THE ACTIVATING, TRANSFORMING, AND CARCINOGENIC EFFECTS OF THE RABBIT PAPILLOMA VIRUS (SHOPE) UPON IMPLANTED TAR TUMORS

By PEYTON ROUS, M.D., and JOHN G. KIDD, M.D.

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

PLATES 36 TO 41

(Received for publication, March 29, 1940)

The virus causing cutaneous papillomas in cottontail rabbits (1) produces remarkable effects when injected into the blood stream of domestic rabbits carrying benign tar tumors (2). It localizes in many of these growths, converts not a few into virus papillomas, makes others proliferate with far greater rapidity than before, alters some with result in tumors of unusual sort, and causes a considerable proportion to take on the character of progressive cancers when otherwise they would remain benign and ultimately disappear. The work now to be recorded has been carried out in the further study of these phenomena. To determine directly the effects of the virus on individual tar tumors, pieces of many of them of various sorts were exposed to it *in vitro* and implanted in the muscles and subcutaneous tissue of the hosts.

Numerous viruses are known to be capable of flourishing within tumors, but none except the one causing rabbit papillomas has been observed to have any important influence upon the growths. Years ago Levaditi and Nicolau demonstrated that extraneous viruses may exist in permanent association with transplanted neoplasms into which they have been experimentally introduced (3). Levaditi and his associates have since shown that herpes, vaccinia (4), rabies (5), and fowl pest virus (6), and the virus of lymphogranulomatosis (7) all have this ability. Virus lymphogranulomatosis gives rise to the corpuscles typical of its action, and herpes, rabies, and vaccinia virus produce their characteristic inclusion bodies. In an early paper Levaditi and Schoen stated that vaccinia infection may at first cause tumors to grow faster, but there is no mention of this phenomenon in later communications, and other workers seem not to have observed it. The virus of fowl pest destroys the cells of transplantable mouse tumors, but has no effect on those of the Brown-Pearce rabbit tumor, though persisting in the growth. Findlay and MacCallum (8) have demonstrated that several strains of yellow fever virus may be carried along in mouse carcinomas, their sole action being to increase necrosis in certain instances. Pownall and Florey (9) reported that the dermotropic variety of the virus of infectious ectromelia retards or destroys tar tumors in mice; but it is uncertain whether this is consequent on virus infection of the cells of the growths or due to the serious damage done to the skin round about them. Rivers and Pearce

(10) have contributed the significant observation that transplanted tumors may accidentally become infected with an extraneous virus. They encountered virus III as a contaminant of Brown-Pearce tumors. It gave no morphological sign of its presence other than a few inclusion bodies. Mellanby (11) found that the tar and benzpyrene tumors of fowls may become infected with the virus causing a chicken sarcoma (chicken tumor I), when a growth of this sort is produced in them, the infection taking place during the period when the virus of the enlarging sarcoma circulates in the blood. It brings about no discernible change in the tar and benzpyrene tumors, is present in only small amounts, and can no longer be procured from the growths after they have been transplanted once or twice. Andrewes and Ahlström (12), studying a rabbit sarcoma which arose secondarily in a connective tissue mass resulting from localization of the Shope fibroma virus in voluntary muscle injected with tar, made numerous tests to learn whether the virus accompanied the sarcoma on serial transplantation. It was not demonstrable either directly or on immunological test. Another sarcoma that appeared under the same circumstances failed to grow on transplantation (13).

Morphological evidence of the formative influence of the rabbit papilloma virus can be discerned in many of the cancers springing from the growths it produces (14); and the persistence and increase of the virus have been demonstrated in two such cancers which were successfully transplanted, one of them to ten successive groups of animals (15). The virus fails to establish itself, however, in Brown-Pearce rabbit carcinomas into which it has been experimentally introduced (16).[1] The benign papillomas it directly causes are themselves susceptible to infection with other viruses. Levaditi and Schoen proved that vaccinia virus will localize out of the blood stream and persist in the papillomas (17); and Syverton and Berry have demonstrated that the papilloma cells and those of the derivative cancers will support two viruses at once in addition to that which is the cause for their pathological state (18). The latter investigators utilized both cottontails and domestic rabbits, when studying the multiple virus infections, and inoculated the growths with herpes and vaccinia, B virus, virus III, and *Virus myxomatosum*, employing as telltales the characteristic cytoplasmic and intranuclear inclusion bodies which these agents produce, and utilizing serological tests as well.

Materials and Methods

For the purposes of the present work tumors elicited with tar were partly or wholly excised and cut fine. Two equal portions of the fragments were placed in Tyrode solution containing virus and ordinary Tyrode respectively at pH 7.2, and after a brief interval they were implanted at corresponding situations in the subcutaneous connective tissue and leg muscles of the animals from which they had come.

The Procedure with Domestic Rabbits.—The domestic rabbits providing tumors were all of brown-gray (agouti) breed, and had been tarred twice a week on the insides of

[1] The Brown-Pearce tumors developing from implanted tissue which had been exposed *in vitro* to the virus grew much less rapidly than did those from material merely exposed to Tyrode solution. In later unpublished experiments of the sort no such antithetic effect of the virus has been noted.

the ears during some weeks or months, with intermissions in the case of a few of those tarred longest.[2] In many instances tarring was kept up for from 1 to 3 weeks after the implantations because of the possibility that the general changes it brought about might favor growth at the new sites. No indication of such influence was discernible in the behavior of the implants.

Early experiments showed that if the tumors were utilized immediately after they had been stripped of tar, abscesses were likely to develop at the implantation sites because of bacteria in the moist, and often macerating, growths. If the tar was removed a few days before the experiment, however, the tumors dried down and could be used with little risk of purulence. Hence in the later work this was regularly done. The growths were punched from the ear with sterilized cork borers, a single blow of the mallet sufficing; and whenever they were large enough a piece was left *in situ*. This was a great desideratum as disclosing the capabilities of the tumors at situations where they were already established. Bacterial infection of the wounds almost never followed, and the holes in the ears healed in rapidly. A median slice of each disc punched out was put into Zenker's fluid for section, and the remaining tumor tissue was trimmed free from normal elements, so far as possible, and its basal portion was sliced off and cut fine in a dish containing a little Tyrode to which sometimes a few drops of the animal's own serum had been added with a view to cell protection. This precaution seemed to make no difference in the results, nor was any to have been expected since previous implantation experiments with virus papillomas and the derivative carcinomas had abundantly shown that Tyrode brought to pH 7.2 with carbonic acid gas is not injurious to tumor cells exposed to it for brief periods.

The hashed material was separated into two lots of equal size, which were taken up separately on the blade of a knife and transferred to two graduated, 15 cc. centrifuge tubes, one containing 1 cc. of Tyrode solution, the other 1 cc. of a virus-containing Berkefeld filtrate of a Tyrode extract of a cottontail papilloma. Sometimes serum had been added to the fluids for the reason just given. The tubes were kept at room temperature for a period ranging from 15 to 25 minutes, and whirled between the hands at intervals to resuspend the fragments. Then Tyrode was added to the 15 cc. mark, they were suspended once more, and sedimented at once by brief centrifugation. The supernatant fluid was pipetted away as completely as possible, and enough new Tyrode was put on so that 8 to 15 fragments, suspended in 1 cc. of fluid, could be injected at one or two places in the host. The spots utilized were the upper extensor muscles of all four legs and the subcutaneous tissue of the axillae and groins. Sometimes the materials were put at two situations in each groin. The skin was slit prior to introduction of the injecting needle, to exclude the possibility that normal epithelium might be carried in on its point, but no precaution was taken to avoid virus infection of the epidermal wound when the needle was withdrawn, and papillomas frequently appeared in the newly healed scar, showing that a superabundance of virus had been present in the injected suspension. There was never any evidence, though, that it reached distant points in the body. The injections were done forcibly to separate the fragments, an aim accomplished as the eventual sections showed; and afterwards some of the virus was rubbed into a freshly scarified patch of skin on the rabbit's side, to determine its pathogenicity. Confluent papillomatosis regularly resulted. The implantations took but a

[2] Horizontal retort tar was used, from the Ostergasfabrik of Amsterdam. It was the gift of Dr. Karl Landsteiner.

few minutes and the control specimens,—which had been held *in vitro* for the same time as the experimental,—were injected first.

Usually there were many benign tar tumors on the ears of the rabbits, and tissue from the four or five largest was submitted to the action of the virus, as was also a piece of the hyperkeratotic epidermis, punched from a spot where no growths were visible, trimmed of cartilage and connective tissue, and hashed in the usual way. Ordinarily the animals were killed within 3 to 6 weeks since our aim was to learn the early changes produced by the virus, as distinct from such alterations as might take place secondarily in the enlarging growths.

The Procedure with Cottontails.—The ears of the cottontails had generally been tarred longer, for several months at least, sometimes for a year or more, and often on both surfaces. Only cancerous growths were utilized. A piece was punched out of one such tumor of each animal, and cut up, treated in the way already described, and implanted at four to six corresponding situations in the leg muscles and subcutaneous tissue. The tumors had usually ulcerated, and abscesses often complicated the outcome.

Results with the Tar Tumors of Domestic Rabbits

The benign tumors evoked in domestic rabbits by tar are of three well defined types (19). We have utilized examples of all three,—the rare frill horns and the more rapidly growing tar papillomas and carcinoids. The tar carcinoids, so designated by Borst (20), might better be called carcinomatoids,[3] to avoid confusion with the argentaffin tumors to which the term carcinoid is currently applied. They are growths having the histology of carcinomas and often ulcerating and extending through the ear and into lymph vessels, yet they are actually examples of spurious malignancy induced by local conditions (19). Their cancerous traits are wholly dependent upon the stimulation provided by continued tarring, and after this has been left off they either disappear or round up into epidermal cysts or become ordinary, benign papillomas. Proof exists (19) that they are intrinsically tumors of the latter sort, with cells notably responsive to stimulating influences and for this reason mimicking cancer cells when tarred.

None of the control implants of 57 tar tumors gave rise to a growth at the new situation (Table I). The tumor tissue either disappeared promptly, or died by keratinization,—with persistence for a while of the keratinized fragments,—or gave rise to minute, keratinizing cysts lined with stratified squamous epithelium, like those arising from implants of ordinary skin. In this respect our findings agree with those of Ferrero (21) who noted the failure of benign tar tumors to grow after implantation in the host rabbit.

The virus regularly "took" on the bits of epidermis punched from skin rendered hyperkeratotic by tarring but devoid of tumors. There resulted

[3] Suggestion of Dr. H. T. Karsner.

nodules of papilloma tissue precisely like those which develop after the transfer to the muscles or connective tissue of fragments of virus papillomas produced by infecting normal skin (22). As in such instances, the growths consisted of more or less spherical cysts, often partially fused or multilocular, frequently patched with gray, and having a solid, lamellated core of dead, keratinized cells surrounded by a layer of living, differentiating

TABLE I

Effects of the Papilloma Virus on the Benign Tar Tumors of Domestic Rabbits

Number of rabbits implanted	Tarred	Interval to implantation	Later tarring	Period of implantation	The implanted tumors		Outcome of implantation	
					Character	Fate on ears	Tyrode-steeped materials	Virus-steeped materials
	days	*days*	*days*	*days*				
7	78	5 to 10	9 to 21	20 to 64	12 benign papillomas	9 excised 1 vanished 1 stationary 1 grew	10 negative 2 tiny cysts	1 negative 1 tiny cyst 10 virus papillomas
					20 carcinomatoids	5 excised 9 vanished 2 dwindled 4 became benign papillomas	19 negative 1 tiny cyst	9 negative 8 virus papillomas 1 carcinomatoid (?) 2 carcinomas
1	91	2	21	59	1 frill horn	Excised	Negative	Tiny cyst
					3 benign papillomas	1 excised 2 stationary	2 negative 1 tiny cyst	1 negative 2 virus papillomas
3	112	275	None	42 to 65	2 frill horns	Excised	Negative	2 negative
					11 papillomas*	10 excised 1 stationary	Negative	7 negative 4 virus papillomas
2	200	92	7	38	8 benign papillomas	6 excised 1 dwindled 1 grew	7 negative 1 tiny cyst	3 negative 3 virus papillomas 2 tar papillomas

* 8 of the papillomas had been slowly retrogressing, 1 was stationary, and 2 were slowly enlarging.

epithelium. Often living papillae existed within the core, and always the proliferating cell layer exhibited the cytological peculiarities characteristic of the virus' action (19). Growth was often extremely rapid, the nodules reaching a diameter of several centimeters within a few weeks.

Pieces of three *frill horns* were exposed to the virus, all of them small tumors which had enlarged slowly. The control fragments of these growths disappeared, whereas those that had been exposed to virus yielded in one instance a virus papilloma and in another a tiny cyst lined with the typical,

frill horn epithelium. Incidentally to previous work (2) we have repeatedly noted the conversion of frill horns into papillomas after the virus had reached them *in situ*. Their cells, which have a highly characteristic morphology, were directly converted into virus papilloma cells, exhibiting the stigmata distinctive of such elements (19).

Of 34 *tar papillomas*, 15 gave no evidence of any effects of the virus, the tissue exposed to it either vanishing, or keratinizing completely, or rounding up into tiny cysts lined with stratified squamous epithelium, like some of the control implants. The tissue of 17 other growths gave rise to nodules of what appeared in the gross to be ordinary virus papillomatosis. As already mentioned, tissue of this sort often proliferates with prodigious rapidity after implantation within the body, and one would expect it to outgrow tar papilloma tissue in mixed grafts unless this latter was greatly stimulated by the virus. Most of the nodules appeared to consist entirely of virus papilloma but microscopic search sometimes disclosed living, proliferating islands of the tar tumor. The two sorts of papillomatosis can be readily distinguished under ordinary conditions owing to their cytological peculiarities (19), but this was not so easy in implantation nodules of mixed composition because the epithelial layers of differing kinds had frequently united and appeared to grade into each other. The longer the interval before the animal was killed, the less often was tar papilloma tissue encountered, for the reason that virus papilloma tissue more and more preponderated.

Fragments of two tar papillomas which had been exposed to the virus established themselves on implantation and grew actively while retaining their distinctive character.

D. R. 3-73 provided both instances (Table I). They were essentially similar. One is illustrated in Figs. 1, 2, and 3. As Fig. 2 shows, each of the several bits of tumor implanted in a leg muscle after exposure to virus gave rise to a papillomatous growth with the traits of the original tar tumor (Fig. 1). The orientation of the epithelial layer was the reverse of that in nodules consisting of virus papilloma tissue. When growing within the body the latter forms cysts filled with keratinized material and enclosed in a rind of living epithelium which differentiates *inwards* (Fig. 3). The opposite held true of the tar papilloma tissue submitted to the influence of the virus; its epithelium formed knobs connected with the host by pedicles, and these knobs keratinized *outwards*, soon becoming surrounded by much dead, squamous material (Fig. 2). The tar papilloma of Fig. 1 had been completely excised and the fragments implanted as controls disappeared.

The results with the other tar papilloma stimulated by the virus differed only to the extent that the piece of tumor left on the chronically inflamed ear continued to grow. The effect of chronic inflammation to bring about a persistence of tar papillomas

which would otherwise vanish has been stressed in a previous paper (2). Each of the numerous fragments implanted after exposure to the virus rounded into a keratinizing knob and grew, whereas the control fragments failed to establish themselves.

Carcinomatoids are to be found only on ears which have recently been tarred (19), and hence none was available in the case of the rabbits utilized for our experiments long after the last tarring. Bits of 20 carcinomatoids in all were subjected to virus infection (Table I). During the days before they were taken, when no more tarring was done, the growths dried down and in some instances began to involute. The virus had no evident effect upon 9 of them, gave rise to nodules of virus papillomatosis in 8, and in 3 caused tumors to appear which were invasive and had the morphology of carcinomas. One of these last consisted in the main of tissue like that of the original carcinomatoid, but much virus papillomatosis was present as well. This complication was negligible in one of the other two tumors and absent from the third. The findings with them were as follows:—

The carcinomatoid of D. R. 4-97 was an ulcerated disc 1 cm. across when about half of it was taken for implantation (Figs. 4 and 5). The remainder dwindled and had vanished when the animal died 20 days later. During this period a nodule 1.5 cm. across, of cancerous tissue devoid of papillomatous characters (Fig. 6), formed in the axillary connective tissue where bits of the virus-infected material had been placed. The control fragments disappeared.

Figs. 7 to 9 summarize events in the case of a growth of D. R. 5-10. The carcinomatoid furnishing the material was 2.3 cm. in longest diameter, a raised, raw disc which had extended, near its middle, to the outside of the ear through lacunae in the cartilage. About one-tenth of the disc was removed for implantation 5 days after the tar had been stripped from the ears after 78 days of tarring. This was resumed for 11 days after the biopsy and then discontinued for good. During the 56 days before the animal was killed the ear tumor gradually vanished. A slice taken at autopsy through the mound which marked where it had once been showed ordinary, stratified squamous epithelium, smooth and slightly hyperplastic, covering redundant connective tissue, with a patch of foreign body giant cells deep in the latter round about a few dying tumor cells (Fig. 8). Nothing was found in the leg where the control fragments had been implanted, whereas the material exposed to virus had given rise to an actively invasive growth 2 cm. in diameter. Multicentric, because of proliferation from the scattered tumor fragments, it was more malignant-looking (Fig. 9) than the original carcinomatoid (Fig. 7). A small, discrete island of Shope papillomatosis was present in the growth at one spot.

The character of the highly invasive, anaplastic, progressively enlarging neoplasms which resulted from the action of the virus upon carcinomatoids leaves no doubt that these had been rendered cancerous. The growths wholly resembled some of those fatal and frequently metastasizing tumors

resulting from the action of the virus upon tar papillomas and carcinomatoids situated on the tarred ear (2). Virus papilloma tissue, after transfer to muscle, occasionally invades and simulates carcinomatosis in the brief period before its epithelium differentiates and forms multilocular cysts. But even at such times its cytology is very different from that of the growths just considered.

The Results with Old Tar Tumors

Facts already reported (19) have shown that the benign tar tumors elicited by tarring the ears of domestic rabbits are all conditional growths devoid of the power to proliferate without the aid provided by local conditions. Tarring induces these conditions, rendering them permanent if it is long kept up, and growths thus aided may persist and enlarge, and occasionally change into cancers. The longer tar is applied the more likely is this to happen. It has seemed possible, in view of such findings, that benign tar tumors might gain the ability after a while to persist without aid, and to grow upon transfer to other situations. To obtain light on this point we have carried out implantation experiments with 19 papillomas which had been present for months.

Three of the animals furnishing the growths had been tarred for 112 days and then kept for 275 days more, during which period certain of their larger papillomas had persisted on the much altered ears, a few growing slowly, a few remaining unchanged, while the majority slowly dwindled. The eleven growths which were most vigorous were utilized for test, and two frill horns as well. The results with the latter have already been mentioned. Neither they nor the papillomas manifested any ability to grow on implantation, and exposure of their hashed tissue to the virus led at most to virus papillomatosis.

Eight tar papillomas were utilized which had persisted for 92 days after a tarring period of 200 days. They came from two animals. Again the control implants failed to grow, but in two instances the fragments exposed to infection with the virus proliferated actively at the new situation and retained the characteristic morphology of tar papillomas. These instances have also been described.

Effects of the Virus on the Tar Cancers of Wild Rabbits

The tar we employed very seldom elicits cancers in domestic rabbits and then only after a year or more; but it frequently calls them forth within a few months in cottontails. Experiments were carried out with some of the tumors thus provided.

The cancers evoked by tar in cottontails are often multiple, especially when it has been applied to both surfaces of the ear, a procedure well sustained, though soon fatal in our experience to most domestic rabbits. The tumors utilized were squamous cell carcinomas, as proven by their morphology, by continued growth of the portions left

behind on the ears, sometimes by metastasis, and by growth of the control implants in certain cases. Ulceration had usually taken place, necessitating the use of the most deep-lying tissue. Occasionally the cancer occupied so much of the organ that amputation was done instead of a punch biopsy. Metastases in lymph nodes at the base of the ear were taken as material in two cases. Despite these precautions abscesses totally destroyed the grafts in three of twelve implanted animals, and complicated the findings in most of the others. Tarring was stopped after the implantations.

Only equivocal results were obtained with the metastatic tissues. In one case abscesses destroyed all of the grafts except one,—which had been exposed to the virus,—and where this had been put a small area of unhealthy carcinomatosis was present in addition to an abscess. In the other animal a single, minute nodule of unhealthy squamous cell carcinomatosis was found,—also at a site where tumor bits exposed to the virus had been placed. This rabbit was not killed until 72 days had elapsed.

One of the remaining seven animals died on the 10th day after implantation. Papillomas could not be seen as yet on the inoculated area on its side, and microscopic sections were not taken, so there is no certainty that the rabbit was susceptible to the virus, since some "normal" cottontails prove refractory on inoculation, presumably because of previous infection (23). The cancer had just begun to grow at all of the implantation sites and no influence of the virus upon it could be discerned. Nor was any evident in the growths of two animals which lived longer and developed papillomatosis where the skin had been inoculated. One of them, killed on the 25th day, had small nodules of moderately anaplastic squamous cell carcinoma, together with small abscesses, at every implantation site. In the other the cancer failed to survive at any of the five control sites, and barely did so at two situations where material exposed to virus had been put, while at the remaining three large nodules of ordinary virus papillomatosis were found at autopsy on the 62nd day. There had been opportunity in this instance for the virus to infect non-neoplastic epidermis included in the tumor fragments.

The virus had remarkable effects upon the cancers of four animals (Table II).

W. R. 100 E.—This animal was subjected to two courses of tarring, on both sides of the ears, throughout two periods totaling 7½ months in all, with an interval of 3 months. The cancer appeared at the end of the second period, grew rapidly on both sides of the ear, and after another 2 months had occupied its whole further half. The tumor was 1 cm. thick on the inner side of the cartilage, fleshy and ulcerated, with a rolled rim, and on the outer side was nearly as thick, nodular, partly ulcerated, but with a subcutaneous extension. A part of this latter was punched out and utilized. The growth grated under the knife, showed numerous yellow, opaque dots, and proved to be a cystic, squamous cell carcinoma (Fig. 10), orderly for the most part and keratinizing, but in some places breaking up into small nests of anaplastic cells. Five implantations were made of the control fragments and of fragments exposed to virus, respectively. When the animal was killed, after 2 weeks, large cancerous nodules had arisen (Fig. 12) wherever the latter had been put (Table II), while nothing was found at one of the control situations, at three others the tumor had barely succeeded in surviving, and at the fifth site a minute cancerous nodule had formed (Fig. 11). There were abscesses amidst some of the large tumors due to the virus-infected material. The cancer on the ear had continued to grow, but it had not metastasized.

In this instance the virus not only stimulated the cancerous tissue, enabling it to proliferate with great rapidity (Table II), but exerted a

TABLE II

Effects of the Papilloma Virus on the Tar Cancers of Cottontail Rabbits

Rabbit No.	Character of implanted material and fate of cancer on ear	Implantation period	Situation of implant	Tumors resulting from tissue exposed to: Tyrode — Size	Tyrode — Constituents	Virus — Size	Virus — Constituents	Virus yield of: Tyrode growths	Virus yield of: Virus growths	Remarks
		days		*cm.*		*cm.*				
100 E	Nodular, fleshy growth covering half of an ear: a differentiating, cystic, squamous cell carcinoma (Fig. 10)	14	Foreleg	0.5	Small abscess and minute cysts lined by unhealthy tumor cells	1.8	Original cancer with increased anaplasia, and *new hybrid cancer*			
			Axilla	0.2	Keratinized cysts and one minute cancer nodule	0.5	*New hybrid cancer*			
	(Growth continued to enlarge)		Groin	0		1.8	*New hybrid cancer*			See Figs. 10–14
			Anterior thigh	0.8	Abscess with a few cancer islands in wall	2.8	Much *new hybrid cancer* in wall of large abscess			
			Posterior thigh	0.5	Abscess with a few cancer islands in wall	1.5	Large nodules of *new hybrid cancer* and small abscess			
1-47 E	Small, fleshy, ulcerated squamous cell carcinoma, moderately anaplastic	19	Foreleg	2.7	Large abscess with some cancer of original sort	2.7	Scattered abscesses in large mass of original cancer	0	±	
			Axilla	1.3	Abscess only	1.3	Abscess only			
	(Growth recurred at edge of punch hole)		Anterior groin	1.5	Abscess only	1.3	Original cancer; *new hybrid cancer*		±	
				1.7	Original cancer	1.7	Original cancer; *new hybrid cancer*			
			Posterior groin	0.6	Cyst lined with unhealthy cancer cells	0				
			Anterior thigh	2.0	Original cancer	2.3	Original cancer	0	0	
			Posterior thigh	1.5	Original cancer	1.4	Original cancer	0		
	Inoculation papilloma on side								+	

TABLE II—*Concluded*

Rabbit No.	Character of implanted material and fate of cancer on ear	Implantation period	Situation of implant	Tumors resulting from tissue exposed to: Tyrode — Size	Tyrode — Constituents	Virus — Size	Virus — Constituents	Virus yield of: Tyrode growths	Virus yield of: Virus growths	Remarks
		days		*cm.*		*cm.*				
1-40 E	Large, fleshy, ulcerated squamous cell carcinoma, keratinizing and cystic (Ear amputated)	26	Foreleg	0.15 0.15 0.2	Original cystic cancer, beginning to invade	2.5	Separate regions of virus papilloma and original cancer; of an intimate mixture of the two; and of a *new hybrid cancer.* Also abscess 0.6 cm. across		++	Serum at death contained antibody in considerable amount
			Axilla	1.3	Abscess only	1.5	Virus papilloma in wall of abscess		++	
			Anterior groin	0		1.7	Virus papilloma. Original cancer lying separate		++ +	
			Posterior groin	0		1.4	Virus papilloma and original cancer closely intermixed		++± +++	Twice tested
			Anterior thigh	3.5	Large abscess with a little cancer in wall	1.3	Virus papilloma and original cancer closely intermixed			
			Posterior thigh	0.4	Original cancer, invading	1.4	Original cancer *New hybrid cancer*	0	±	
	Inoculation papilloma on side								++++	
61 N	Subepidermal tumor consisting of three different types of squamous cell carcinomatosis (Figs. 18-20) (Growth continued to enlarge)	72	Foreleg	0		4 × 3	Virus papilloma		+	See Figs. 15-20
			Axilla	0		1.3 2.0	Some cancer of original sorts but mostly virus papilloma Mostly cancer of all three original types; a little virus papilloma tissue		± 	
			Groin	0.1	Cyst lined with dead epithelium	1.3	Mostly original cancers but some virus papilloma			
			Thigh	0.2	Original cancer, unhealthy	5 × 3.5	Original cancers. Virus papilloma in about equal amount		0 0	
	Original cancer as above								0	
	Inoculation papilloma on side								+±	

formative influence upon it (Figs. 12 and 14), rendering it unlike both the original tumor (Fig. 10) and the small control nodule (Figs. 11 and 13), which were similar histologically. The result was a new, hybrid cancer, as one might call it, exhibiting the stigmata indicative of the virus action (Fig. 14).[4] The cells were considerably larger than those of either the parent tumor or the control nodule, and they increased still more in size as differentiation took place, ballooning instead of flattening and granulating prior to keratinization. The nuclei were also larger and as they enlarged further their chromatin marginated, coarse parakeratotic granules sometimes forming next them. The original tar tumor had been cystic, and the growing control implant was becoming so, but the cancers changed by the virus had almost completely lost this tendency (Fig. 12). The result of these changes was a carcinoma of comparatively coarse cytology, very active and highly aggressive. No such tumor has resulted from the intramuscular implantation of virus papilloma tissue in our numerous experiments of the sort; for though the papilloma, when gaining a foothold, may simulate cancer, as already mentioned, its epithelial layer soon rounds up into keratinized cysts like those of Fig. 3. The new, hybrid tumors of W. R. 100 E were evidently the outcome of the combined influence of the virus and of the principle actuating the original tar carcinoma, whatever that may have been.

The cancer to be next discussed grew actively at every control situation. There was thus introduced a difficulty which had not entered into the experiments with the benign tar tumors of domestic rabbits. In their case the consistent failure of the control implants to establish themselves brought with it a certainty that any proliferation of the material exposed to virus could be referred to the influence of the latter. But no such "open and shut" results could be expected with tumors capable of growing at the new situations without aid. Any stimulating effect of the virus upon them would find expression merely in relative rate of enlargement, and differences in this respect would diminish in proportion as the control grafts grew more rapidly. If the cells composing these were doing their utmost in the way of proliferation the virus could scarcely stimulate them further. And there was another complication,—the grafts exposed to virus inevitably contained cells which escaped contact with it because lying under the surface of the

[4] We have dealt with these stigmata in previous papers (*J. Exp. Med.*, 1936, **64,** 401; 1940, **71,** 469). No one of them is peculiar to tumors influenced by the papilloma virus, nor do such growths always show them, as the present work makes clear; yet when found together they are pathognomonic of the virus' action. A scrutiny of several hundred tar tumors of domestic rabbits and cottontails has failed to disclose any growth presenting the same association of cytological features, though many had a striking superficial similarity to virus papillomas.

tissue fragments. Hence some of the new tumors could not but be mixed growths, made up in part of the descendants of cells like those forming the control nodules. Whether such conditions obtained in the following instance cannot be said. But certain it is that the grafts exposed to virus grew no faster than did the controls, which enlarged with very great rapidity. Yet the virus "took" on the tar cancer cells, as proven by recovery of it from two of the implantation tumors, and by the hybrid morphology of one of them.

W. R. 1-47 E (Table II), tarred for not quite 4 months on both surfaces of the ears, had on the inside of the right an ulcerated, discoid tumor 1.4 cm. across and 4 mm. high, which had extended through to the outer side as a firm, subepidermal mound. It was nearly all taken for the experiment, but the remaining fragment enlarged during the 19 days before the rabbit was killed. The control material and that exposed to virus were implanted at six sites each. Abscesses developed at some of them and cancerous nodules at others. The virus had not evidently influenced the size or the morphology of the new tumors save at one situation where a new hybrid carcinoma nearly resembling that of W. R. 100 E was present, together with malignant tissue of the original sort. The recovery of virus will be considered further on.

In the remaining two instances the presence of virus papillomatosis in the implantation growths complicated the findings.

W. R. 1-40 E.—A large cancer was present on the outside of one ear of this animal, after they had been tarred for 4 months on both sides. It was 4 cm. across, 1.5 cm. high, raw and fungating, had extended through the cartilage and given rise to a big, ulcerated mound on the inner side. The ear was amputated and the neoplastic tissue next the cartilage was taken for the experiment. Section showed the growth to be a keratinizing, cystic, squamous cell carcinoma.

When the animal was killed 26 days after implantation, nothing was found at two of the control sites, abscesses at two more, with a small amount of surviving cancerous tissue at one of them, while at the remaining two, tiny nodules had formed of cancerous tissue like that of the original growth (Table II). Large tumors were present at all of the virus sites. One growth consisted of virus papillomatosis in the wall of an abscess, but four of the other five tumors consisted of cancer and papilloma intimately intermixed. The fifth tumor was composed of cancerous tissue like that of the original growth and of a new hybrid cancer nearly resembling that found in W. R. 100 E.

W. R. 61 N was tarred throughout two periods of many months each, at first on both sides of the ears. After 21 months in all, 3 months after the last tarring, the animal had an ulcerated growth 1.5 cm. across but only 4 mm. thick, projecting equally on each side of the ear. On the outer side several firm, blunt prongs extended toward the base of the organ in the subcutaneous tissue. The thickest part of the tumor was procured by punching, and the tissue next the cartilage was selected for the implantations. Section showed two distinct cancers in the material, one a keratinizing, cystic squamous cell carcinoma, the other having the same general character but with bizarre cells, many of them enormous and multinucleate. Embedded in the neoplastic tissue were numerous hair follicles, with melanoblasts amidst their cells.

The portion of the tumor left behind grew steadily during the 72 days before the animal was killed. At death it was 4.5 cm. across, thicker than before, and the extending prongs had broadened into a firm subcutaneous mass. Sections showed three distinct cancers, differing sharply in type, the third tumor being a squamous cell carcinoma with exceptionally small cells. It had doubtless been present in the implantation material as the results with this showed. There were no metastases.

Nodules had appeared rapidly at the four sites where material exposed to virus had been put, whereas at two of the control sites nothing could be found at autopsy, and, at the other two, tiny nodules (Table II). One of these proved to be a completely keratinized cyst (Fig. 16), the other a little cyst walled with degenerating cancerous tissue and containing debris (Fig. 15). The implantation growths from the material exposed to virus were by comparison of great size, from 1.3 to 5 cm. in diameter. One consisted wholly of virus papilloma while the others were mixtures of cancer and virus papilloma in varying amounts, the latter being almost absent from one of two axillary nodules (Table II). No hybrid cancer had developed but all three types of malignancy found in the original growth at autopsy were represented (Figs. 18 to 20); and at most situations the papilloma tissue had formed separate, discrete nodules consisting of the usual creamy or gray cysts enclosing keratinized epithelium (Fig. 17). The separation of papilloma and cancer enabled us to make comparative tests for the presence of virus in the two sorts of tissue.

The implantation growths developing in W. R. 1-40 E from material exposed to the virus were close-textured and appeared entirely cancerous in the gross, but the microscope showed in most instances a mixture with virus papilloma tissue. The cancer was largely of the original type, with a new hybrid carcinoma at some situations. One nodule consisted wholly of the two. In W. R. 61 N the virus papilloma tissue had the form of discrete nodules. They were largely melanotic, a condition which pointed to an origin from the hair follicles present in the implanted fragments of the tar tumor. This latter contained no melanoblasts.

The virus stimulated the cancer cells in both these instances, and in one altered them morphologically.

The Recovery of Virus from Implantation Growths

Virus of high pathogenicity, such as we employed, produces vigorous papillomas in domestic rabbits, but from them it can only exceptionally be got again; and it has never been procured from the derivative cancers. In view of these negative findings we made no attempt in the present work to recover virus from the tumors due to the implantations in domestic rabbits.[5]

[5] By testing many cottontail papillomas Shope procured some strains of the virus which produced in domestic rabbits growths from which it could be recovered (*Proc. Soc. Exp. Biol. and Med.*, 1935, **32,** 830). The strains gave rise only to indolent papillomas, after a long incubation period, and hence were unsuited to the present work.

The conditions are different in cottontails. Virus can be recovered in abundance from their papillomas when these are not so disordered that neutralizing antibody from the blood extravasates into them (24). This quite frequently happens, however, with result that no virus can be got from the growths; and so pronounced is the extravasation of antibody into the derivative cancers,—which regularly fail to yield virus,—that often extracts of the malignant tissue prove capable of neutralizing the latter in considerable quantity *in vitro* (25). None of the antibody is present in animals carrying tar cancers (16), and it appears gradually when virus papillomas are produced in normal rabbits (26). In view of these facts there seemed to be a possibility that virus would be obtainable from some of the malignant tumors resulting from the implantations in cottontails, if the animals were killed early. This has proved to be the case. Unfortunately no tests were made in the instance which provided the most favorable conditions,—that of W. R. 100 E, which developed large cancers within 2 weeks after implantation.

The implantation tumors were removed with due precautions for asepsis, and cut up and placed in 50 per cent glycerin, after slices of them had been taken in Zenker's fluid. Virus papilloma tissue and cancer tissue lying separate in the same mass were handled with different instruments and glycerinated separately. A few weeks later 10 per cent Tyrode extracts were made of the materials and inoculated into checkerboard squares on each of three normal domestic rabbits, which were bandaged afterwards in such way as to prevent any transfer of the inocula (26). The following symbols are employed in Table II to report the findings: ++++ = confluent papillomatosis, +++ = semiconfluent papillomatosis, ++ = many discrete papillomas, + = a few papillomas, ± = 2, 3, or 4 discrete papillomas, ± = 1 papilloma. The results of the inoculations were recorded at frequent intervals until 35 days had elapsed, after which no more growths appeared.

One of the implantation cancers of W. R. 1-47 E,—killed after 19 days,—yielded no virus; but from two others a little was got. The papilloma on the side yielded scarcely more, and the tumors deriving from the control implants gave none. One of the growths from which virus was obtained had a hybrid morphology indicative of its influence. No tests were made to learn whether antibody had appeared in the blood of the animal, as sometimes happens even so early.

The blood of W. R. 1-40 E, killed 26 days after implantation, contained antibody in considerable amount, as disclosed by complement fixation tests. Only one of the tumors was wholly cancerous. It yielded virus, but merely a trace as compared with the abundance obtained from the papilloma on the side of the animal. Most of the growths consisted of mixtures of cancer and virus papilloma tissue, and they yielded virus in amounts roughly corresponding to the proportion of the latter. In one growth the two were so sharply separated that both could be tested; and both yielded virus, more coming from the papilloma tissue. Only one of the control implants provided sufficient material for test. No virus was obtained from it.

The findings with W. R. 61 N were enlightening in several respects. The inoculation

growth on the side yielded only a moderate amount of virus, one of the large implantation growths which consisted wholly of virus papilloma tissue yielded still less, and none at all could be got from the discrete, virus papilloma nodules forming part of another large implantation mass which consisted for the rest of cancer (Fig. 17). This latter gave no virus. A little was got from another tumor consisting mostly of cancer but with some virus papilloma tissue intermixed. None of the control implants provided enough tissue for test, but the original tar cancer on the ear, which had continued to enlarge, failed to yield virus. Serum tests were not made, but the rabbit had carried large papillomatous masses for more than 7 weeks and the likelihood is that its blood contained antibody in considerable amount.

The results with the growths of W. R. 1-40 E and W. R. 61 N show that papillomas lying deep are less likely to yield virus in quantity than are those on the skin surface, local circumstances altering cases much in this respect. From one of the actively growing intramuscular papillomas of W. R. 61 N no virus whatever could be procured, although it was got from another implantation tumor in which cancer predominated. In general the cancers yielded far less virus than did the associated papillomatous tissue. The reasons will be discussed further on.

Implantation of a Tar Carcinoma of the Domestic Rabbit

The tar we employed does not elicit cancer in domestic rabbits until after many months have gone by, and only one growth of the sort was available for implantation tests.

The cancer animal had been tarred throughout three periods, totaling 5 months, with long intervals between them. After 21 months in all, 6 months after the last tarring, a persisting papilloma was noted to have ulcerated, and a gland at the base of the ear was found to be large and firm. During the next month the tumor grew with great rapidity, destroying part of the ear, and numerous large, firm nodules developed under the jaw and further down the neck in the lymph glands. The enlarged gland at the base of the ear was now excised, and the neoplastic tissue which had almost entirely replaced it was hashed, exposed to virus, and implanted in the usual way. When the animal died 58 days later, abscesses had developed in some of the metastases along the neck and others had coalesced into huge masses. Nothing was found at one of the situations where cancer tissue unexposed to virus had been placed, but at the other four large tumors were present, as also at all five spots where the material exposed to virus had been put, some of the growths being 5 cm. in diameter. There had been no significant difference in their rate of enlargement. The original tar tumor was an anaplastic, squamous cell carcinoma, very unhealthy and necrotizing early.

In this instance vigorous papillomas appeared where the skin had been inoculated, yet the virus caused no morphological alteration or stimulation of the cancerous tissue exposed to it.

DISCUSSION

The Findings in Domestic Rabbits

In a previous paper we have reported that the benign, epidermal tumors elicited by tarring the skin of domestic rabbits are wholly conditional neoplasms, dependent for their persistence either on continued tarring, on the chronic changes this eventually brings about in the supporting tissues, or on inflammation and maceration incidental to crowding, when the growths are large and many (2, 19). The present results accord with these findings. Not one of 57 benign but vigorous tar tumors transplanted within the host to situations well suited to the growth of implanted cancers and virus papillomas was able to establish itself. The tumor cells actually proved less capable in this respect than the normal cells accidentally transferred with them. Living hair follicles in good condition, sebaceous glands and minute cysts lined with ordinary squamous epithelium were not infrequently met at sites from which all neoplastic elements had disappeared.

The hyperplastic epidermis punched from places on the tarred ears where no tumors were visible and implanted after exposure to the virus regularly yielded nodules of virus papilloma tissue. No "anomalous tumors" developed such as arise fairly often when the virus localizes in areas on tarred ears which are devoid of growths (2); but the conditions of the present experiments were not favorable to their occurrence. Even when the virus localizes abundantly in the ears and gives rise to innumerable papillomas of typical sort where previously there was only hyperplasia, the anomalous growths are never many, whence one can conclude that the cells capable of forming them must be relatively few. The chances were greatly against the exposure of such cells to virus infection when the skin was cut into half-millimeter pieces—the average size of the hashed fragments employed in the experiments.

On many of the tar tumors the virus seemed to have no stimulating effect, these failing to grow at any implantation site. This cannot have been due to lack of pathogenicity of the inoculum since it regularly acted upon fragments of the tarred skin of the same animals. Destruction of the grafts by bacterial infection can be invoked to explain some failures, notably in the case of tissue procured from ulcerated carcinomatoids,—which as a group gave a high proportion of negative results,—but this will not cover the findings with orderly, "healthy looking" growths. At not a few sites aggregates of keratinized scales showed that the graft had died by differentiation, while at others tiny cysts remained, lined with stratified squamous epithelium. In these instances bacterial action cannot have

caused the failures. We have repeatedly noted that some of the tar tumors which were present on the ears at the time when the virus localized in these organs behave as if wholly uninfluenced by it, altering not at all or disappearing while everywhere else over the entire surface an active, crowded, virus-induced proliferation is going on (2). Several of the cottontail cancers of the present work, which grew after implantation, were not perceptibly altered by the virus. One must conclude that the cells of certain tar tumors are refractory to its influence. Whether they become infected with it is uncertain.

The positive yield of the implantations was small as compared with that when the virus takes effect in benign tar tumors situated on the ears. There the conditions favor its association with cells already neoplastic,—a fact clearly evident in the frequency with which it affects the tar tumors when it localizes at only a few spots,—and virus papillomatosis obscures its stimulating and altering influence far less often than when pieces of tar tumors, containing normal as well as neoplastic cells, are submitted to its action. However carefully the fragments are trimmed, normal elements can seldom be excluded from them; for non-neoplastic epidermis is often present in the clefts between the fingers of papillomas, and hair follicles extend down both into them and the carcinomatoids. A considerable proportion of the papilloma nodules arising on implantation may have derived from such components. Sometimes though, a conversion of tar papilloma cells into virus papilloma cells may have occurred; for the change has been repeatedly noted in sections of tar tumors removed from the ears shortly after the localization of intravenously injected virus. In whatever way the new virus papilloma tissue arose, it rapidly outgrew any surviving tar tumor tissue, and soon so preponderated as to hide the latter.

A few of the tar papillomas retained their characteristic morphology though impelled to grow by the virus. The possibility has been ruled out by previous work (2) that the carcinogenic effect of the latter upon tar tumors is due merely to conversion of the growths into virus papillomas, with malignant changes so rapidly ensuing in the latter as to be telescoped into a few days instead of taking place after months or years, as under ordinary circumstances (27). The results of the implantations exclude this possibility also. One can readily tell where virus papilloma has been present in muscle or connective tissue, since on dying it leaves laminated, keratinized pearls of distinctive form, which are only very slowly resorbed. Microscopic search of the cancers which arose at spots where carcinomatoids infected with the virus had been implanted yielded no such evidence that they had been preceded by virus papillomatosis.

Many of the growths which result from the changes taking place in tar tumors after virus has localized in the ears have no duplicates amongst either the tar or virus tumors but exhibit a blend of their characters. They are the anomalous tumors already referred to. The implantations in domestic rabbits did not yield any of these hybrid or mongrel growths,—a fact scarcely surprising in view of how few tumors, other than virus papillomas, were obtained in all; but several hybrid growths arose from the implanted fragments of cottontail cancers that had been exposed to virus infection. They will be discussed further on. Only the carcinomatoids of domestic rabbits, not the tar papillomas, grew as cancers after implantation, but this is not expressive of a rule since we have repeatedly seen tar papillomas on the ears undergo alteration to rapidly growing cancers as result of infection with the virus (2),—a transformation followed by repeated biopsies. Yet it was also noted that carcinomatoids are more likely to be rendered malignant in this way. The possibility has to be considered in such relation that the carcinomatoids which become actively cancerous after infection with the virus are actually ineffective carcinomas, incapable of maintaining themselves without aid. Yet since the carcinomatoids as a group have proved to be mere phases of tar papillomatosis (19), and since tar papillomas themselves not infrequently become cancerous under the influence of the virus, there would seem to be no reason to assume the existence in domestic rabbits of an additional category of benign growths. The carcinomatoids obviously have cells far more sensitive to the stimulus of tarring than those of tumors of the same essential character which retain the papilloma form. They mimic true cancers as the latter do not though submitted to the same conditions (19). At the time when the papilloma virus infects their cells these are already invading and destroying and are more or less anaplastic. All that the virus need do to render them truly cancerous is to assure their continued activity in the state they have already assumed, in the same way that it assures the progressive growth of certain tar papillomas though causing no morphological alterations in them. Whatever the fact, the statement of a previous paper (14) that "one cannot suppose anaplastic squamous cell carcinomatosis in either rabbits or man to be maintained by agents that act as adjuvants to the essential cause of the neoplastic condition" must be withdrawn.

Many of the tar papillomas of mice yield cancers at once when stimulated by injury, or cut up and implanted (28). The malignant growths then arising may be the outcome of secondary changes in the papilloma cells brought about by injury,—which frequently precipitates a cancerous change in virus papillomas (27),—or they may conceivably be due to the liberation

of malignant cells already present in the apparently benign growths. Our implantation experiments with rabbit papillomas which had long been tarred or had long persisted, were undertaken as bearing upon these alternatives. They yielded no evidence of any increased capacity of the papillomas to grow on implantation, nor of the existence of latent cancer cells. Yet needless to say, such cells may be present in the growths later on, since most of the tar carcinomas of rabbits arise from preexisting papillomas.

The Findings in Cottontail Rabbits

The virus failed to influence some of the cottontail cancers perceptibly, just as it failed with some of the benign tar tumors of domestic rabbits. Other cancers it impelled to grow rapidly, often altering their form. It did not stimulate those that were inherently capable of growing at a great rate, though sometimes it altered them.

The actuating factor in cottontail cancers, whatever this may be, causes them to proliferate at differing rates. Other things being equal, one would expect the stimulating effect of the virus to be most evident upon slow-growing tumors; and this was the experimental finding. In two instances (W. R. 100 E, 61 N—Table II) the virus was the factor determining success at the new sites, the control grafts doing practically nothing. Its inability to hasten the enlargement of cancers capable of growing quickly without its aid can be explained by assuming that the malignant cells were already urged to their maximum. This would cover the case of the tar carcinoma of a domestic rabbit which the virus failed to influence. It was certainly present in the implantation nodules of one of the fast-growing cottontail cancers (W. R. 1-47 E) which gave no sign of stimulation, for they not only had a morphology indicative of its presence but yielded it on extraction.

Very little virus was recovered from the cancerous implants, considering the great changes that it induced in them (Table II). More was usually got from tumors containing some virus papilloma tissue, and still more from growths consisting wholly of the latter. Yet even from these less virus was procured than from papillomas produced on the skin of the animal by the same inoculum. No virus whatever was obtained from one of the papilloma nodules of W. R. 61 N, though another yielded it (Table II). The observed differences were doubtless due mostly to differences in the local conditions affecting the extravasation into the growths of virus-neutralizing antibody; for such extravasation often wholly prevents the recovery of the virus from skin growths known to contain it (24). Cancers ordinarily provide conditions more favorable to extravasation than do papillomas (25), and hence one would expect them to yield less virus.

But a possibility exists that even the small amount obtained from the cancerous implantation tumors really came from virus papilloma tissue situated in parts of the growths which could not be submitted to microscopic scrutiny because extracted for test. The best evidence for the presence of the virus in the implantation cancers lay in their altered character.

General Considerations

On reviewing the results as a whole one perceives that the effects of the virus on the implanted tar tumors fell into five categories:

1. In many instances it changed the tumors to virus papillomas.
2. It brought about the formation of hybrid tumors, morphologically expressive of the combined influence of the virus and of the unknown factor responsible for the neoplastic state of the tar tumor cells. The hybrid condition was sometimes, though not always, accompanied by an increased rate of tumor growth.
3. It converted some previously benign tumors into cancers showing no morphological sign of its presence.
4. It enabled some tumors to establish themselves and grow, while retaining their characteristic features, and it hastened the proliferation of others capable of establishing themselves without its aid.
5. It failed in some instances to influence the tumor cells perceptibly.

These findings corroborate and extend observations made upon tar tumors *in situ* (2). The influence of the virus ranges all the way from apparently complete domination of the tar tumor cells, with result that they become virus papilloma cells, to equally complete ineffectiveness. Often, as in the anomalous tumors several times mentioned, the alterations in the neoplasm appear to be the expression of some balance of power struck with the intrinsic cause or causes for the tar tumors. Judging from the behavior of the latter these causes are generally less forceful than the virus. Hence, perhaps, the frequency with which it becomes the dominating influence.

Our recent work with the virus has nearly all been directed to the problem of its relation to the carcinomas originating from the papillomas it engenders. Are they due to some other cause than the virus, acting upon cells which the latter has prepared? Or does the virus persist in them and, though not their cause, exert a modifying effect upon them? Or is the virus, or a variant of it, the actuating principle in the malignancy?

Something can now be said on these matters. The presence of the virus has been demonstrated in two cancers deriving from the papillomas experimentally induced with it in domestic rabbits (15, 29). One of the growths

has been transplanted to thirteen successive groups of animals thus far, and serological tests have shown that the virus regularly persists in the tumors and increases in amount as they enlarge in the new hosts.[6] The present work proves, like experiments already reported (2), that the virus is able to influence tumors due to some cause unrelated to it antigenically (15), namely tar papillomas and carcinomas. It has proved itself capable of goading these to more rapid proliferation, of altering their form and rendering some cancerous when otherwise they would have remained benign and eventually have disappeared. In initiating and maintaining malignancy in benign tar tumors it acts as the effective cause for cancer. Since it can stimulate and alter some of the squamous cell carcinomas elicited with tar in its native host, the cottontail rabbit, there is every reason to suppose that it can influence the cancers of similar histological sort, derived from cells that it has itself rendered papillomatous. Actually there are signs of its influence in many such cancers (27).

Yet one cannot conclude that the virus is more than an adjuvant influence in the malignant growths. It would seem to be merely this in the tar tumors, though frequently decisive for their character and fate.[7] None of a hundred and more virus materials provided by the naturally occurring papillomas of cottontails has ever on test given rise to anything but benign papillomas of identical morphology, when rubbed into scarified normal epidermis. To function as the cause for the carcinomas deriving from such growths it must either have undergone variation, with result that it expresses itself in altered cellular ways, or else the conditions of its partnership with the cell,—a partnership which ordinarily results in neoplastic growths of stereotyped character,—must have changed, or the state of the cell which is its medium of expression must have altered (2). In recent papers we have brought evidence favoring the view that the malignancy is due to virus variation (30, 25).

The demonstration that the virus may exert driving and formative effects upon cells rendered neoplastic by another means brings up the possibility that under natural conditions intercurrent infection with viruses may sometimes stimulate and alter tumor cells which otherwise would never disclose themselves. The influence of bacterial infection to enhance the malignancy

[6] This finding can scarcely be deemed remarkable in view of the ability of virus papilloma cells and those of the derivative cancers to support a multiple infection with wholly alien viruses (Syverton, J. T., and Berry, G. P., *Am. J. Path.*, 1938, **14,** 633).

[7] The virus recovered from the implanted cottontail cancers of the present work gave rise only to papillomas on inoculation: it had not become a directly carcinogenic virus in its influence upon scarified normal epithelium.

of human tumors has long been recognized, though its scope is still undetermined. Rivers and Pearce's discovery that virus III had entered the Brown-Pearce rabbit tumor they were working with, and flourished under the circumstances of further transplantation, sufficiently shows that an extraneous virus can reach and infect cancers.[8] But the literature reviewed at the beginning of the present paper indicates that viruses which exert no proliferative influence upon cells under ordinary circumstances have none upon tumors into which they are introduced. Furthermore the neoplastic viruses thus far studied have proved to be remarkably selective in their action. Each is pathogenic only for species nearly related to that from which it came, and each causes cells of but a single sort to become tumor cells. The rabbit papilloma virus will not persist on introduction into the Brown-Pearce rabbit carcinoma (16), a growth presumably originating in a hair follicle (31), and inducing no immunity against the virus. The virus of chicken tumor I, which causes sarcomas when injected into connective tissue, is devoid of influence upon the sarcomas of connective tissue origin elicited with chemical carcinogens, and soon disappears from them (11). All this speaks for the great rarity of secondary virus infection as a precipitating cause for tumor growth under natural conditions, if indeed it ever acts in such a way.[9]

In some recent experiments bits of tar cancers from cottontail rabbits were exposed *in vitro* to Tyrode and to the Shope fibroma virus (32) respectively, with implantation afterwards in the leg muscles and subcutaneous tissue of the host. The general procedure was that used in the work with the papilloma virus, and the proliferative strain of virus (OA strain of Andrewes and Shope (33)) was employed. Its effects were tested upon two or three cancers of each animal. It gave rise to characteristic fibromas where the fragments exposed to it had been implanted, and the cancerous tissue included in the growths proliferated more actively in some instances and with more anaplasia

[8] Andrewes (*J. Path. and Bact.*, 1940, **50,** 227) has recently encountered virus III as a damaging contaminant in infectious fibromas of rabbits, growths due to another virus. In addition to producing inclusion bodies, the virus III sometimes caused focal necroses and early retrogression of the fibroma. Andrewes found it also in a repeatedly transplanted sarcoma which had arisen where an infectious fibroma had been present.

[9] McIntosh has lately reported that a strain of the virus of contagious dermatitis of sheep, maintained in rabbits for 3 years and producing proliferative cutaneous lesions of the skin of such animals, failed to affect tar tumors when injected intravenously (McIntosh, J., *16th Ann. Rep. Brit. Empire Cancer Campaign*, London, 1939, 33). He has confirmed our finding that the Shope papilloma virus, thus introduced, causes such tumors to grow more rapidly and in many cases to become malignant. Brunschwig, Tschetter, and Hamann tried to induce cancer in rabbit ears previously treated with benzpyrene, by injecting extracts of human warts intravenously. The results of their experiments were equivocal (*Am. J. Cancer*, 1940, **38,** 50).

than that of the control implants. The difference might have been great had not most of the cancers exerted a strong desmoplastic influence of their own. Some of them evoked at the control situations a connective tissue reaction almost as lively as that due to the virus, though differing in detail; and it was obviously favorable to growth of the tumor.

The carcinoma cells proliferating amidst the fibroma tissue frequently showed the eosinophilic granulation which Shope and others have described (32) as occurring in the epidermis overlying superficial fibromas produced with the virus. Ordinarily these latter retrogress in cottontails after some weeks, and they do so even sooner in domestic rabbits, though tar injections will cause them to enlarge for a much longer time, sometimes with secondary dissemination (12). It had been hoped that the months of preliminary tarring whereby the cancers had been evoked in our implanted cottontails might have so shaped conditions that the fibromas would grow progressively; but this was not the case. Retrogression began within the ordinary period.

The interpretation of the phenomena occurring when the papilloma virus localizes in tarred ears will be the theme of a later paper.

SUMMARY

The effects of the rabbit papilloma virus upon tar tumor tissue are widely various, as the present paper and previous ones attest. It enables some of the benign tar tumors of domestic rabbits (papillomas, carcinomatoids) to establish themselves after implantation,—which they are unable to do under ordinary circumstances, being dependent upon favoring factors; and it may drive them to active proliferation without altering their morphology. Some growths it fails to influence and some it converts into virus papillomas. Often, however, it brings about cytological changes which are indicative of a combination of its influence with that of the undetermined factor motivating the original tumor. The resulting neoplasm exhibits a blend of characteristics.

The virus makes some benign tar tumors become cancerous forthwith, the malignancy developing without intermediate virus papillomatosis. It can be readily imposed upon some of the squamous cell carcinomas which tar elicits in its natural host, the cottontail rabbit, and it may drive such tumors to proliferate faster, or alter them morphologically, or do both. Its stimulating effect is especially pronounced in the case of those tar cancers that are slow-growing.

Since the virus can influence tar cancers markedly, one can scarcely suppose it to be devoid of effect upon the cancers of the same type which derive from the papillomas it has itself engendered. Other implications of the work are discussed.

BIBLIOGRAPHY

1. Shope, R. E., *J. Exp. Med.*, 1933, **58,** 607.
2. Rous, P., and Kidd, J. G., *J. Exp. Med.*, 1938, **67,** 399; **68,** 529.
3. Levaditi, C., and Nicolau, S., *Compt. rend. Soc. biol.*, 1922, **87,** 498.
4. Levaditi, C., and Nicolau, S., *Ann. Inst. Pasteur*, 1923, **37,** 443.
5. Levaditi, C., Schoen, R., and Reinié, L., *Ann. Inst. Pasteur*, 1937, **58,** 353.
6. Levaditi, C., and Haber, P., *Compt. rend. Acad. sc.*, 1936, **202,** 2018. Levaditi, C., Schoen, R., and Reinié, L., *Compt. rend. Soc. biol.*, 1937, **124,** 711. Levaditi, C., and Schoen, R., *Compt. rend. Soc. biol.*, 1937, **125,** 607.
7. Schoen, R., *Compt. rend. Soc. biol.*, 1938, **128,** 135; *Ann. Inst. Pasteur*, 1938, **60,** 499.
8. Findlay, G. M., and MacCallum, F. O., *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1937, **30,** 507.
9. Pownall and Florey, H. W., *12th Ann. Rep. Brit. Empire Cancer Campaign*, London, 1935, 105.
10. Rivers, T. M., and Pearce, L., *J. Exp. Med.*, 1925, **42,** 523.
11. Mellanby, E., *12th Ann. Rep. Brit. Empire Cancer Campaign*, London, 1935, 99; *J. Path. and Bact.*, 1938, **46,** 447.
12. Andrewes, C. H., and Ahlström, C. G., *J. Path. and Bact.*, 1938, **47,** 87.
13. Andrewes, C. H., Ahlström, C. G., Foulds, L., and Gye, W. E., *Lancet*, 1937, **2,** 893.
14. Rous, P., Beard, J. W., and Kidd, J. G., *J. Exp. Med.*, 1936, **64,** 401.
15. Kidd, J. G., Beard, J. W., and Rous, P., *J. Exp. Med.*, 1936, **64,** 79. Kidd, J. G., and Rous, P., *J. Exp. Med.*, 1940, **71,** 813.
16. Kidd, J. G., Beard, J. W., and Rous, P., *J. Exp. Med.*, 1936, **64,** 79.
17. Levaditi, C., and Schoen, R., *Compt. rend. Soc. biol.*, 1936, **122,** 736.
18. Syverton, J. T., and Berry, G. P., *Am. J. Path.*, 1938, **14,** 633.
19. Rous, P., and Kidd, J. G., *J. Exp. Med.*, 1939, **69,** 399.
20. Borst, M., *Z. Krebsforsch.*, 1924, **21,** 341.
21. Ferrero, V., *Arch. sc. med.*, 1926, **48,** 67.
22. Rous, P., and Beard, J. W., *J. Exp. Med.*, 1934, **60,** 701, 723, 741.
23. Rous, P., Kidd, J. G., and Beard, J. W., *J. Exp. Med.*, 1936, **64,** 385.
24. Kidd, J. G., *J. Exp. Med.*, 1939, **70,** 583.
25. Kidd, J. G., and Rous, P., *J. Exp. Med.*, 1940, **71,** 469.
26. Kidd, J. G., Beard, J. W., and Rous, P., *J. Exp. Med.*, 1936, **64,** 63.
27. Rous, P., and Beard, J. W., *J. Exp. Med.*, 1935, **62,** 523.
28. Cramer, W., *Brit. J. Exp. Path.*, 1929, **10,** 335. Mottram, J. C., *Am. J. Cancer*, 1934, **22,** 801; *J. Path. and Bact.*, 1936, **42,** 79.
29. Kidd, J. G., and Rous, P., *J. Exp. Med.*, 1940, **71,** 813.
30. Rous, P., *Am. J. Cancer*, 1936, **28,** 233.
31. Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, **37,** 601.
32. Shope, R. E., *J. Exp. Med.*, 1932, **56,** 793, 803.
33. Andrewes, C. H., *J. Exp. Med.*, 1936, **63,** 157. Shope, R. E., *J. Exp. Med.*, 1936, **63,** 173. Andrewes, C. H., and Shope, R. E., *J. Exp. Med.*, 1936, **63,** 179.

EXPLANATION OF PLATES

All of the sections were stained with eosin and methylene blue.

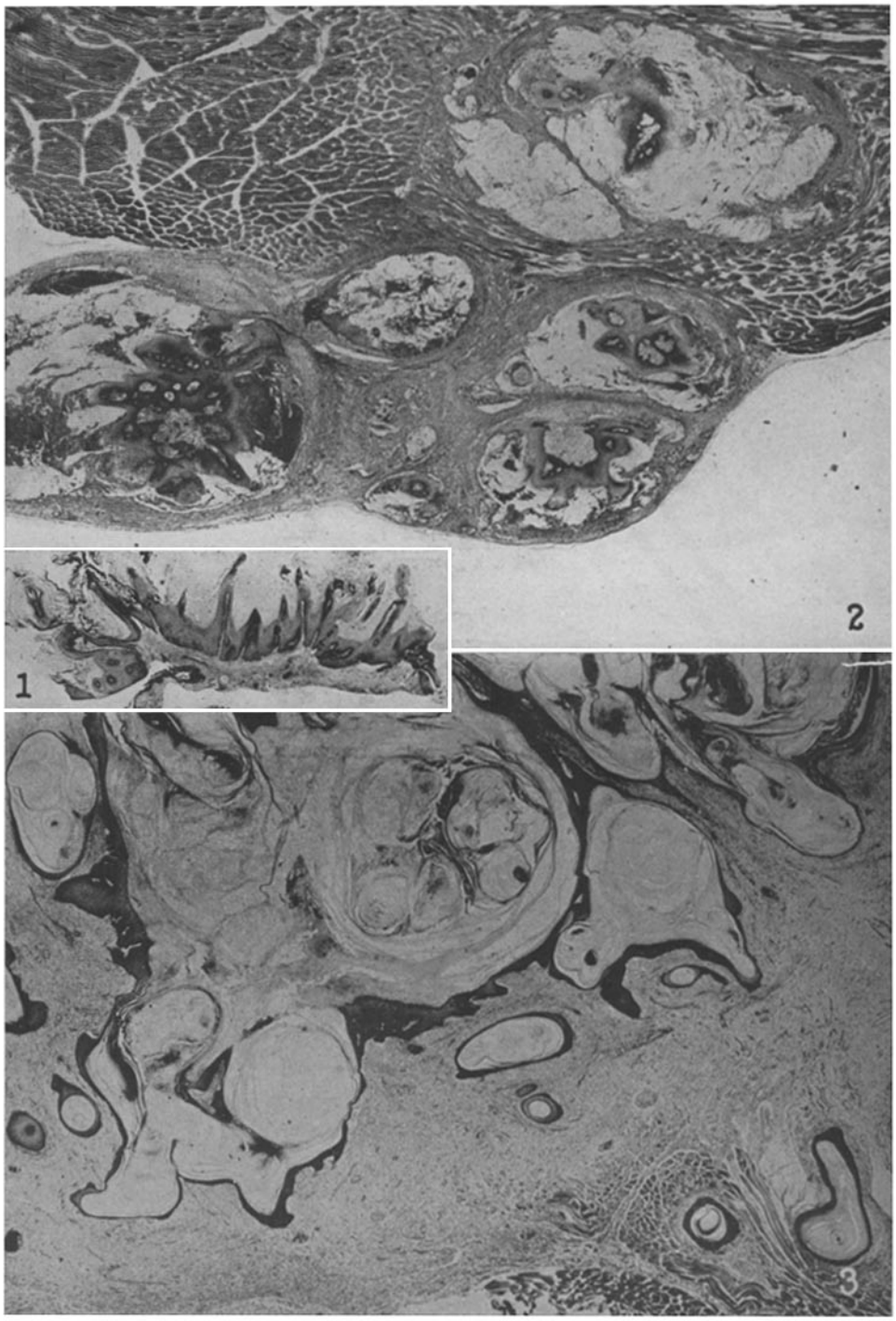
PLATE 36

Figs. 1 and 2. Effects of the Virus on a Tar Papilloma of the Domestic Rabbit.—

FIG. 1. Cross-section of a growth excised from the ear of D. R. 3-73. × 12.

FIG. 2. Results of implanting in the leg muscle bits of the tumor of Fig. 1, which had been exposed to the virus. Each fragment has given rise to a growth with the morphology of the original tumor. The proliferating epithelium has rounded into knobs connected with the periphery by connective tissue pedicles, and keratinization has taken place *outwards*. Animal killed on the 38th day. No trace was found of the tumor bits used for the control implantations. × 12.

FIG. 3. For comparison with Fig. 2. Part of a nodule consisting of virus papilloma tissue, which resulted from the intramuscular implantation of bits of another tar papilloma exposed to virus. The epithelium has rounded into cysts and is keratinizing *inwards*, as always with virus papillomas under such conditions. × 12.



Photographed by Joseph B. Haulenbeek

(Rous and Kidd: Effects of papilloma virus upon implanted tar tumors)

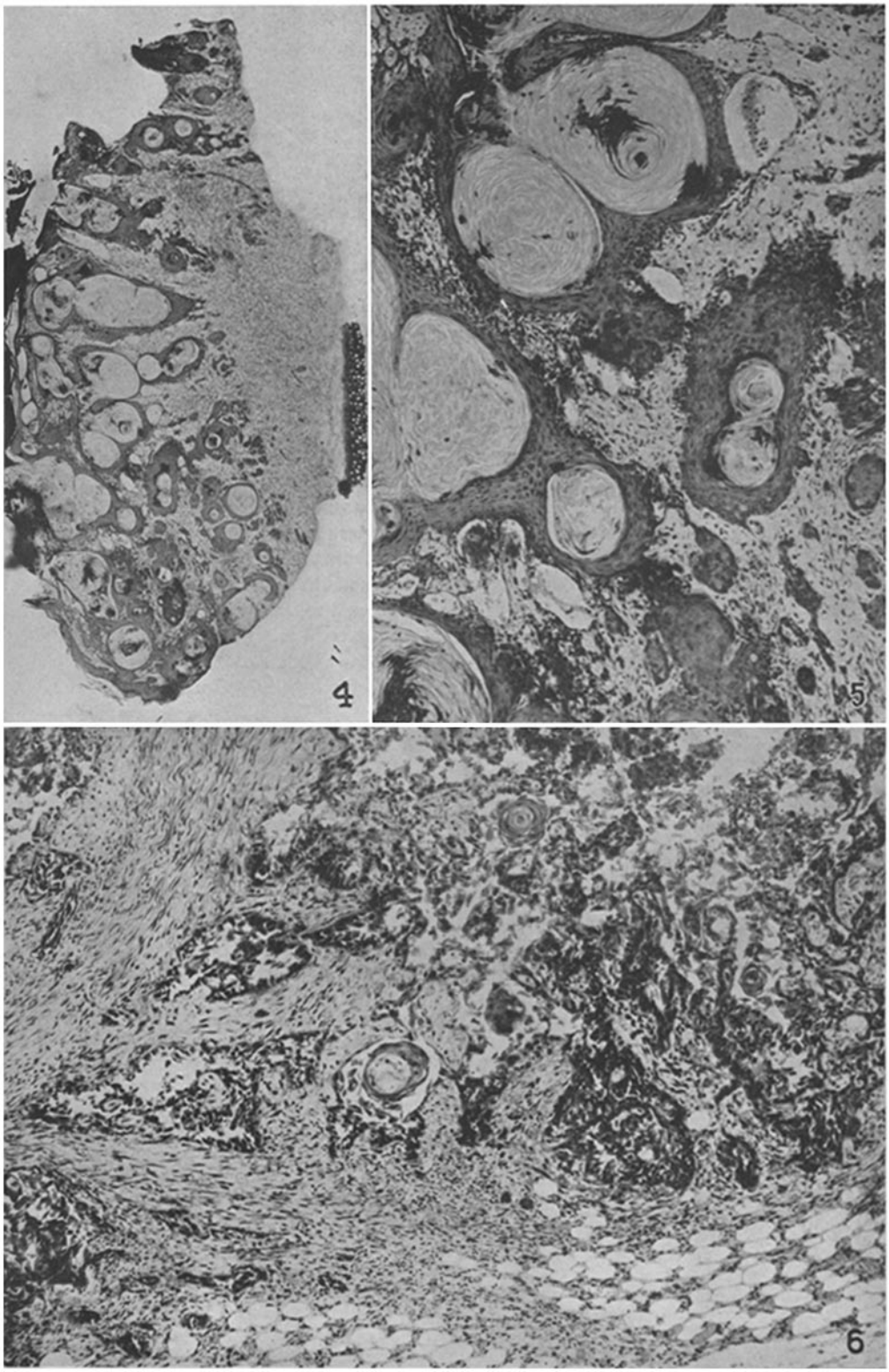
PLATE 37

Effects of the Virus on a Tar Carcinomatoid of the Domestic Rabbit.—

FIG. 4. Cross-section of the piece of tumor removed from the ear of D. R. 4-97. About half of the growth was left behind, and this disappeared in the course of the 20 days before the animal died. Tarring of the other ear had been continued. × 20.

FIG. 5. Part of the specimen at a higher magnification. It looks like a squamous cell carcinoma. × 80.

FIG. 6. Periphery of the nodule, 1.5 cm. in diameter, which resulted from implantation in the subcutaneous tissue of fragments of the tumor, which had been exposed to the papilloma virus. The growth is actively malignant and more anaplastic than the original carcinomatoid. At the lower left-hand corner of the photograph its cells can be seen invading the fatty tissue. There is no sign that it has been altered by the virus in the direction of papillomatosis. The control fragments had completely vanished. × 80.



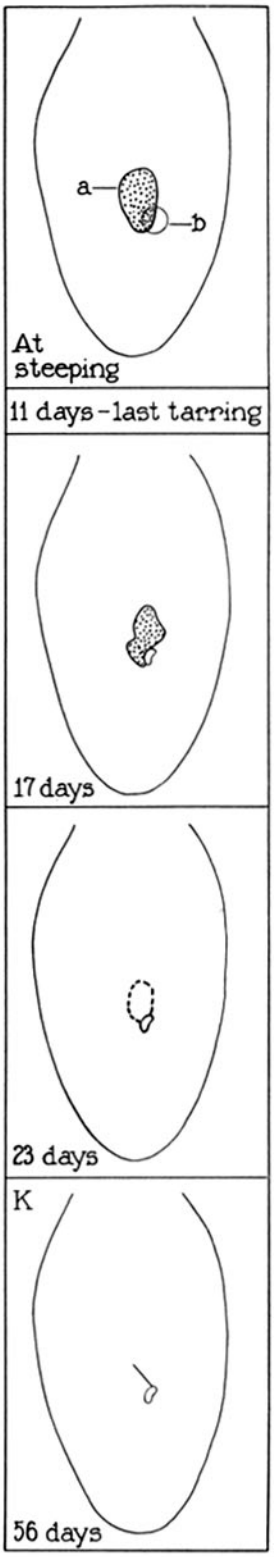
Photographed by Joseph B. Haulenbeek

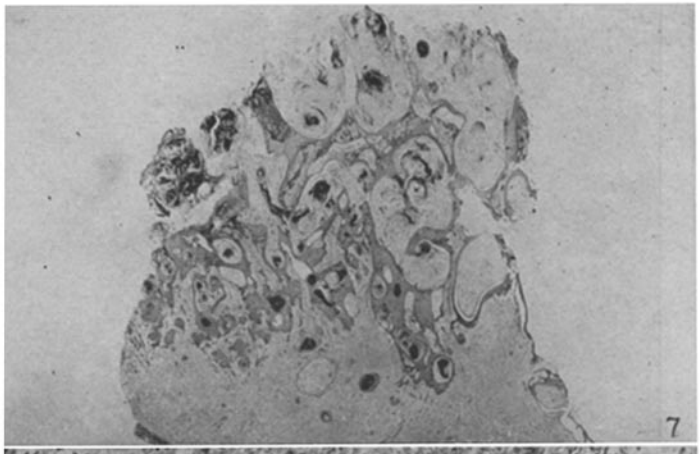
(Rous and Kidd: Effects of papilloma virus upon implanted tar tumors)

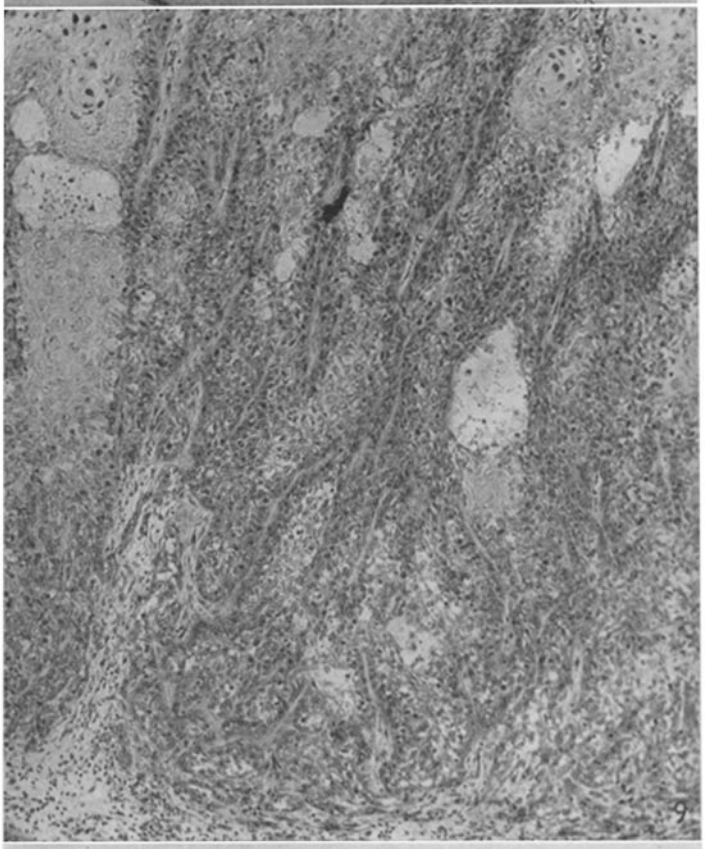
PLATE 38

Effects of the Virus on a Tar Carcinomatoid of D. R. 5-10.—

FIGS. 7 to 9. The growth (*a*) was a raised, raw disc, 2.3 cm. across, when a small part of it was punched out, as indicated by the circle in the outline drawing. Some of the near-by tissue was removed incidentally. No tarring had been done for 10 days and the neoplastic tissue was rounding up into cysts. Fig. 7 (× 6) shows in cross-section the tumor material procured. It was cut fine and implanted in the usual way, tarring was resumed for 11 days more, and on the 56th day the animal was killed. The carcinomatoid was gone by then, leaving a mound covered with smooth epidermis next the small hole in the ear. A slice was taken through this mound, as indicated by the heavy line in the lowest drawing. Sections showed it to consist entirely of connective tissue save at one small spot (arrow) where were dying carcinomatoid cells surrounded by foreign body giant cells (Fig. 8; × 5). The overlying epidermis was devoid of downgrowths. Nothing was left where the control fragments of tumor had been implanted. Those exposed to virus, on the other hand, had given rise to an actively invasive, multicentric cancer, 2 cm. across (Fig. 9; × 30).







Photographed by Joseph B. Haulenbeek

(Rous and Kidd: Effects of papilloma virus upon implanted tar tumors)

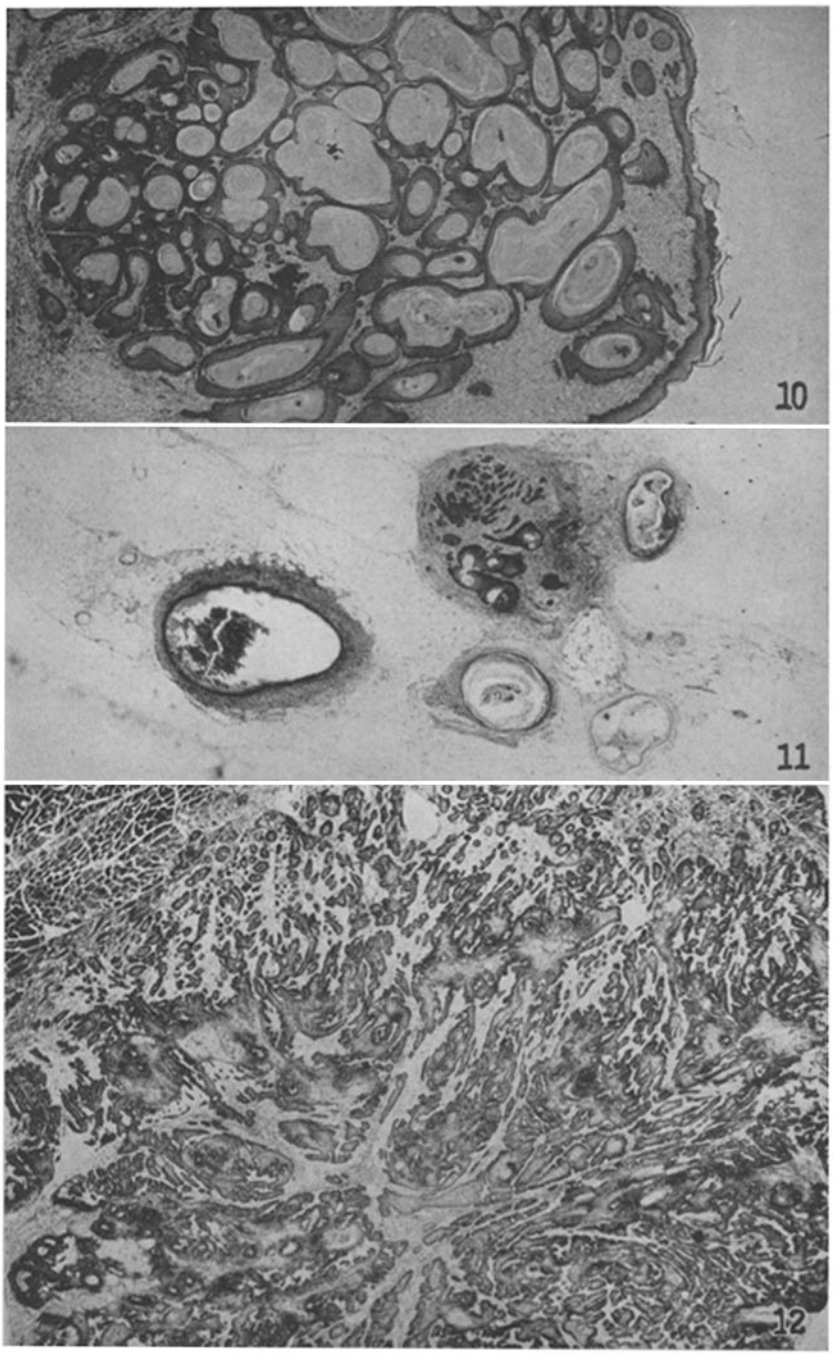
PLATE 39

Effect of the Virus on a Tar Carcinoma of Cottontail Rabbit W. R. 100 E (Table II).—

FIG. 10. Section of the biopsy specimen utilized for the test. The growth is a cystic, squamous cell carcinoma of orderly character. × 18.

FIG. 11. Cross-section of the only nodule found at any of the five situations where control fragments of the growth had been implanted. It was 0.2 cm. across. All except one of the bits of tumor tissue are dying or dead, after having formed keratinized cysts. This one has started to grow, and the tongues of neoplastic cells have begun to form cysts with a thick outer layer of living epithelium, like those in the original neoplasm. (For higher magnification see Fig. 13.) × 14.

FIG. 12. Part of one of the large growths found where the cancer fragments exposed to virus had been put. It is a very aggressive carcinoma, wholly unlike the original tar tumor. (See Fig. 14.) × 14.



Photographed by Joseph B. Haulenbeek

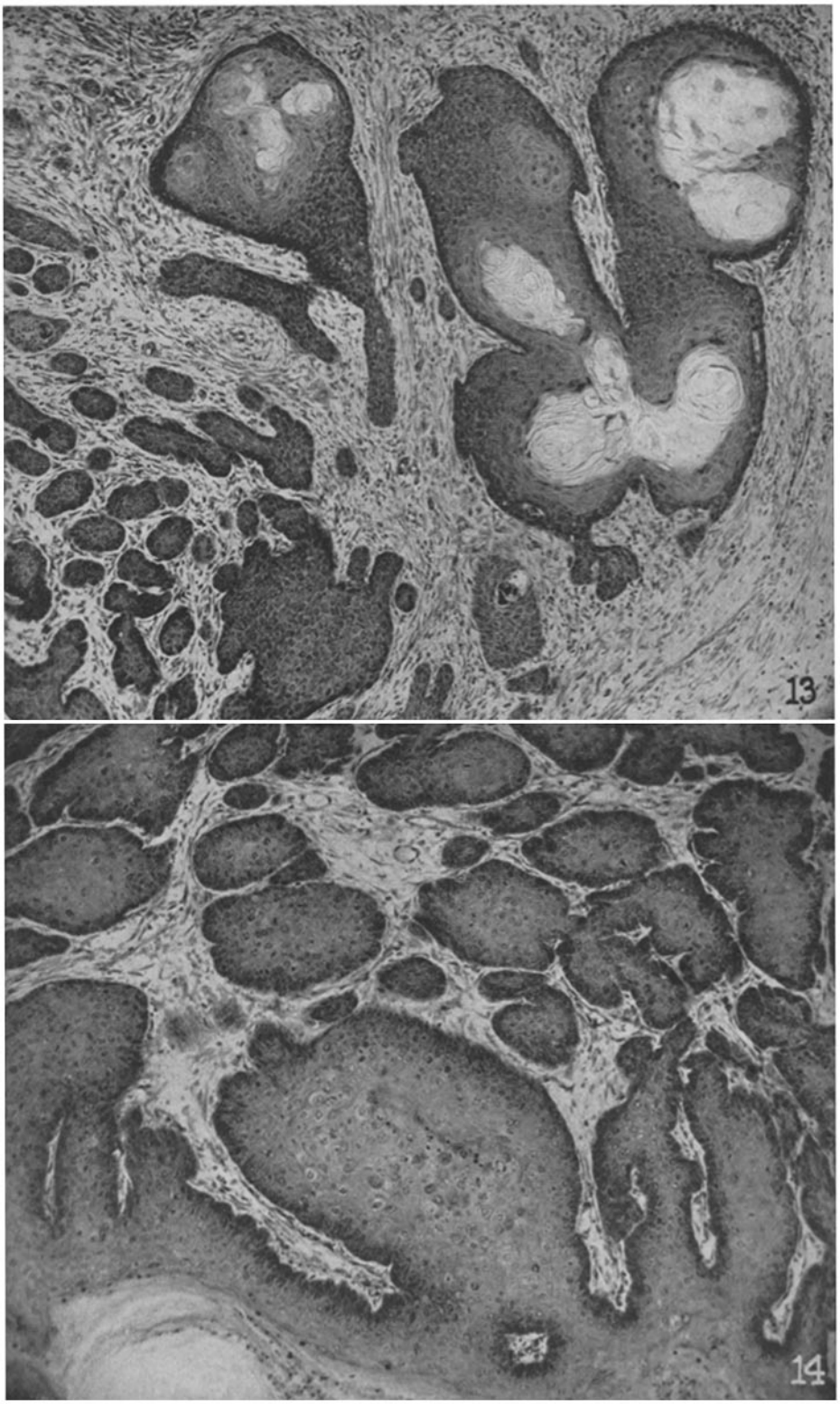
(Rous and Kidd: Effects of papilloma virus upon implanted tar tumors)

PLATE 40

The implantation growths of Figs. 11 and 12 at high magnification,—to show the alterations brought about by the virus.

FIG. 13. Growing portion of the control nodule of Fig. 11. The tumor resembles the original cancer cytologically. × 95.

FIG. 14. The implantation cancer of Fig. 12. The tongues of carcinomatous tissue are much coarser than those in the control nodule, and the cells are larger and exhibit stigmata indicative of the influence of the virus. × 95.



Photographed by Joseph B. Haulenbeek

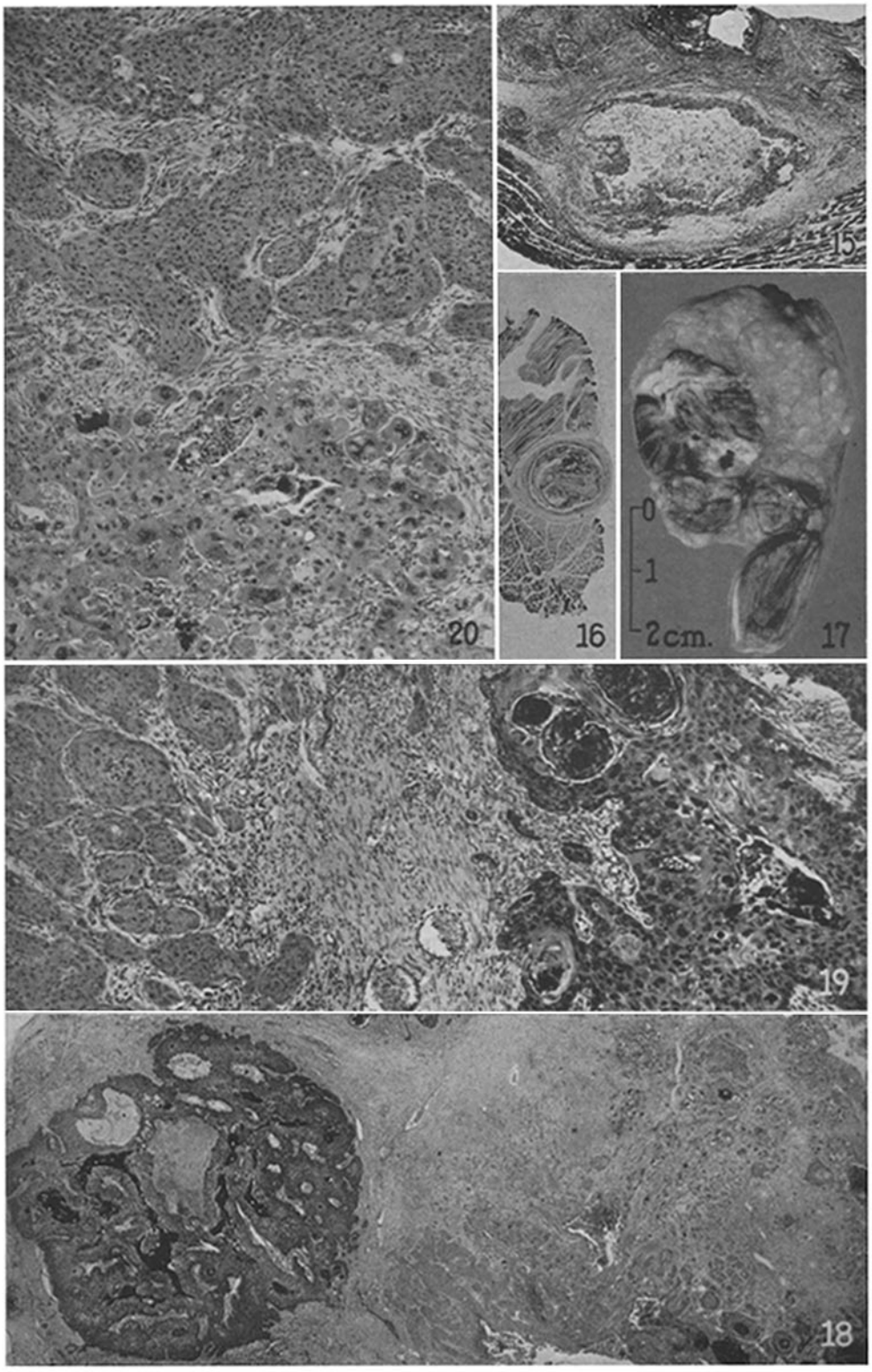
(Rous and Kidd: Effects of papilloma virus upon implanted tar tumors)

PLATE 41

Stimulating Influence of the Virus on the Tar Cancers of Cottontail Rabbit W. R. 61 N (Table II).—

FIGS. 15 to 20. The tumor on the ear was composed of three distinct types of carcinomatous tissue. In the hashed material used for implantation, some ordinary hair follicles were present. When the animal was killed 72 days later living cancer was found at only one of the four spots where the control implants had been put, and at this one as a degenerating layer on the wall of a minute cyst (Fig. 15; × 16). At the other three spots the growth had either wholly disappeared or died by keratinization (Fig. 16; × 12). The material exposed to virus on the other hand had given rise to large growths, three of them containing a great deal of cancerous tissue, with virus papilloma as well, while the fourth was composed wholly of the latter. The two kinds of growth were mostly separate, as in Fig. 17 (natural size). In the growth pictured the papilloma tissue consisted of well demarcated cysts filled with keratinized material, markedly striated and much of it melanotic, whereas the adjoining cancer was pale, irregularly lobulated, and dotted with necroses. From neither component could any virus be obtained. Another tumor was almost wholly cancerous (Fig. 18; × 12), and all three types of carcinoma present in the ear tumor were found in it (Figs. 19 and 20; × 74).

In this instance the virus greatly stimulated the cancerous cells but did not alter their morphology. The papillomatous tissue resulting from its action on the implanted fragments must have been derived from the hair follicle epithelium included in the cancerous tissue, since this latter contained no melanoblasts.



Photographed by Joseph B. Haulenbeek

(Rous and Kidd: Effects of papilloma virus upon implanted tar tumors)