were removed, diced, washed in saline, and kept in 50 per cent glycerol-Locke's solution at about 4°C. 10 per cent extracts of the domestic rabbit growths were then rubbed into scarified areas of the skin of four normal rabbits, which had been prepared with turpentine and



TABLE V  
Comparative Yield of Virus from Papillomas of Domestic and Cottontail Rabbits Produced by the Same Virus Strains

Papillomas procured from		Virus strain used to produce papillomas W.R.	Pathogenicity tests*											
Species	Rabbit No.		14th day				21st day				35th day			
			a	b	c	d	a	b	c	d	a	b	c	d
Domestic rabbits	6-11	1-52	0	0	0	0	±	+	±	+	+	±	+	±
		2-95	0	0	0	0	0	0	0	0	0	0	0	0
	6-15	1-52	0	0	0	0	±±	±±	±±	±±	±±	±±	±±	±±
		2-95	0	0	0	0	0	0	0	0	0	0	0	0
6-09	1-52	0	0	0	0	+	±±	±±	±±	±±	±±	±±	±±	
	2-95	0	0	0	0	0	±	0	0	+	±	±	±	
6-12	1-52	0	0	0	0	±±	±±	±±	±±	±±	±±	±±	±±	
	2-95	0	0	0	0	±	±	0	0	±	±	0	±	
Cottontail rabbits	1	1-52	±±±±	±±±±	±±	±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±
		2-95	±±±±	±±	±±	±±	±±±±	±±±±	±±±±	±±	±±±±	±±±±	±±±±	±±±±
	2	1-52	±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±
		2-95	±±	±±±±	±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±
	5	1-52	±±±±±	±±±±	±±±±	±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±
		2-95	±±±±±	±±±±	±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±
	7	1-52	±±±±±	±±±±	±±	±±±±	±±±±±	±±±±±	±±±±	±±±±	±±±±±	±±±±±	±±±±	±±±±±
		2-95	±±±±	±±±±	±±±±	±±	±±±±±	±±±±	±±±±	±±±±	±±±±±	±±±±±	±±±±	±±±±±
8	1-52	±±	±±	±±	±±	±±±	±±±	±±±	±±±	±±±	±±±	±±±	±±±	
	2-95	±±±±	±±	±±±±	±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	±±±±	
10	1-52	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	
	2-95	±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	±±±±±	

\* 10 per cent saline extracts of the papillomas were rubbed into scarified skin areas which had not been treated four times with a mixture of turpentine and acetone.  
a-h = test rabbits.



W.R. 1-28	1:20,000	++	0	±	+++	+	±	3-26	±	±	0	0	0
								3-27	++	+±	0	0	0
								3-28	+±±	±	0	0	0
								3-29	+++	+±±	0	0	0
W.R. 1-28	1:20	++++	++++	++++	++++	++++	++++	3-30	++++	++++	++++	++++	±
								3-31	++++	++++	++++	++++	0
								3-32	++++	++++	++++	++++	++++
								3-33	++++	++++	++++	++++	++++
Normal controls.....													
								3-34	0	0	0	0	0
								3-35	0	0	0	0	0
								3-36	0	0	0	0	0
								3-37	0	0	0	0	0

10 cc. of the materials was injected intraperitoneally, as indicated, on the 1st and 8th days of the experiment. Serum was procured for test on the 15th day.  
 2 units of complement in all tubes.  
 Antigen W.R. 1-70, 1:120.  
 \* 10 per cent saline suspensions of the papillomas were rubbed into scarified skin areas which had been treated four times with a mixture of turpentine and acetone prior to inoculation.

acetone, and 10 per cent extracts of the wild rabbit papillomas were similarly inoculated into similarly prepared skin areas of another group of four test rabbits.

The results of the experiment are shown in Table V. As was to have been expected, there was a striking difference in the amount of virus procured from the papillomas of cottontail and domestic rabbits. On the 14th day after inoculation all of the areas inoculated with the papilloma extracts from the cottontails showed numerous papillomas, and by the 35th day most of them were covered with confluent papillomatous masses. There was no detectable difference in the yield of virus from the papillomas produced by the two strains of virus as evidenced by number of growths arising and time of appearance. The domestic rabbit papilloma extracts, on the other hand, contained relatively little active virus, as attested by the longer incubation period and the smaller number of growths, and the differences in yield observed in the preceding experiment were again apparent. Virus was demonstrable in extracts of all of the growths produced by virus strain 1-52, whereas it was found in the growths of only two of the four rabbits inoculated with strain 2-95 and in smaller amount than from the papillomas produced by strain 1-52. The papillomas produced by the extracts of the domestic rabbit growths did not differ in morphology from those produced by the cottontail materials.

*Comparative Antigenicity of Extracts of Domestic Rabbit and Wild Rabbit Papillomas.*—When extracts of cottontail or domestic rabbit papillomas are injected intraperitoneally into normal rabbits, an antibody specifically directed against the papilloma virus is elicited (5) which can be demonstrated in the blood by either the neutralization or the complement fixation test. Is the antibody response called forth by the papilloma extracts proportional to the amount of demonstrable virus in the extracts? To obtain an answer to this question the antibody response elicited by the injection of five different domestic rabbit papilloma materials was compared with that obtained by the use of a highly infectious, wild rabbit papilloma material.

*Experiment 6.*—The glycerolated papillomas of D.R. K 1-90, 2-31, and 2-32 (produced by strain W.R. 1-72), and D.R. 3-96, 4-01, and 4-02 (produced by strain W.R. 1-28), were used in this experiment. The infectivity of some of these materials had been previously tested (see Experiments 2 and 3). Weighed portions of the papillomatous tissue were ground with sand and suspended in saline to make 5 per cent suspensions. The sand was allowed to settle out and all of the crude supernatant material was removed for test. In addition a 5 per cent saline suspension of the highly infectious cottontail rabbit papillomas of W.R. 1-28 was prepared in the same way and a portion of it was also diluted to 1:20,000. Previous experiments had shown that this latter dilution would be about as infectious as the 5 per cent suspensions of the domestic rabbit papillomas. 10 cc. of each suspension was then injected intraperitoneally into groups of four normal domestic rabbits after the skin of the belly had been slit to prevent infection of it, and 1 week later the injections were repeated

with freshly prepared suspensions. After another week had elapsed all of the rabbits were bled from an ear vein for serum, as were also four normal domestic rabbits. A portion of each suspension was tested for infectious virus by inoculation into scarified areas on three domestic rabbits, previously prepared by four applications of turpentine and acetone. The sera were tested for their capacity to fix 2 units of complement in mixture with a 1:120 saline extract of cottontail papillomas (W.R. 1-72).

The results of the experiment are summarized in Table VI. The infectivity tests showed that the 1:20 suspensions of domestic rabbit papilloma tissue and a 1:20,000 suspension of the wild rabbit material elicited about the same number of papillomas on inoculation into normal domestic rabbits. The suspension of cottontail papilloma tissue at a dilution of 1:20 was highly infectious. The sera from the rabbits injected with the domestic rabbit papilloma material or with the wild rabbit suspension diluted 1:20,000 fixed complement in dilutions of from 1:2 to 1:8, whereas that of those receiving the wild rabbit papilloma suspension diluted 1:20, and hence containing a thousand times as much of the virus, fixed complement in a dilution of 1:128 in three of the four test rabbits. No antibody could be detected in the normal serum controls. The experiment shows that the antibody response to intraperitoneal injection of crude suspensions of either domestic or cottontail rabbit papillomas closely parallels the content of infective virus of the suspensions.

#### DISCUSSION

The experiments here reported show that active virus is present in extracts of many papillomas of domestic rabbits which seem to contain none when tested by the ordinary methods. Even the virus strains most liable to "masking" on inoculation into domestic rabbits yielded a considerable proportion of growths from which virus could be obtained again. The amount obtained, however, even with the most pathogenic extracts, was always small when compared with that recovered from comparable papillomas of cottontail rabbits,—a fact evident not only from the small number of growths which resulted from inoculation of the extracts into prepared skin, but from the antibody response after the intraperitoneal injections of suspensions of the papilloma tissue of domestic and wild rabbit papillomas (Table VI). It has been known for some time that the antibody response of domestic rabbits carrying virus-induced papillomas falls far short of that of cottontails carrying comparable growths. This is not due to an inferior capacity of domestic rabbits to develop antibody, for they react as markedly as cottontails to the injection of fixed amounts of virus (12). There would seem to be no doubt that far less of this agent in infective or antigenic form exists in the growths of domestic rabbits than in those of cottontail rabbits.

By further improvements in technique it may be possible to demonstrate virus more readily and in a greater proportion of papillomas than have yielded

it in the present work. There is no way of knowing at the moment whether eventually virus can be obtained from every domestic rabbit papilloma. None has yet been got from a considerable proportion of the papillomas produced with some strains of virus (*e.g.*, that of W.R. 2-95, Table IV), a fact the more remarkable because the growths failing to yield it have often a notably great proliferative vigor.

It may well be that such papilloma virus as can be obtained in the infective state from domestic rabbit papillomas,—and those of wild rabbits for that matter,—represents an excess over the amount implicated in the proliferation of the growths, this being bound in some way to the cell structure and hence innocuous. Such a state of affairs is not unusual, for the yield of identical viruses from different diseased species and tissues varies enormously. Tobacco mosaic virus, for example, can be recovered in quantity from tobacco, but in comparatively small amounts from spinach (13). The conception that recoverable virus is excess virus in the papillomas finds some support in the singular difference in yield from growths produced with the same inoculum in individual domestic rabbits, a difference not always to be explained by the presence of neutralizing antibody (*vide* Experiment 3). Possibly differences exist in the virus-cell relationships from rabbit to rabbit, some proving more favorable to the increase of virus than do others. However this may be, it seems certain that the antibody response of the individual animal to the papilloma that it carries represents mainly, if not entirely, a response to active virus,—whence one may conclude that, if there is such a thing as bound or masked virus, it is so closely bound as not to function in any considerable degree as an antigen, unless indeed it calls forth some antibody of special sort which has not yet attracted attention.

#### SUMMARY

By preliminary preparation of the skin in ways that render it hyperplastic the presence of infective virus can be demonstrated in extracts of domestic rabbit papillomas which yield no growths when inoculated by the ordinary methods and which for this reason have been supposed to contain no virus. The amount of virus recovered by the method outlined in the present work, however, is small when compared with the yield obtained in most instances from comparable cottontail rabbit papillomas. The yield is greatly influenced not only by the virus strain used to produce the growths but by the individual rabbit host. Although virus has been obtained from papillomas produced in domestic rabbits by all of the virus strains tested, a total of 21 thus far, only about one-fourth of these strains are readily to be procured again from the growths they cause and the others are demonstrable only in hosts in which the conditions are favorable for reasons unknown.

An experimental comparison of the capacity of suspensions of papilloma

tissue from domestic and cottontail rabbits to elicit specific antibodies has shown that the titers attained are approximately proportional to the amount of infective virus demonstrable in the suspensions. The findings as a whole indicate that far less virus exists in infective or antigenic form in the papillomas of domestic rabbits than in those of cottontail rabbits.

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