OBSERVATIONS ON THE RELATION OF THE VIRUS CAUSING RABBIT PAPILLOMAS TO THE CANCERS DERIVING THEREFROM

I. The Influence of the Host Species and of the Pathogenic Activity and Concentration of the Virus

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Plates 24 to 26

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The carcinomas deriving from the papillomas induced with a virus in domestic rabbits (1) have this virus as their primary cause. The present paper is concerned with the carcinogenic effect of various strains of the virus,—meaning thereby the infective materials procured from the "spontaneous" papillomas of different cottontail rabbits,—and with the influence of the concentration of the inoculum, and the method whereby it is introduced. In corollary certain unusual tumors will be described which have followed upon the papillomatosis engendered by the virus in a cottontail rabbit.

Virus Concentration as Affecting Cancer Incidence

Many domestic rabbits have been inoculated into scarified skin areas of standard size with serial dilutions of virus or with serum mixtures in which it had been partially neutralized incidentally to titration tests (2). Some of these animals have been retained for months, until malignancy developed. The records made, of the time of appearance and situation of the cancers, show that the more concentrated the inoculum the sooner and oftener, in general, did cancer appear.

The method of inoculation has already been detailed. The various materials were rubbed into scarified squares about 4 cm. across, situated on the belly and lower sides of the test animals. The emerging growths ranged from solitary or scattered papillomas, punctate

in origin, to masses of confluent papillomatosis occupying the entire square. All concentrations of virus beyond a certain minimum gave rise forthwith to growths of the latter sort, these appearing at about the same time, looking alike, and behaving in the same way. Inocula containing slightly less virus than the minimum mentioned caused crowded, discrete papillomas which became confluent masses after a few days, a negligible interval when compared with the many months that often elapsed before cancer appears at even the most favorable site. During the long precancerous period, the scattered or solitary papillomas produced by still more dilute virus frequently enlarged to a diameter of several centimeters or united to form a broad mass of precisely the same appearance as if the area had been papillomatous from the beginning; and throughout this period all of the growths of any one rabbit behaved in the same way with rare exceptions, all enlarging, becoming stationary, or retrogressing together. It was to have been expected that the larger the area involved primarily in papillomatosis the more often in general would cancer occur, and needless to say this was the case. But a further fact presented itself: Cancer appeared soonest and most often, generally speaking, in those areas of confluent papillomatosis which had resulted from the most concentrated inoculum,—this although other areas had been covered with confluent growths of precisely similar aspect, for the same length of time. Some illustrative instances follow:

1. A "departiculated" (3) 25 per cent extract of virus W. R. 1240 was prepared (4 cc. of Tyrode to every gram of glycerinated cottontail papilloma), a portion was diluted to 10 per cent, and part of this was passed through a Berkefeld filter V, with further dilution of the filtered and unfiltered materials in multiples of 10. All the fluids were then inoculated into squares on domestic rabbit K 9-5 (Table I). The symbols employed to record the character of the growths that arose as result of the inoculations were: +−, one or two discrete papillomas; +, a small number of discrete papillomas; ++, many discrete papillomas; +++, semiconfluent papillomatosis; ++++, confluent papillomatosis. Tracings were made of the growths at intervals of a few days or weeks, but the system of plus marks employed in the tables gives a better index of how they were constituted. They often increased in size greatly, but their number did not do so after the first few weeks. Most of the records made during the long precancerous period have been omitted from the tables.

The cancers that developed from the papillomas were ulcerated, fungoid or depressed, invasive and destructive tumors, arising in the papilloma. All of them

progressed, most metastasized, and they regularly led to death of the animal. The histological findings confirmed the clinical diagnosis.

Table I shows that where the three most concentrated materials had been inoculated the growths were primarily confluent, that by the 47th day those from several of the other inocula had become semiconfluent, and that at the 96th day five confluent masses of approximately the same size were present, with two others, semiconfluent and somewhat smaller. Thereafter all retained the same general aspect and relative proportions until the 7th month when ulcerating, invasive cancers appeared in the two masses due to the most concentrated inocula.

2. Rabbit 3-74 (Table II) was inoculated with mixtures of departiculated virus W. R. 632 and the partially neutralizing sera of papillomatous rabbits. A control mixture with Tyrode solution was also employed in duplicate. The latter gave rise promptly to diffuse papillomatosis, whereas the most pathogenic of the serum mixtures produced growths that remained semiconfluent during many weeks. After some months, however, this difference disappeared, and when all the masses were measured on the 186th day, six of them had practically the same size and an identical appearance. By the 247th day ulcerative, spreading cancers had appeared in two of them, namely those deriving from the inocula which had given rise forthwith to confluent growths. These were the control mixtures with Tyrode. By the 275th day another cancer had established itself, as result of the inoculum in which the virus had undergone least neutralization.

3. D. R. 4-91 (Table III). Mixtures were made of virus suspension with serum specimens taken from three normal jack rabbits (*Lepus californicus*, Gray) and an animal (rabbit IV) of this species which had a "spontaneous" papilloma (4). This last specimen neutralized the virus almost completely, while those from the other individuals proved somewhat less favorable to it than the Tyrode of a control mixture. By the 41st day, however, the growths from four of the inocula were confluent, and from that time on their aspect was identical. Cancer did not put in an appearance until the 300th day, and then only where the virus-Tyrode mix ture had been introduced.

4. D. R. 3-16 (Table IV) was inoculated with graded dilutions of virus 632. The growths produced by the 1 per cent and 10 per cent virus were luxuriant from the first, and indistinguishable in their character, but those from the lower dilutions appeared later and never reached the same size. By the 428th day cancer had appeared in the mass due to 1 per cent virus, and by the 447th day in that due to the 10 per cent preparation.

Additional instances of the phenomenon here illustrated could be provided were this necessary. In two of the tabulated experiments the virus was not diluted with Tyrode but instead was partially neutralized with immune serum. This has the same effect as dilution: it acts to cut down the number of effective virus entities, not to alter their pathogenic capabilities (2). The tables show that the

Cancer Incidence as Determined by Virus Concentration

TABLE I. Test rabbit D. R. K 9-5, inoculated with graded dilutions in Tyrode of virus W. R. 1240.

Virus strength	23 days	47 days	96 days		201 days
				cm.	
25%	++++	++++	++++	5 x 4	++++C
10%	++++−	++++	++++	5 x 4	++++C
" filtered	++++	++++	++++	5 x 4	++++−
1%	++	+++−	++++	6 x 4	++++
" "	++−	+++−	++++	4 x 4	++++
0.1%	+	++−	+++	3 x 2	++++
" "	+	+++−	+++	4 x 2	++++−
0.01%	0	+−	+	2 x 1	+

TABLE II. Test rabbit D. R. 3-74, inoculated with virus W. R. 6-32 mixed with Tyrode and with the sera of papillomatous domestic rabbits.

Virus	Inocula		Rabbit No.	24 days	41 days	186 days		247 days		275 days
	Tyrode	Serum								
	cc.	*cc.*					*cm.*		*cm.*	
	0.5	—	—	++++	++++	++++	4 x 3	++++C	4 x 3	++++C
	"	—	—	++++	++++	++++	4 x 3	++++C	3 x 3	++++C
	0.3	0.2	V	+++	+++	++++	4 x 2.5	++++	4 x 2.5	++++C
	—	0.5	"	+++−	+++	++++	5 x 2.5	++++	4 x 2	++++
6⅔%, 0.5 cc.	0.3	0.2	VI	++−	+++−	++++	4 x 2	+++	4 x 2	++++
	—	0.5	"	+−	+	+	4 x 1.5	+	6 x 2	+
	0.3	0.2	VII	++	+++	++++	3 x 3	++++	3 x 3	++++
	—	0.5	"	0	+−	+−	1.5 x 1	+−	1.8 x 1.8	+−
	0.3	0.2	VIII	0	+−	+−	2 x 1.5	+−	1.5 x 1.5	+−
⅔%, 0.5 cc.	0.5	—	—	++−	++	++++	3.5 x 2	+++	3.5 x 1	+++

TABLE III. Test rabbit D. R. 4-91, inoculated with virus mixed with Tyrode and with the sera of normal and papillomatous jack rabbits.

Virus	Inocula		Rabbit No.	14 days	19 days	26 days	41 days	300 days	
	Tyrode	Serum							
	cc.	*cc.*							*cm.*
6%, 0.5 cc.	0.5	—	—	++−	++++−	++++	++++	++++−C	4 x 4
	0.3	0.2	I	+	+++	++++	++++	++++	6 x 2.5
	"	"	II	+	++	++++−	++++−	++++−	4 x 2
	"	"	III	+	++−	+++	++++−	++++	5 x 2.5
	"	"	IV	0	0	0	+−	+−	2 x 2
	—	0.5	I	+−	+	+++	++++−	+++	3.5 x 3.5
	—	"	II	+	++	++++−	++++	++++−	4 x 2
	—	"	III	+	+++	++++	++++	++++−	5 x 2.5
1%, 0.5 cc.	0.5	—	—	+−	+−	+−	+−	+++	3.5 x 2.5
	—	0.5	I	0	0	+	++−	0	
	—	—	II	0	0	0	+−	0	
	—	—	III	0	+−	+	+	+	4 x 2

TABLE IV. Test rabbit D. R. 3-16, inoculated with graded dilutions of virus W. R. 6-32.

Virus strength	16 days	24 days	45 days	241 days		428 days		447 days
					cm.		*cm.*	
per cent								
10	+++	++++	++++	++++	5 x 2	++++	5 x 2	++++C
1	++++	++++	++++	++++	5 x 2	++++C	5 x 2	++++C
0.1	0	+	+++	+++	4 x 2	+++	4 x 2	+++
0.01	0	0	+−	+−	1.5 x 1.5	+−	2.5 x 1	+−
0.001	0	0	+−	+−	2.5 x 2	+−	2 x 2	+−
0.0001	0	0	0	0		0		0

0 = negative. + − = 1 or 2 papillomas. + = 3 to 8 papillomas. ++ = many scattered discrete papillomas. +++ = semiconfluent areas of papillomatous proliferation. ++++ = confluent papillomatous proliferation covering inoculation site.

C = cancer present.

concentration of virus employed to produce confluent papillomatosis notably influenced the incidence of the cancers. The reasons for this become plain when one considers the composition of the virus-induced growths.

Composition of the Papilloma as Determined by Virus Concentration

The papillomas result from the multiplication of those cells which become infected with the virus at the time of inoculation, no discernible involvement of neighboring elements taking place. It follows that the growths are inevitably multicentric in origin and composite in character, save in those presumptive instances in which the initial infection has involved but one cell. Often the appearance of the solid growths resulting from broadcast inoculation of scarified areas gives no hint of their composite nature. But in not infrequent cases portions of the mass are gray with pigment while others are pink (Fig. 1). The virus infection frequently discloses the existence of local differences in the skin that are imperceptible while it is normal, the young, multicentric growth being spotted or stippled in various shades of gray (Fig. 2). If the centers of proliferation are numerous and crowded, the growth may assume a pepper and salt aspect, or be evenly gray, a color which darkens as the projecting mass keratinizes and dries. The diversity of hue is due to the inclusion of stimulated melanoblasts in certain of the proliferating cell aggregates, as not in others.

Successive tracings of confluent papillomas, notably of those assuming this form secondarily, have disclosed the fact that many of the clefts separating them into peaks, or larger subdivisions, are the boundary lines between all hosts of differing lineage, deriving from different cell-virus associations, that is to say. These clefts may persist for many months. Often after a time cell families of greater vigor than their neighbors proliferate at the expense of the latter, pressing these to one side, perhaps extending more deeply into the connective tissue (Fig. 3) and forming usually discrete, squat domes or onion-shaped growths (Fig. 4). These are often nonpigmented or only slightly so, in contrast to the rest of the mass, and they are notably liable to cancerous change, as we have often observed, a matter which will be discussed further on. To what their proliferative vigor is due,

whether to the influence of exceptionally active or numerous virus entities, or to a special susceptibility of the cells primarily infected, or to some unusual suitability of virus entity and individual cell, cannot at this writing be said. Whatever the reason, it is plain that some cell-virus associations are especially favorable to proliferation. The more concentrated the virus introduced into a given expanse of scarified skin the greater should be the number of such associations; and the greater in consequence the likelihood of cancer.

In another way the virus concentration may influence cancer incidence, namely by bringing about a crowded condition within the proliferating mass. Where competing cell families are most numerous crowding will be most pronounced, the epithelium most disordered, the supporting connective tissue most disturbed, and bacterial infection most frequent—conditions all that further malignant change (1).

An important inference for experimentation follows from the observations as given: If one wishes to obtain cancers as soon as possible after inoculation one should introduce concentrated virus into considerable skin areas. Malignancy will ensue only after months of proliferation at the earliest and generally where the papilloma is oldest. Growths that have arisen by enlargement from one small inoculated spot contain but a small proportion of old papillomatous tissue as compared with others of the same size that have been confluent from the beginning. It is no accident that cancers appearing in growths of the first sort are usually situated near their center; for here the papilloma is oldest, and favoring local disturbances have been present longest.

The Carcinogenic Activity of Virus Materials of Differing Derivation

The more vigorously the papillomas grow the sooner and oftener does cancer occur. Recognizing this we have ordinarily discarded those virus materials which were prone to produce retrogressing or indolent papillomas. In comparing the carinogenic potentialities of the more pathogenic strains, not only must the method of inoculation and the concentration of virus be taken into account, but many other influences. The papillomas of Dutch belted rabbits grow especially well, and hence they are more favorable to malignancy than agouti rabbits; the general character of the skin of animals of the same breed

has some influence; and so too has the local skin character, cancer developing much more frequently in black haired regions than in white ones of the same individual. Certain interferences with the papilloma precipitate the malignant change (1), and to these we have often resorted. Because of these complicating factors and the variety of our experiments, it is difficult to analyze them as a whole. This much is clear from them however, that every virus material which gave rise to papillomas that grew progressively in several animals for many months proved carcinogenic in one or more of them, irrespective of the animal breed and of whether the inoculation had been into scarified areas or by tattooing into a number of small spots. Chart 1 records the incidence and time of appearance of cancer after inoculation of the four virus materials most extensively used. They were all inoculated as 3 per cent or 10 per cent extracts of glycerinated papilloma tissue. To narrow the conditions of comparison, only those animals are entered in the chart which were of agouti or Dutch breed, inoculated in one of the two ways just mentioned, and as result carrying papillomas which had grown progressively during the first 3 months, without later tendency to retrogress. This entailed the omission of a large proportion of the data.

Cancer never asserted itself clinically before the 4th month after inoculation, at earliest after 132 days, in an animal not charted because it had been inoculated with a strain of virus (W. R. 18) employed in a very few rabbits. Of the viruses figuring in the chart, W. R. 538+638 had the shortest incubation period, giving rise to papillomas within 7 to 14 days, and W. R. 632 the longest period, 11 to 25 days, W. R. 738 and W. R. 1240 occupying intermediate places (papillomas after 10 to 19 days). In most instances the growths produced with W. R. 538+638 were progressive, whereas many caused by W. R. 632 disappeared after a few weeks and a large proportion of the others retrogressed subsequently. This material was extensively used because of its suitability for experiments on the antiviral power of the blood. Retrogression was considerably less frequent with strains W. R. 738 and 1240.

When Chart 1 is scrutinized it will be seen that the more active the virus material, as evidenced by incubation period and subsequent course of the papillomas, the earlier did cancer appear. The fre-

quency of malignancy varied directly with the vigor of the growths, in accordance with our previous experience, but on this point the chart provides no information. Several materials have proved carcinogenic besides those tabulated. Virus W. R. 18 which gave rise to the earliest cancer thus far noted was as actively papillogenic as virus 538+638, if not more so. Virus 738 still caused cancer after passage through a snowshoe rabbit (*Lepus americanus*, Erxleben).

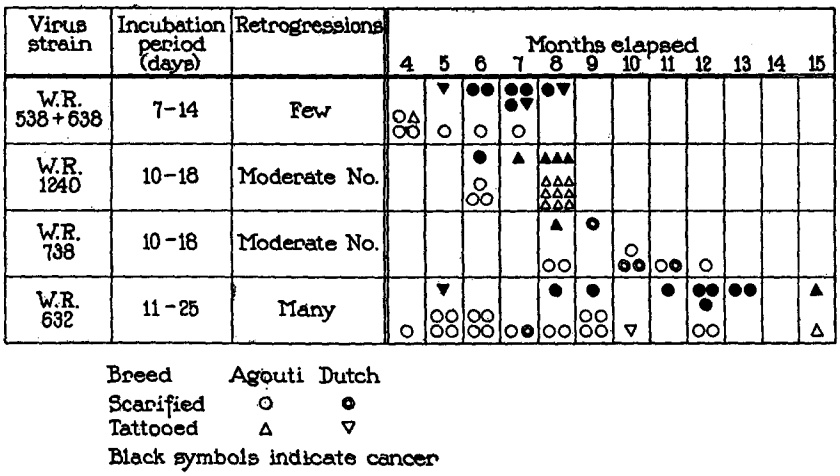

CHART 1. Virus strain and cancer incidence. Each symbol stands for the growth in one rabbit, and only those rabbits have been considered in which the papillomas grew well. A black symbol means the occurrence of cancer in the month designated, and a white symbol that no malignancy had appeared in the existing papillomatous tissue to the time of record, which was either that of last examination or of death.

The Incidence of Cancers in Cottontail Rabbits

From one to three rabbits of every shipment of 24 coming from our source of supply in southern Kansas has had somewhere on its skin a virus-induced papilloma or several of them; and this although the dealer has endeavored to retain all animals carrying such growths. He traps many thousand rabbits yearly, and it is to his interest to send us those having ulcerated lumps in association with papillomas, but of the several thus far received none has proved cancerous, nor has

Shope encountered any in the several hundred cottontails he has utilized. Malignancy is evidently a rare event, but it does occur, for Syverton and Berry have described an instance (5), and we have now noted one. Theirs was a metastasizing squamous cell carcinoma first noted developing from a "spontaneous" growth 85 days after the cottontail came under observation.

In our laboratory five cottontails with notably vigorous growths have been maintained for periods of 14 to 23 months and four more for from 9 to 11 months. The growths were due in most instances to carcinogenic virus strains. Of the three animals surviving 23 months, one ultimately developed both a carcinoma and a metastasizing sarcoma. It had received especially active virus.

W. R. 26 was inoculated by the tattooing method with 5 per cent virus 538+638 at two spots 2 mm. across near the tip of each ear. Scharlach R in olive oil was injected beneath one of each pair of the discrete growths that soon appeared, and this was done twice again at intervals of a few weeks. The growths thus stimulated became somewhat the larger, and remained so later. By the 8th month all were enormous, radish-shaped cutaneous horns, tough and black, with turgid, darkly pigmented, fleshy bases (Fig. 5), and such they continued to be throughout almost another year. Then the horny masses began to flake away (Figs. 6 and 7), the pigmentation of their discrete, slaty bases was gradually replaced by a purply pink hue, and discrete protuberances of rounded or football shape, ranging up to 1.4 cm. in diameter, formed at several places on the tense margins of the growths on one of the ears (Fig. 6). Biopsy of such a protuberance yielded the picture of an orderly, cellular fibroma, with almost no mitoses; and during the next 2 months they all dried into brown, nut-like masses as if their circulation had been cut off. Meantime the cutaneous horns became irregularly striated, they dried down to the ear level and largely flaked away, and at some points a narrow, raised, very firm ring of new tissue appeared about them, separated from them by a narrow cleft. The ring was largest about the biggest horn, which was now buff-colored and apparently calcified in its basal portion, with a slight thickening and scabbing directly beneath it on the under side of the ear. Biopsy showed the ring to consist of keloid-like tissue with cancellous bone in its midst. During the ensuing and final month, a discrete nodule, apparently of the same keloid tissue, about 3 mm. across and 2 mm. high, developed some 6 mm. away from the largest horn on the other ear, toward the base. The animal died after 683 days in all. At this time nearly all of the horny material had recently come away, leaving flat ulcers. The microscope showed beneath these on both ears, slightly thickening them, an unencapsulated spindle cell sarcoma, which in some regions contained neoplastic giant cells. It had penetrated to the inner side of one ear, occupying the scabbed region already mentioned as opposite the buffy,

dead papilloma, but it had caused only a slight protrusion there. The discrete, small nodule near the horn on the other ear consisted of sarcomatous tissue. There were numerous small metastases replacing but not greatly enlarging the lymph glands at the base of both ears, a larger metastasis 6 mm. across in a salivary gland adjacent to one of them (Fig. 11) and numerous secondary growths up to 4 mm. across in the lungs. At all these situations the growth was a simple, spindle cell sarcoma. Remarkably little living papilloma persisted where it had grown vigorously for so long; but at one of its original sites where a proliferating small remnant of it was still present an indubitable squamous cell carcinoma (Fig. 10) had almost entirely replaced it. The cancer was only about 1 cm. across, very shallow,—nowhere more than 2 mm. thick,—and it was situated well away from the sarcoma. Its cells were not joined to those of the papilloma, but had engulfed some of the melanotic pigment of the latter where they were destroying it, and at one point they had penetrated into the new-formed cancellous bone.

In this instance repeated biopsies had failed to disclose the existence of either the sarcoma or the carcinoma, and these growths gave so little indication of their character during life that they were lost for experimental purposes. Furthermore they had caused such local destruction that it was no longer possible to tell where they had arisen. This was notably true of the sarcoma: one cannot even be certain whether its presence in both ears was consequent on metastasis from a primary situation in one of them. During the long precancerous period the papillomas obviously acted as local irritants, notably where calcification had taken place, and both keloid and bone formation resulted. That the sarcoma was another result of the irritation seems likely; and it is conceivable that the carcinoma may have originated in this way as well, from epidermal cells adjacent to the papilloma but not themselves infected with the virus. The histological findings did not suggest carcinoma sarcomatodes.

Two snowshoe rabbits with papillomas consequent on virus inoculation into scarified areas have been under observation for more than a year, with no malignant change as yet. Judging from the behavior of the growths, the animals are only moderately favorable to the virus.

DISCUSSION

The differences found in the carcinogenic activity of the virus materials can all be referred to differences in their ability to produce and maintain actively proliferating papillomas of the usual sort. It

seems probable that any strain of the Shope virus capable of giving rise to vigorous growths in domestic rabbits will prove carcinogenic through their agency.

The similarity of the growths produced by the various inocula was remarkable. There was no morphological indication of any qualitative difference in their pathogenicity, much less that any of them contained virus entities which were distinctively carcinogenic. Yet the wide range in their carcinogenic effect depended on more than a mere numerical difference in their content of papilloma-producing entities. Some of the virus materials provided entities which gave rise individually to vigorous growths, and others only such as caused indolent, retrogressing ones,—a fact very evident in the behavior of the papillomas of punctate origin resulting from the inoculation of high dilutions of virus into broad areas of skin. All of the cottontails supplying the materials came from a region of the Southwest in which the papillomatosis is endemic (Kansas, Iowa, Oklahoma, Texas). The size of this region, the existence of the disease there for a very long period (it has been known for more than 50 years), and its chronicity in the individual rabbit would lead one to look for virus variants. But certainly none of major calibre has been encountered thus far in the study of the "spontaneous" growths or those purposely induced with the virus.

The composite character of the virus-induced papillomas has already been discussed to some extent in its bearing upon carcinogenesis. The fact that most of the growths consist essentially of aggregates of competing cells, deriving from a greater or less number of primary cell-virus relationships, must be kept in mind during any consideration of how the cancers come about. It provides yet another reason, besides those already recorded, for the local origin of the malignancy. As already remarked, some of the cell-virus relationships formed upon broadcast inoculation of a skin area are more favorable than others to an eventual cancerous change. Why this should be the case is not yet clear.[1]

[1] In this connection the fact deserves mention that a variety of tumors, including cancers, originate from the epidermal cells of tarred skin after the virus is brought into association with them by intravenous injection (Rous, Peyton, and Kidd, J. G., *Science*, 1936, **83**, 468).

The dominant cell aggregates from which cancer arises tend to be nonpigmented or to become so before it occurs, a fact difficult to reconcile with the observation that the incidence of cancer is greatest in the papillomas produced on pigmented skin and themselves pigmented. Yet the same holds true of the tar cancers, these occurring oftenest on pigmented skin, though themselves nonpigmented or soon becoming devoid of pigment (6).

The infrequency with which cancer develops from the papillomas of cottontail rabbits cannot be laid to any peculiarities of their fabric which tend to prevent malignancy in general, for carcinoma can be readily induced by tarring their skin. In three of nine cottontails with ears tarred three times a week for 11 to 15 months,[2] squamous cell cancers have occurred on both ears at nearly the same time in each case, metastasizing to the lungs in one of them, to a lymph gland at the base of one ear in another, and in all proving so destructive locally as to force amputation of the organs for conservation of the material. None of a more considerable group of domestic rabbits tarred in the same way for a much longer period with result in large papillomas has developed an indubitable, progressive cancer.

An obvious reason for the failure of virus-induced papillomas to become cancerous in cottontails is to be found in the lack of vigor of the growths, a feature generally attended by a negative outcome in domestic rabbits. Cottontails are notably resistant to the virus despite the fact that it maintains an endemic disease in them. From 1 to 3 out of every 10 cottontails experimentally inoculated with active virus prove insusceptible, and in more than half of the others retrogression of the induced growths sooner or later occurs, though they are often vigorous to begin with. In the few remaining animals papillomatosis persists until death, that is to say for periods up to nearly 2 years, in our experience. After the growths have become established they remain stationary in size for many months as a rule, though still proliferating. We have yet to observe papillomas in a cottontail that grew so large as to prove fatal, a frequent happening in domestic rabbits. The virus and the wild rabbit are evidently habituated to each other, as is often the case when the association between a parasite

[2] Horizontal retort tar of the Oster-Gasfabrik of Amsterdam, kindly provided by Dr. Karl Landsteiner.

and its host species has endured for a long time According to Theobald Smith symbiosis is the eventual result of such a state of affairs (7). The ability of the Shope virus to lie latent over long periods in cottontails (8), as not in domestic rabbits, seems worth recalling in this connection.

SUMMARY

All the strains of the Shope virus thus far tested which give rise to vigorous, progressively enlarging papillomas in domestic rabbits, function as carcinogenic agents by way of these growths. The more pathogenic the virus as evidenced by the brevity of its incubation period and the vigor of the papillomas produced, the sooner and oftener does cancer occur. The number of virus entities contained in the inoculum notably influences the outcome, cancer appearing most frequently in those confluent, papillomatous masses which have resulted from the greatest concentration of the virus material under test. The papillomas experimentally induced by the ordinary inoculation methods are essentially aggregates of proliferating cell families, each the outcome of some primary cell-virus association. Some of these associations are followed more frequently by cancer than others are in the same animal.

Cottontail rabbits, the natural hosts of the virus, are notably resistant to its sustained activity, as compared with domestic rabbits. Though often growing rapidly at first, the papillomas of cottontails soon become relatively inert in most cases, and they usually retrogress, and rarely undergo malignant change. In an instance here reported both a squamous cell carcinoma and a metastasizing sarcoma appeared at the base of some papillomas due to experimental inoculation, which had existed on the ears of a cottontail for nearly 2 years.

The meaning of the phenomena is discussed.

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EXPLANATION OF PLATES

PLATE 24

FIG. 1. Particolored papillomatous expanse resulting from a broadcast inoculation of Shope virus into the scarified skin of the abdomen of a brown-gray domestic rabbit (21st day). Considerable areas of the growth are gray or black, while others are nonpigmented. The normal skin had given no indication of the differences now apparent. ×½.

FIG. 2. Pigmented and nonpigmented, proliferating cell aggregates consequent on the inoculation of various dilutions of virus into the skin of a domestic rabbit (27th day). Where the aggregates are numerous and crowded, as in the confluent mass on the right of the photograph,—which resulted from the least dilute material,—they are difficult to discern individually. ×¾.

FIG. 3. Side view of a papillomatous expanse produced by the inoculation of a wild rabbit (90th day). The growth has been raised with the aid of the finger and a slide in order to show better a discrete, nonpigmented nodule which has proliferated with especial vigor and extended below the general level. The rest of the mass is sooty, but reflections from its surface, notably in the region beneath the edge of the slide, tend to confuse the picture. ×¾.

FIG. 4. Papillomatous masses consequent on tattooing the virus material into two small spots on the side of the same rabbit (90th day). One mass consists of numerous dark gray peaks, all of about the same height, and the other contains some similar ones of various sizes, but all these latter have been pushed aside by a rapidly enlarging, almost nonpigmented, dome-shaped growth. ×¾.

FIG. 5. Cutaneous horns (8th month) due to tattooing the Shope virus into two spots on each ear of cottontail W. R. 45. The larger growth on each ear had been injected with Scharlach R some weeks previously. ×½.

PLATE 25

FIGS. 6 and 7. Same growths as in Fig. 5. The dry cutaneous horns have mostly come away (20th month). Fig. 6 shows the rounded fleshy protuberances that had formed at the border of the remaining low papillomas, and Fig. 7 the calcification of one of the horns on the other ear (arrow).

FIG. 8. Growth of Fig. 6 (22nd month). The protuberances have dried down, puckering and folding the ear. Only the tip can be seen of one of them (A), which is separated from the other by a furry strip (seen vertically in Fig. 6). A ring of firm tissue has formed around the base of the nearer growth, and a few milli-

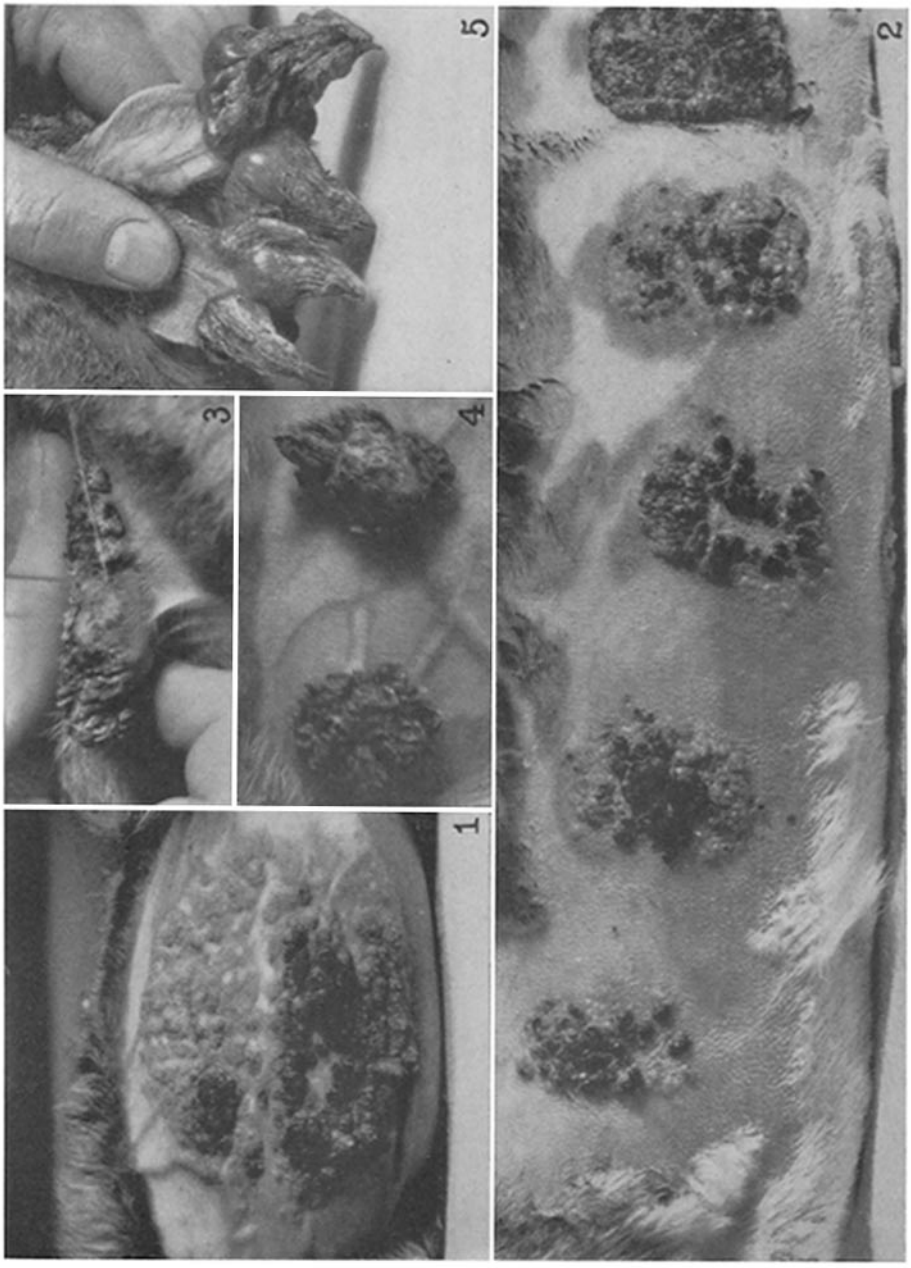
meters away is an intracutaneous nodule (B). Both were found to be sarcomatous at autopsy.

FIG. 9. Remains of the calcified horn of Fig. 7 with a thickening next its base (arrow), which proved to be sarcomatous.

PLATE 26

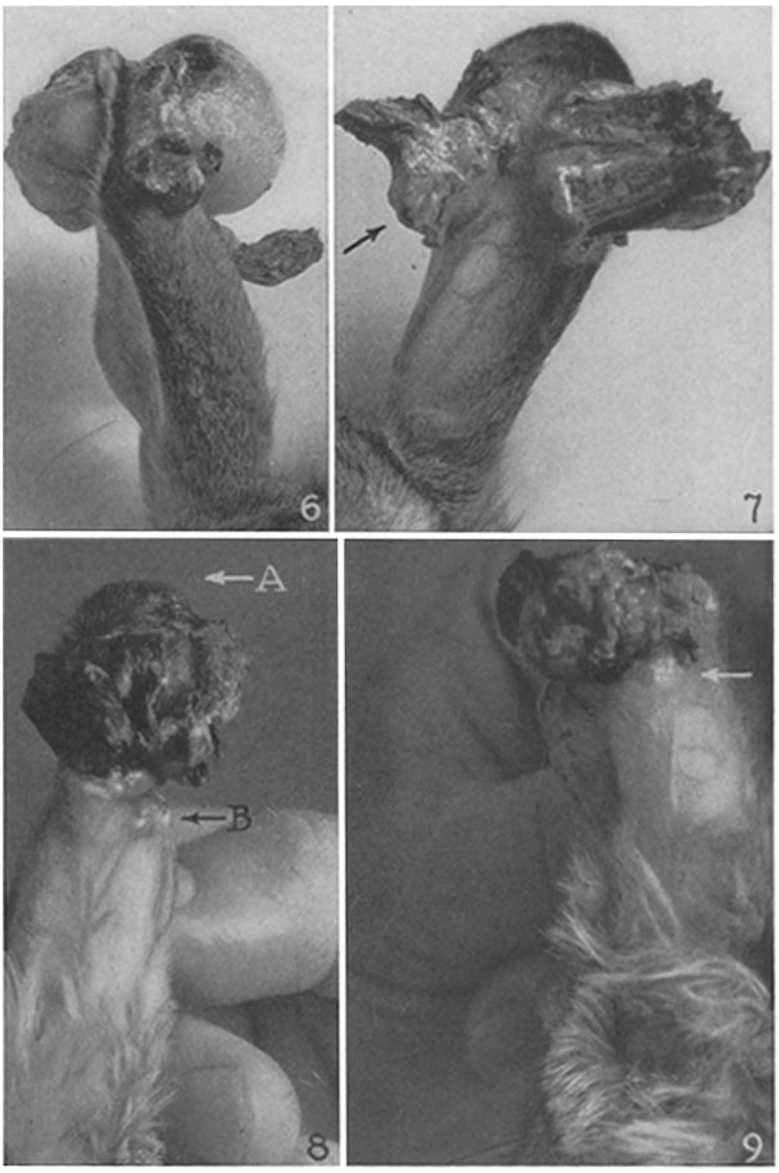
FIG. 10. Section of the thickening of Fig. 9. Some new-formed bone can be seen at the center of the picture, with the intact epidermis above. A squamous cell carcinoma is invading the deep tissue. ×90.

FIG. 11. A metastasis from the sarcoma of Figs. 9 and 10. It has replaced the lymph node at the base of one of the ears and invaded the adjacent salivary gland. ×90.



Photographed by Joseph B. Haulenbeek

(Rous *et al.*: Cancers derived from papilloma virus. I)



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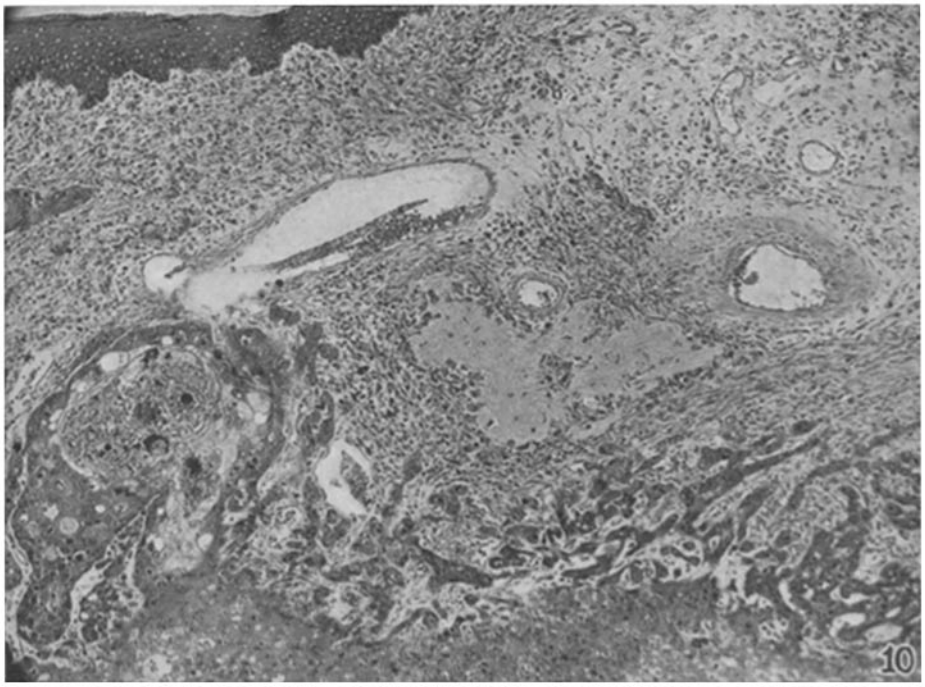

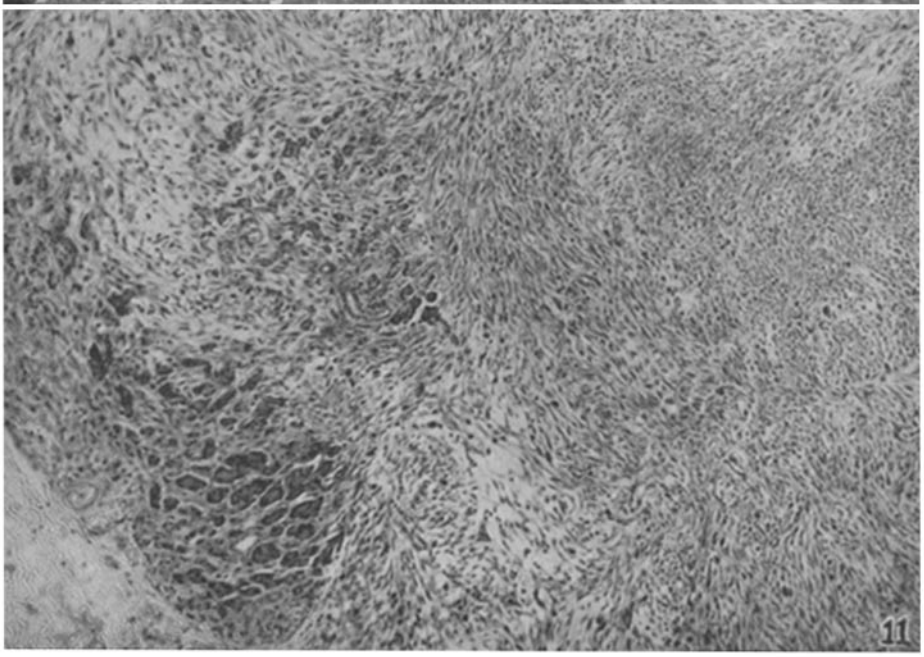

Photographed by Louis Schmidt

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