

## SEROLOGICAL REACTIONS WITH A VIRUS CAUSING RABBIT PAPILLOMAS WHICH BECOME CANCEROUS

### I. TESTS OF THE BLOOD OF ANIMALS CARRYING THE PAPILLOMA

By JOHN G. KIDD, M.D., J. W. BEARD, M.D., AND PEYTON ROUS, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

#### PLATE 5

(Received for publication, April 29, 1936)

The cutaneous papillomas induced in domestic rabbits with a virus procured from western cottontails (1) have the immediate attributes of tumors (2), and they frequently become cancerous (3). The problem of the relation of the virus to the neoplastic activities is complicated by a singular difficulty. Though readily obtained from most of the naturally occurring papillomas of cottontails, it cannot ordinarily be recovered from the far more vigorous growths induced with it in domestic rabbits. Recently indeed, Shope (4) has secured virus strains with which the disease can be serially transmitted in such animals, and it should be possible to make direct tests for the presence of these in any cancers that may develop as result of their action. But the large material thus far studied by us,—comprising more than 150 instances of malignant change in all,—has been provided by the inoculation of domestic rabbits with virus strains irrecoverable from either the papillomas or the cancers, by any of the various means thus far employed. For this reason recourse has been had to a serological method to determine the presence of the virus.

Shope noted that rabbits carrying the papilloma proved more or less resistant on reinoculation, and that their sera exerted a neutralizing influence when mixed with the virus *in vitro* (1). We have titrated the antiviral power, investigated the conditions of its development, and sought for it in the blood of animals carrying tumors of various sorts. The findings will be recorded in two associated papers.

*General Method*

A method whereby sera could be compared was worked out first.

The virus acts only where brought in contact with injured epidermis, but it is capable of surviving for long periods outside of the body. When many neighboring areas of scarified skin are inoculated they must be protected for a few days, until healing has taken place, in order to exclude all risk of the transfer of virus material from one site to another. This can be readily accomplished by shaving only the immediate areas utilized, placing on each after inoculation a sterile gauze square which fits neatly amidst the fur, mooring these firmly in place with adhesive, and covering all with a large gauze dressing and a many-tailed bandage. In no instance amongst hundreds of tests carried out in this way, often involving more than a score of inoculations into each animal, has a papilloma developed under conditions suggesting that the virus had undergone accidental transfer.

Each inoculation area was a square 3 to 4 cm. across, separated from its neighbors by a furry zone 1 cm. or more wide. It was soaped and shaved, rinsed well in running tap water, and allowed to dry for several hours at room temperature. On the ventral surface of an adult domestic rabbit there is room for two rows of five or six squares, to either side of the midline, and for an additional row of four or five squares immediately next them on each side (Fig. 1). The skin higher on the sides is somewhat thicker and more difficult to scarify, and its fur soon grows again. Hence it is less favorable, both to infection with the virus and to visibility of the young papillomas. Differences in situation as possibly affecting the results were excluded by inoculating the test mixtures and their controls at corresponding places on the right and left, and systematically varying the situations of the inocula from animal to animal.

A strip of sterile sandpaper that could be shifted within a clamp on a handle was used for scarification, with fresh sandpaper for each square. Sterile, dry, Perfection glass test tubes were employed to rub the test mixtures in, each scarified area receiving 3 drops of one of these before the next area was abraded. As soon as all the squares had been inoculated a gentle blast of air warmed by an electric coil was directed upon them to dry them, and then they were dressed as already described. Each animal received all the inocula before the next was dealt with. 2 to 6 rabbits were employed for the tests of every experiment.

The dressings were removed when healing was complete, after 7 to 10 days. The papillomas usually began to appear within 2 to 3 weeks, and their number, character, and rate of development were recorded at intervals of 3 to 5 days until it was plain that no further, pertinent data could be obtained. Fig. 1 shows the range of lesions encountered, from solitary discrete growths to confluent

papillomatosis, the latter being consequent on the action of the virus upon many adjacent cells. Confluent growths could be perceived sooner than scattered ones, and often some of the latter appeared 1 or 2 weeks later than others of the same inoculated square; but skin that was still negative after 6 weeks invariably remained so. The findings were recorded with the aid of the following symbols: + —, one or two discrete papillomas; +, a small number of discrete papillomas; ++, many discrete papillomas; + + +, semiconfluent papillomatosis; + + + +, confluent papillomatosis. Different observers were found to record the lesions identically, yet throughout the testing the same individual did this work. The recording system made no provision for the rate of enlargement of the lesions, nor for their ultimate fate, but these later phenomena were largely determined by the character of the individual test rabbits, and primary virus neutralization was the subject under study.

#### *Results of Diluting the Virus*

On several occasions a virus-containing extract of the glycerinated tissue of a "spontaneous" papilloma has been diluted with Tyrode in multiples of ten, and inoculated into rabbits according to the method described. The 10 per cent extract and the 1 per cent usually gave rise to confluent or semiconfluent proliferation, whereas the higher dilutions yielded scattered papillomas in numbers roughly proportionate to the decrease in virus amount. The most active materials gave rise to only 1 or 2 discrete growths at a dilution of 1 in 100,000, and none was ever obtained at 1 in 1,000,000. Numerous tests carried out incidentally to other experiments have yielded like findings. The virus always acted within a few weeks or not at all. Many of the animals receiving titration mixtures and kept for more than a year have been repeatedly examined with this point in view. The brief delay in the appearance of some of the discrete papillomas of punctate origin can be accounted for by virus infection of but a few cells or a single cell; for the papilloma enlarges by intrinsic proliferation, and the time required for it to become visible will inevitably vary with the number of cells from which it derives. The late-appearing papillomas differed in no essential respect from the generality.

Some areas that remained negative after inoculation with virus dilutions that might conceivably have yielded growths were tarred or injected with Scharlach R in olive oil, and others were painted repeatedly with xylol, to further the action of virus that might have lain latent in the absence of such stimulation. No growths ever developed.

In other experiments having the same object, areas about 12 by 4 cm. in diameter on the sides of susceptible rabbits were infiltrated with active virus extract, by intradermal injections about 2 cm. apart, all at the same level; and subsequently at intervals of 2 weeks the infiltrated skin was traumatized by tattooing to provide the injury necessary to render the virus effective. Again no growths were got save at the points, marked with dye, where the injecting needle had originally been thrust in, and at points where tattooing was done on the day of injection. From all this it seems certain that virus experimentally introduced into the skin of domestic rabbits does not ordinarily lie latent there. It certainly does so on occasion in the cottontail, its natural host. In an animal of this sort, tarred repeatedly on the ears, numerous characteristic, pigmented growths appeared at 2 out of 3 sites on the sides where virus had been inoculated many months previously with negative results. No tar was put on the sides at any time, nor was there any sign there of the diffuse hyperkeratosis consequent on tarring.

*Neutralization Tests with the Sera of Domestic Rabbits Bearing Papillomas*

*General Technique.*—Blood specimens were taken from an ear vein into tubes coated with paraffin to prevent hemolysis, and the serum was taken off 24 to 48 hours later and centrifuged to remove the cells. The clot had stood several hours at room temperature and for the rest of the time in the ice box.

The virus materials utilized for the work reported in our previous papers regularly gave rise to growths that enlarged progressively, during the first weeks at least. To broaden the observations it was desirable to employ a material which caused papillomas that retrogressed not infrequently yet which was carcinogenic under favorable conditions. Fortunately one was available, the papillomatous tissue of W.R. 6-32, which had been hashed fine and stored in 50 per cent glycerine. When a fluid of standard virus strength was desired, 4 cc. of this glycerinated tissue was thrown down in a graduated centrifuge tube by spinning at a fixed speed; the amount of sediment was read off; it was suspended in 8 parts of Tyrode solution; thrown down again, ground with sand, made to the required dilution by bulk, usually 6 per cent; and finally spun long at high speed. The central portion of the fluid column was drawn off through a needle, spun again, and its central portion aspirated in the same way. The "dearticulated" test material thus obtained was invariably clear, and it had always the same power to produce papillomas, as shown by dilution tests. Such tests were carried out a few days prior to the experiments and again 10 weeks later, that is to say 2 weeks

before the final serum examination. On both occasions virus dilutions of  $10^{-4}$  gave rise to a few growths scattered on the area of inoculation, but  $10^{-5}$  to none.

Two mixtures were made of virus with each serum in differing proportions. After incubation at  $37^{\circ}\text{C}$ . for 2 or 4 hours all were inoculated into 3 to 6 normal brown-gray rabbits shaved in squares, with corresponding virus-Tyrode controls. Some of these rabbits proved more favorable than others, their papillomas appearing sooner and growing more vigorously. The variety thus introduced into the findings did not alter their implications, since each animal served as its own control.

The experiment which follows was devised to test whether the power of the blood to neutralize virus varies with the amount of papillomatous tissue developing. Incidentally an effort was made to determine whether the presence of large growths had any influence on the course of small ones appearing somewhat later.

*Experiment 1.*—8 brown-gray rabbits of from 1940 to 2850 gm. were paired according to size, weight, and similarity of skin texture. All were bled 5 cc. for serum, and then 10 per cent virus was tattooed into areas 2 mm. across at 3 widely separate points at the same horizontal level on each side. An electric tattooing machine was used. In addition the animals of one group were inoculated broadcast over an abdominal area about 12 by 8 cm.

The growths developing on the sides were traced and charted at intervals of a few days, and the character of the abdominal growths was also recorded. On the 33rd, 42nd, and 68th days after inoculation serum was again procured, and on the last of these days 4 of the rabbits, of differing antiviral power at the most recent test, were reinoculated with two different strains of active 10 per cent virus (W.R. 18 and W.R. 8-76), into scarified areas about 10 cm. across on the sides.

In Chart 1 the skin areas covered by the growths resulting from the tattoo inoculations are shown to scale. The confluent abdominal masses occupied the entire inoculation area in every case, but their heights are indicated diagrammatically by groups of vertical lines joined at the base. The antiviral power of the serum of the host, as expressed in terms of its power to neutralize, is given in hatched columns. An arrow surmounting a column means that neutralization of the virus suspension employed was complete, and hence that the serum titer was probably greater than is shown. The positive or negative outcome of the reinoculations on the 68th day is recorded as R + or R -.

For the titrations just prior to inoculation, portions of the individual sera were mixed in equal parts with 10 per cent virus and 1 per cent virus respectively; for the tests on the 33rd and 42nd days  $6\frac{2}{3}$  per cent virus was added to an equal part of serum diluted 2:5 with Tyrode and to whole serum respectively (columns x and y); and for the final titration, 68 days after inoculation, similar mixtures with 6 per cent virus were made.

As already stated the standard virus fluid yielded one or two discrete papillomas

when diluted to  $10^{-4}$  with Tyrode, but no growths whatever at  $10^{-5}$  on inoculation in standard quantity on a 4 cm. square. It was arbitrarily assumed that at  $10^{-4}$  one infective virus unit was present in the inoculum, and a 6 per cent virus fluid was deemed to contain 600 units. If it was rendered inactive by serum the latter was adjudged to have a titer of 600 or more. The titer on incomplete neutralization was computed from the total number of plus marks recorded for any one inoculum in all the test rabbits receiving this, as compared with the total for the virus-Tyrode control. If, for example, the latter yielded an average of + + + for each of 3 test rabbits, that is to say 9 pluses in all, and the serum-virus mixture yielded + for each animal, or 3 pluses in all, the neutralizing power of the serum was recorded as 6/9 of 600 units or approximately 400. This method of computation was artificial, but it yielded figures that served as a rough index to the differences actually observed. It was not applicable when the control mixtures gave rise to confluent growths, since all concentrations of virus beyond a certain minimum produced these.

The confluent and semiconfluent papillomatous masses resulting from broadcast inoculation of the abdomen appeared between the 14th and 19th day, and by the 33rd day they measured about 6 by 10 cm., and were 5 to 15 mm. high. By the 42nd day they were 10 to 25 mm. high, becoming redundant, and on the day of the last serum test, they were 1 to 3 cm. in height,—enormous, folded, fissured masses with dry tops and fleshy bases. The general health of the animals remained excellent, however, like that of the companion group, save in the case of 3-26, which died of an intercurrent disease on the 37th day.

The tattoo papillomas appeared late, between the 19th and 23rd day in both groups of animals, and at the 68th day they were barely 2 cm. across in the most favorable rabbit (D.R. 3-19). The total skin area involved in papillomatosis and the actual bulk of papillomatous tissue were from the beginning far greater in the group of rabbits with abdominal growths.

Chart 1 depicts the results of this experiment. None of the sera obtained just prior to inoculation had antiviral power. 33 days later this power was pronounced in the case of the animals which had developed confluent papillomatous masses on the abdomen in addition to the small tattoo growths, whereas it was still slight or lacking in the rabbits carrying the latter only. It is conceivable that the difference may have been due in part to the very different amounts of virus introduced into the 2 groups by way of the scarified skin. But a primary immunization induced in this way cannot account for the progressive increase in the antiviral power as the papillomas enlarged. By the 68th day, or long before that, the sera of the group with broad abdominal masses were capable of neutralizing completely a 6 per cent virus extract, 600 or more units of virus, when mixed therewith in

equal parts. The specimens from the contrasting group of animals with but little papillomatous tissue were relatively ineffective.

The tattoo papillomas appeared and developed at the same rate in both groups, being to all appearances uninfluenced by the antiviral power of the blood.

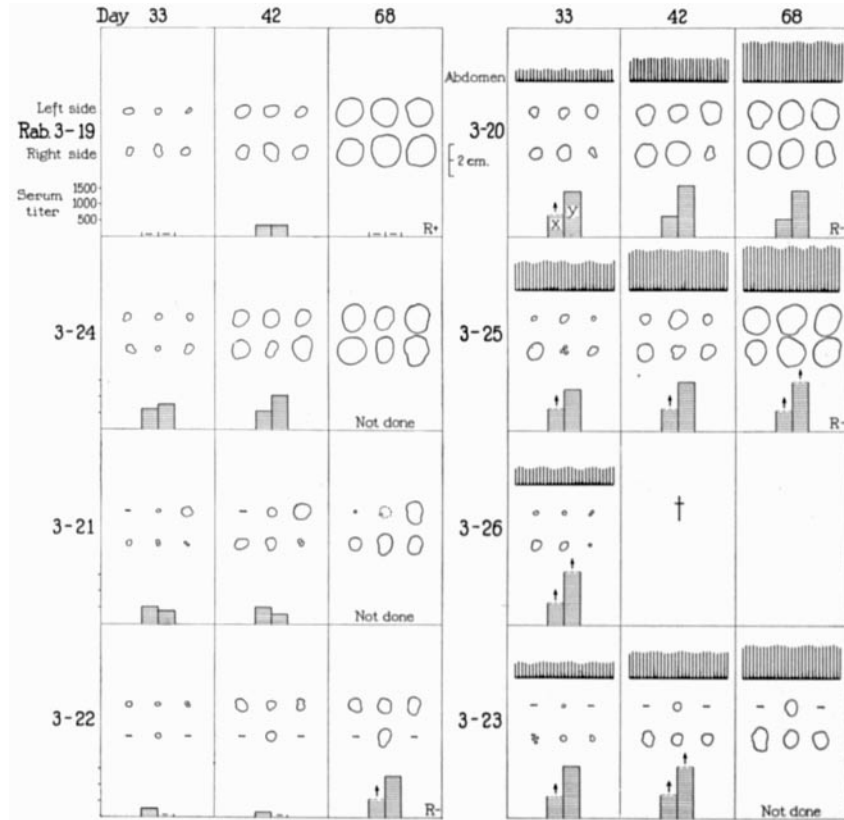


CHART 1. Comparison of antiviral serum titer and papilloma course.

To procure more data a second experiment was carried out along the same lines, but with the addition of intradermal inoculations of the virus. The papillomas resulting from such inoculations are inconstant in occurrence, and punctate in origin, developing only where the injection needle has been thrust through the skin. They tend to appear after those from tattoo inoculations and considerably later than

the confluent masses engendered by rubbing in virus broadcast. For all these reasons they seemed especially fitted to disclose whether the neutralizing power of the blood influences the course of events after the cells have become infected with the virus.

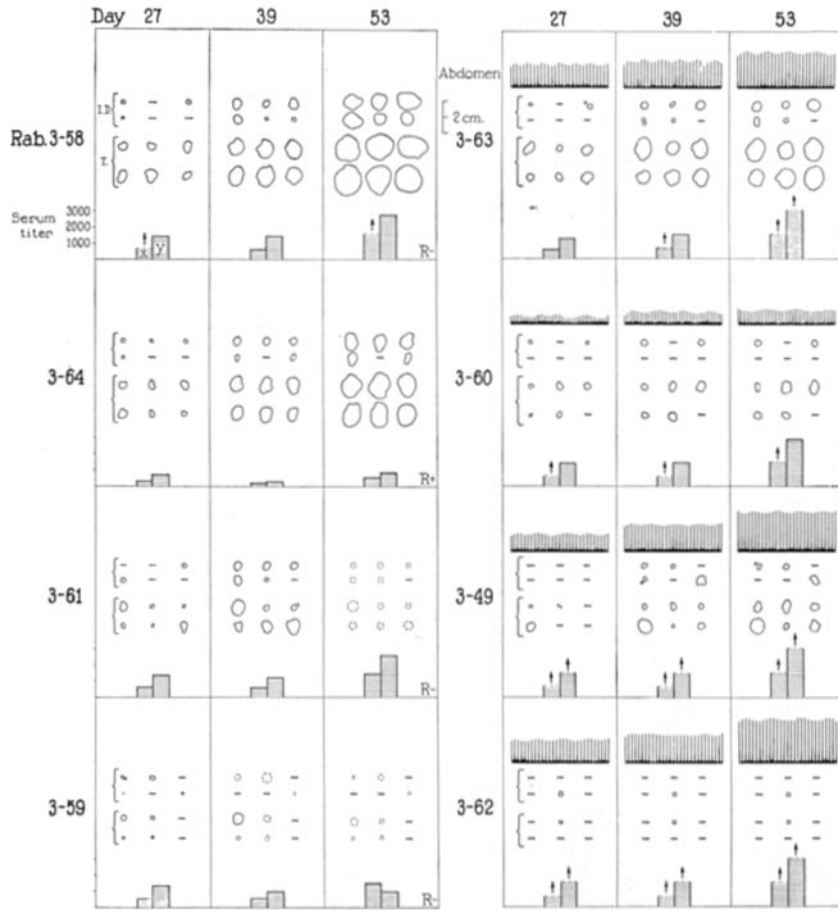


CHART 2. Comparison of antiviral serum titer and papilloma course.

*Experiment 2.*—In this test the abdominal areas inoculated with virus were notably large, 10 by 12 cm. across. A 5 per cent extract of virus 6-32 was used and both groups of animals received 3 tattoo inoculations in a line on each side, and 3 intradermal inoculations of 0.05 cc. each of fluid immediately above, at a distance of about 4 cm. Serum specimens were procured on the day before the experiment



was begun, and again on the 27th, 39th, and 54th days. On the 64th day 4 of the animals were reinoculated intradermally with 0.3 cc. of a 5 per cent virus extract at 4 sites.

For the preliminary neutralization test the sera were incubated in equal quantity with 5 per cent virus, and 0.5 per cent virus, respectively; for the titrations on the 27th and 39th days 6 per cent virus was mixed in equal parts with whole sera and with sera diluted 2:3 with Tyrode; and for the last titration, on the 54th day, 6 per cent virus was again used, in two mixtures with serum diluted 2:3 and 1:4 with Tyrode.

In 3 rabbits the growth on the abdomen appeared at about the 15th day, occupying the whole field of scarification, and it soon became redundant, folded, and 2 to 3 cm. in thickness. In the fourth animal (3-60), on the other hand, it appeared at about the 20th day, as numerous, discrete and semiconfluent papillomas which slowly attained a height of  $2\frac{1}{2}$  mm.

The tattoo papillomas appeared at about the 20th day in every rabbit except 3-62, in which they never developed. The growths from intradermal inoculation became perceptible later, in several instances between the 27th and 39th day, as was to have been expected because of their origin from relatively few cells.

The sera procured just prior to inoculation of the animals were wholly devoid of neutralizing power for the virus, as in the previous experiment. By the 27th day, however, this power was in most instances considerable (Chart 2), and thereafter it underwent a further great increase in 6 of the 8 individuals. The exigencies of charting have made necessary a reduction to half height of the columns representing serum titer. The actual differences in antiviral power in the 2 groups of animals were nearly as pronounced as in Experiment 1.

The sera of the group in which a large amount of papillomatous tissue developed acquired much the greater antiviral power, though this was not true of every animal. It may be pointed out in this connection that rabbit 3-19 of Experiment 1 carried the largest tattoo papillomas of any of its group, 6 growths each nearly 2 cm. across on the 68th day; yet its serum as tested at this time had no perceptible effect to neutralize the virus (Table I), although previously a slight one had been demonstrable; and the final reinoculation resulted in growths. It follows that the presence of a considerable quantity of papillomatous tissue is compatible with a lack of antiviral power. On the other hand there are animals in which this power becomes outspoken although the papillomas responsible for it are small and may endure but a brief time (rabbits 3-22, Chart 1, and 3-59 and 3-61, Chart 2—

animals all of which proved completely resistant on reinoculation). Shope has reported (1) the presence of partial resistance 6 days after the papillomas had first appeared and 14 days after the initial virus inoculation. He had rubbed virus into one entire side of the animals, after appropriate scarification.<sup>1</sup> In rabbits 3-59 and 3-61 of Chart 2 the papillomas were only a few millimeters across at most on the 27th day, yet the antiviral power was already well established.

The intradermal inoculations of Experiment II did not engender

TABLE I  
*Neutralization Tests with the Serum of a Rabbit (D.R. 3-19) Bearing Large Tattoo Papillomas (68th Day after Inoculation)*

6 per cent virus.....	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.
Serum D.R. 3-19.....	0.5 cc.	—	0.2 cc.	—
Tyrode.....	—	0.5 cc.	0.3 cc.	0.5 cc.
Test rabbit				
21 days				
1	++-	++-	++	++
2	+++	+++	+++-	+++
3	+++-	+++-	+++	++-
27 days				
1	++-	++	++	+++-
2	+++	+++	+++-	+++
3	++++-	++++	++++	++++-
34 days				
1	++++-	+++	+++-	++++-
2	++++	++++	++++	++++
3	++++	++++	++++	++++-

The plus marks record the character of the papillomatosis produced by the mixtures (see text) as noted on the days given.

growths regularly, nor did the tattooing for that matter; but they appeared in sufficient number for our purposes. The differences in their number and course seem at first sight to parallel the antiviral differences, both the tattoo and intradermal papillomas being more numerous and becoming somewhat larger in the group with the weaker antiviral power; but when the individual instances are scrutinized, one sees clearly that the phenomena cannot be explained by the influence of this power. In rabbit 3-59 with a notably low serum titer the growths all retrogressed between the 39th and 53d days, whereas in

<sup>1</sup> Personal communication from Dr. Shope.

3-63 with a high titer they progressed, as also in 3-58. In 3-61 retrogression took place, although the serum titer was less than in several other animals in which progressive enlargement occurred. Evidently some undetermined factors exercised a decisive influence in these cases. The possibilities are so various that extensive tests with a large variety of controls will be required to determine whether the antiviral power of the blood has any influence whatever upon the papillomatous proliferation, once this has started. Certainly the presence of such power does not suffice to exclude the appearance and development of punctate growths at sites of intradermal inoculation (rabbits 3-49 and 3-63, Chart 2), much less to prevent a vigorous enlargement of growths already well established. There can be no doubt, on the other hand, that the success of reinoculations with virus is conditioned by the state of the blood. 4 animals of each experiment were reinoculated on the 64th and 68th days, and in one instance only in each case did papillomas result,—in an individual with serum devoid of neutralizing power (Chart 1, 3-19)<sup>2</sup> and in another in which it was slight (Chart 2, 3-64). Shope found 2 papillomatous animals susceptible to reinfection 76 days after primary inoculation. Large growths had resulted from the latter in one of these instances, yet the reinfection gave rise rapidly to growths that seemed equally vigorous. In the other rabbit but few papillomas appeared after reinoculation, and these late, on the broad area into which active virus had been rubbed.<sup>1</sup>

The partially neutralizing sera did not cause the papillomas to appear later than on ordinary dilution with Tyrode, nor to grow more slowly or be different. It was plain that the effect of the serum was to cut down the number of effective virus entities, not to alter their individual pathogenic capabilities.

#### DISCUSSION

The results of rubbing serial dilutions of the Shope virus into areas of scarified skin resemble in some significant respects those from the

<sup>2</sup> The serum of rabbit 3-19 was tested again after the lapse of 426 days in all. At this time it bore 8 papillomatous masses, of which 6 had been largely replaced by cancers. Its serum now completely neutralized a 0.5 per cent extract of active virus, when mixed therewith in equal parts, and it almost neutralized a 1 per cent extract, its calculated antiviral titer being 90.

seeding of dilutions of bacteria upon agar plates. The discrete papillomas forming in the one case, like the colonies in the other, are the outcome of cell proliferation from individual centers; and the number of effective virus entities can be appraised by the number of growths engendered, just as can the number of other bacteria by the colonies to which they give rise. The inability of the Shope virus to lie latent in the skin of domestic rabbits adds to the reliability of the results, growths appearing soon after inoculation or not at all.

The rabbit sera that partially neutralized the virus might have been expected to cause a delay in the appearance of the papillomas or to alter their character; but nothing of the sort occurred. Instead there occurred merely a reduction in the number of effective virus entities, as expressed in the number of growths engendered. Some entities had been cancelled, but those which remained pathogenic caused growths differing no whit from papillomas due to diluted virus. In this connection the results of attempts to attenuate the virus of poliomyelitis may be recalled, these having brought about in general merely a reduction in the amount of active virus, not an essential change in its character (5).

The blood of domestic rabbits in which virus-induced papillomas are developing nearly always acquires some power to neutralize the virus *in vitro*, whereas that of normal animals is devoid of effect. In only one normal rabbit amongst many has any neutralizing influence been encountered and then it was but slight (rabbit 1, Table IV of Paper II). The absence of antiviral principles from the blood of normal domestic rabbits speaks against the possibility that the papillomatosis now endemic in western cottontails is consequent on an escape of the virus into this species from domestic rabbits in which it has become symbiotic by reason of long association; and the notably vigorous papillomatosis produced by the virus on inoculation into domestic breeds as well as the unfailing susceptibility of all normal individuals would seem to exclude this possibility finally.

The tests with the sera of rabbits carrying tar papillomas and Brown-Pearce tumors reported in Paper II, demonstrate the specificity of the neutralizing power manifested by the blood of animals carrying papillomas induced by the Shope virus. The latter evidently acts as antigen, increasing in amount as the papilloma enlarges in domestic rabbits, although its presence cannot be demonstrated directly.

The blood of the domestic rabbits that we studied acquired antiviral power within a few weeks after papillomatosis appeared, the amount of this power being in general roughly proportional to the skin area involved in the growth. There were some striking exceptions however. The blood of some hosts with small, transient papillomas became antiviral at an early period (Chart 2, rabbits 3-59 and 3-61), while that of one individual developing relatively large growths proved wholly devoid of demonstrable influence (Chart 1, 3-19). It is difficult to suppose that differences in the response of individual hosts to an antigen can account entirely for these phenomena. A possibility exists that the amount of antigen set free from the papilloma may vary with the local conditions. Some papillomas are fleshy, whereas others are dry almost to their base, their epithelium rapidly keratinizing and eventually desquamating. The layer of proliferating, virus-infected cells is supported by very narrow connective tissue cores having vessels that often become blocked. In consequence of these conditions the opportunities for resorption from the mass may differ largely from individual to individual. The thought suggests itself that there may be viruses whose association with cells is so close that they only very exceptionally come away into the host organism in sufficient quantity to elicit an antiviral response on its part. However this may be, the fact remains that some virus-induced papillomas of considerable size call forth little or no humoral response on the part of the host, while other, very small ones cause the blood to become notably antiviral. Puzzling phenomena of similar sort have been often observed in the serological testing of chickens with tumors due to viruses; and they may be due to like conditions.

Reinoculation with the Shope virus was successful in the case of 2 rabbits whose blood had slight or no antiviral power, but it failed in the case of 6 in which this power was more marked (Charts 1 and 2). In a recent experiment the sera of 5 domestic rabbits, in which virus-induced papillomas had retrogressed one year before, were tested for antiviral power by the inoculation of scarified areas with virus of proven activity. Such power was present in all these cases (and in that of rabbit 3-19 after 426 days, as already mentioned), but the titer was low. Nevertheless, reinoculation with active virus gave a negative result in 4 of the rabbits, while in the fifth, with very little antiviral power, it caused 2 tiny growths which soon retrogressed.

No evidence was obtained that the antiviral state of the blood had any effect upon the virus, once the latter had infected the epidermis, but on the contrary there was much to show that it was devoid of influence. Similar observations have been made in the case of a chicken sarcoma caused by a virus (Chicken Tumor I), and an ability of the proliferating cells to protect this virus has been demonstrated experimentally (6). Such protection is known to occur in the case of other viruses as well (7), and the assumption seems reasonable that it is responsible for the continued growth of the rabbit papillomas in hosts with blood of strong antiviral power.

In some of the inoculated animals the papillomas appeared slowly and soon retrogressed, whereas in other individuals with blood of much stronger neutralizing power for the virus they enlarged steadily (rabbits 3-59 and 3-61 of Chart 2 as compared with rabbits 3-58 and 3-63). Primary differences in the suitability of the cells for the virus (8), differences in soil that is to say, will go far to explain such instances. Some rabbits are primarily more susceptible to the action of the virus than are others of the same breed; and the papillomatous growth is generally most vigorous in those individuals whose skins react most pronouncedly to the stimulation of Scharlach R. Observations to be recorded in a future paper by one of us support the view that the host sometimes reacts against the cells of established growths, with result that these retrogress, a happening which is frequent in the case of transplantable tumors. Such an occurrence would explain the slow appearance and uncertain course of the papillomas that appeared at the sites of tattoo and intradermal inoculation some time after papillomatosis had become established over broad abdominal areas (Chart 2).

#### SUMMARY

A method has been devised for serological tests with a virus producing rabbit papillomas that become carcinomatous. The discrete character of the growths caused by the virus when suitably diluted fits it notably for quantitative experimentation. It shows no tendency to lie latent in domestic rabbits though it does so on occasion in cottontails, the natural hosts. Sera which partially neutralize it do not alter its character, or attenuate it, but merely cut down the number of its effective entities.

The serum of normal domestic rabbits is ordinarily devoid of neutralizing influence on the virus, but that of animals carrying the papillomas usually exhibits neutralizing power soon after these appear. The rate at which this power increases depends in general upon the amount of papillomatous tissue developing, but exceptions to the rule occur, the presence of fairly large growths being compatible with a lack of such powers in demonstrable amount. Even when the antiviral power is great it has no evident influence on the course of established papillomas, other factors determining whether these enlarge or retrogress. It acts to prevent successful reinoculation of the animal, however.

## BIBLIOGRAPHY

1. Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.
2. Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1934, **60**, 701, 723, 741.
3. Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1935, **62**, 523.
4. Shope, R. E., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 830.
5. Flexner, S., *Science*, 1935, **82**, 420.
6. Rous, Peyton, *J. Exp. Med.*, 1913, **18**, 416. Fischer, A., *Z. Krebsforsch.*, 1927, **24**, 580.
7. Rous, Peyton, McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1935, **61**, 657.
8. Kidd, J. G., Beard, J. W., and Rous, Peyton, *Proc. Soc. Exp. Biol. and Med.*, 1935, **33**, 193.

## EXPLANATION OF PLATE 5

FIG. 1. Results of rubbing mixtures of virus and sera from rabbits carrying papillomas into scarified areas of skin. On one of the squares no growths have appeared; on several there are discrete papillomas in large or small number; and on yet others the growths are semiconfluent or confluent. The confluent proliferation was due to a control mixture with Tyrode.



Photographed by Joseph B. Haulenbeek

(Kidd *et al.*: Virus causing rabbit papillomas. I)